European Medicines Agency

ASSESSMENT REPORT

FOR

IDflu

Common Name: Influenza vaccine (split virion, inactivated)

Procedure No. EMEA/H/C/000966

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 Sanofi Pasteur SA submitted on 3 December 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for IDflu, through the centralised procedure under Article 3 (2) (b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 21 June 2007. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of significant technical innovation.

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The applicant applied for the following indication:

9 microgram strength:
Prophylaxis of influenza in adults up to 59 years of age, especially in those who run an increased risk of associated complications.

The use of IDflu should be based on official recommendations.

15 microgram strength:
Prophylaxis of influenza in individuals 60 years of age and over, especially in those who run an increased risk of associated complications.

The use of IDflu should be based on official recommendations.

Scientific Advice:
The applicant did not seek scientific advice at the CHMP.

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

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

Rapporteur: **Gonzalo Calvo Rojas** Co-Rapporteur: **Tomas P Salmonson**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 3 December 2007.
- The procedure started on 26 December 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 14 March 2008. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 14 March 2008. In accordance with Article 6(3) of Regulation (RC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- The BWP discussed IDflu during their meeting on 14-16 April 2008 and adopted a BWP report to the CHMP.
- During the meeting on 21-24 April 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24 April 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 22 July 2008.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 5 September 2008.
- The BWP discussed IDflu during their meeting on 15-17 September 2008 and adopted a BWP report to the CHMP.
- During the CHMP meeting on 22-25 September 2008, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 12 November 2008
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the CHMP List of Outstanding Issues to all CHMP members on 1 December 2008.
- The BWP discussed IDflu during their meeting on 8-10 December 2008 and adopted a BWP report to the CHMP.
- During the meeting on 15-18 December 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to IDflu on 18 December 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 17 December 2008.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Human influenza viruses, the disease, and its prevention.
Influenza is a contagious respiratory illness caused by influenza viruses. The infection can cause mild to severe illness, and even can lead to death. Uncomplicated illness is characterized by the abrupt onset of respiratory symptoms, such as fever, myalgia, headache, malaise, and non-productive cough. The illness usually resolves after about one week for the majority of persons. However, the viral infection can cause primary influenza viral pneumonia, exacerbate underlying medical conditions (such as diabetes, pulmonary or cardiac disease), or lead to secondary bacterial pneumonia, all conditions which can lead to death.

Influenza viruses cause disease among persons in all age groups, but the risks for complications, hospitalizations, and deaths are higher among persons aged >65 years, young children, and persons with chronic respiratory or cardiovascular diseases or other serious underlying condition, where the infection lead to severe complications of the condition.

Influenza is a globally important health problem, and epidemics of influenza typically occur annually during the fall or winter months. Although the incidence of influenza each season is very variable, usually range from 5 to 20% of the population.

Influenza viruses are members of the Orthomixoviridaes family. This family is characterized by including a number of enveloped viruses whose genome is made up of several segments of linear, negative sense, single-stranded RNA. Within the family, influenza viruses are classified in three virus types (A, B and C) based in the absence of serological cross-reactivity of the two major internal proteins (Nucleoprotein and Matrix protein). Influenza A and B viruses are the two types that cause epidemics in humans. Influenza A viruses are further classified into subtypes on the basis of the two major surface proteins of the virus: the hemagglutinin (H) and the neuraminidase (N). To date, 16 different hemagglutinin subtypes (named H1 to H16) and 9 different neuraminidase subtypes (named N1 to N9) have been identified. Influenza A viruses can be further broken down into different strains. The current subtypes of influenza A viruses circulating in humans are H1N1 and H3N2. Influenza B viruses are not divided into subtypes, but also can be further broken down into different strains.

Influenza virus infection induces both humoral and cellular immune responses, and a large body of data shows that both elements play a role in prevention of infection and in viral clearance during infection. The protective role of anti-HA antibodies has been widely demonstrated. In fact, anti-HA antibodies can neutralize the virus and inhibit hemagglutination induced by the virus in vitro. It has been repeatedly shown that in general high hemagglutination inhibition (HI) titres correlate directly with protection, and in fact, there are EMEA criteria based on this test, which should be fulfilled yearly for inactivated vaccines whenever the strain composition of the vaccine is changed. Studies in animal models and human have also demonstrated that CD8 (CTL) (cytotoxic T lymphocytes) cells are involved in protection and recovering from infection, but the contribution of CD4 mediated immune response in protection /recovery is much less well known. Thus, different elements contribute to the protective immune response but the exact contribution of each of them and the identification of a clear-cut surrogate parameter for protection are far from clear.

Antigenic variation is a characteristic of the virus, and involves primarily the two external glycoproteins of the virus: the HA and the NA. The mechanism by which small changes (mutations) are introduced continually over time in these two genes is denominated “antigenic drift." The mutations are introduced during the replication of the viral genome in the infected cell, and if a new antigenic variant (with mutations in the HA and/or NA) is generated and this variant is not recognized by the body's immune system mounted against a previous infection, the variant will expand in the

human population. Thus, every season the composition of the vaccine needs to be adapted in accordance with the circulating strains.

Vaccination against influenza virus is an important public health measure and is the primary strategy for preventing influenza infections and related severe complications. However, the immune response to vaccination in elderly is comparatively lower with respect to younger adults, highlighting the need for more immunogenic and effective vaccines for this population.

About the product
IDflu is an intradermal influenza vaccine (split virion, inactivated) propagated in fertilized hens' eggs with a Micro-Injection System for delivery the vaccine via the intradermal route. The aim of the intradermal (ID) vaccination is to allow for the presentation of the vaccine antigens to a large number of dermal or interstitial dendritic cells which are able to induce an efficient immune response. In addition, the intradermal route of administration likely presents a lower risk of local neurovascular injury due to the short size of the needle.

The manufacturing process used to develop the intradermal influenza vaccine is based on the Applicant's intramuscular seasonal influenza vaccines process with an addition of a concentration step to obtain a concentrated monovalent bulk in order to formulate a lower volume vaccine for intradermal use. Two vaccine dosages are formulated from the concentrated monovalent bulk to target two populations, adults and the elderly. The indication is: Prophylaxis of influenza in adults from 18 to 59 years of age, especially in those who run an increased risk of associated complications (9 microgram strength) and prophylaxis of influenza in individuals 60 years of age and over, especially in those who run an increased risk of associated complications (15 microgram strength).

The vaccine is a suspension for injection in pre-filled syringe with a Micro-Injection System which should allow easy fast and reproducible injection by the intradermal route. The Micro-Injection System features an integral micro-needle which protrudes 1.5 mm from the proximal end of the glass syringe, a needle penetration depth limiter to ensure correct needle placement and a needle shielding system that protects the needle after injection hence reducing the risk of inadvertent needle-stick injury.

The active substances of IDflu are the purified influenza virus antigens of type A (H1N1), type A (H3N2) and type B strains. The composition of the influenza strains will be those officially recommended for the season.

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The eligibility to the centralised procedure was based on demonstration of significant technical innovation, relating to the use of the intradermal route and the novel intradermal delivery system.

2.2 Quality aspects

Introduction

IDflu is an intradermal influenza vaccine (split virion, inactivated) propagated in fertilized hens' eggs with a Micro-Injection System for delivery the vaccine via the intradermal route. Two vaccine dosages are formulated from the concentrated monovalent bulk (i.e. the drug substance) to target two populations, adults (9µg hemagglutinin/dose) and the elderly (15µg hemagglutinin/dose). The intradermal influenza vaccine contains antigens from each of the three influenza virus strains Type A (H1N1), Type A (H3N2) and Type B in phosphate buffered saline (PBS) solution. The nominal dose of product is 0.1 ml. The composition of the influenza strains will be the officially recommended ones by the appropriate regulatory bodies.

The vaccine is a colourless and opalescent suspension for injection in pre-filled syringe (type I glass) with a Micro-Injection System. The Micro-Injection System is a pre-filled, ready-to-use syringe. It features an integral micro-needle which protrudes 1.5 mm from the proximal end of the glass syringe,

a needle penetration depth limiter to ensure correct needle placement and a needle shielding system that protects the needle after injection hence reducing the risk of inadvertent needle-stick injury.

Drug Substance

The concentrated monovalent bulk is a concentrated aqueous suspension of inactivated, split viral particles that were propagated in embryonated eggs and purified by zonal centrifugation. The reference viral strains used to prepare the concentrated monovalent bulks are selected based on the annual recommendations made by the World Health Organization (WHO) and are supplied by WHO Collaborative Centers. The influenza strains are derived as follows:

- Influenza type A seed strains: since the early 1970s, type A strains are prepared by genetic reassortment using the field strains chosen by WHO experts and an A/PR8/34 or PR8-like master strain to ensure a satisfactory growth in embryonated eggs.
- Influenza type B seed strains: type B strains are field isolates because no master B strain has yet been found that improves the growth performance of influenza B viruses in egg-based production systems.

For 2006-2007 manufacturing campaign used for validations and Phase III clinical studies, the reference viral strains were provided by World Health Organization (WHO) Collaborative Centers and were as follows:

- Strain A/New Caledonia/20/99 IVR-116 (H1N1): reassortant strain between A/New Caledonia/20/99 and IVR-6 (A/Texas/1/77)
- Strain A/Wisconsin/67/2005 NYMC X-161 (H3N2): reassortant strain between A/Wisconsin/67/2005 and A/PR/8/34
- Strain B/Malaysia/2506/2004: non-reassortant strain

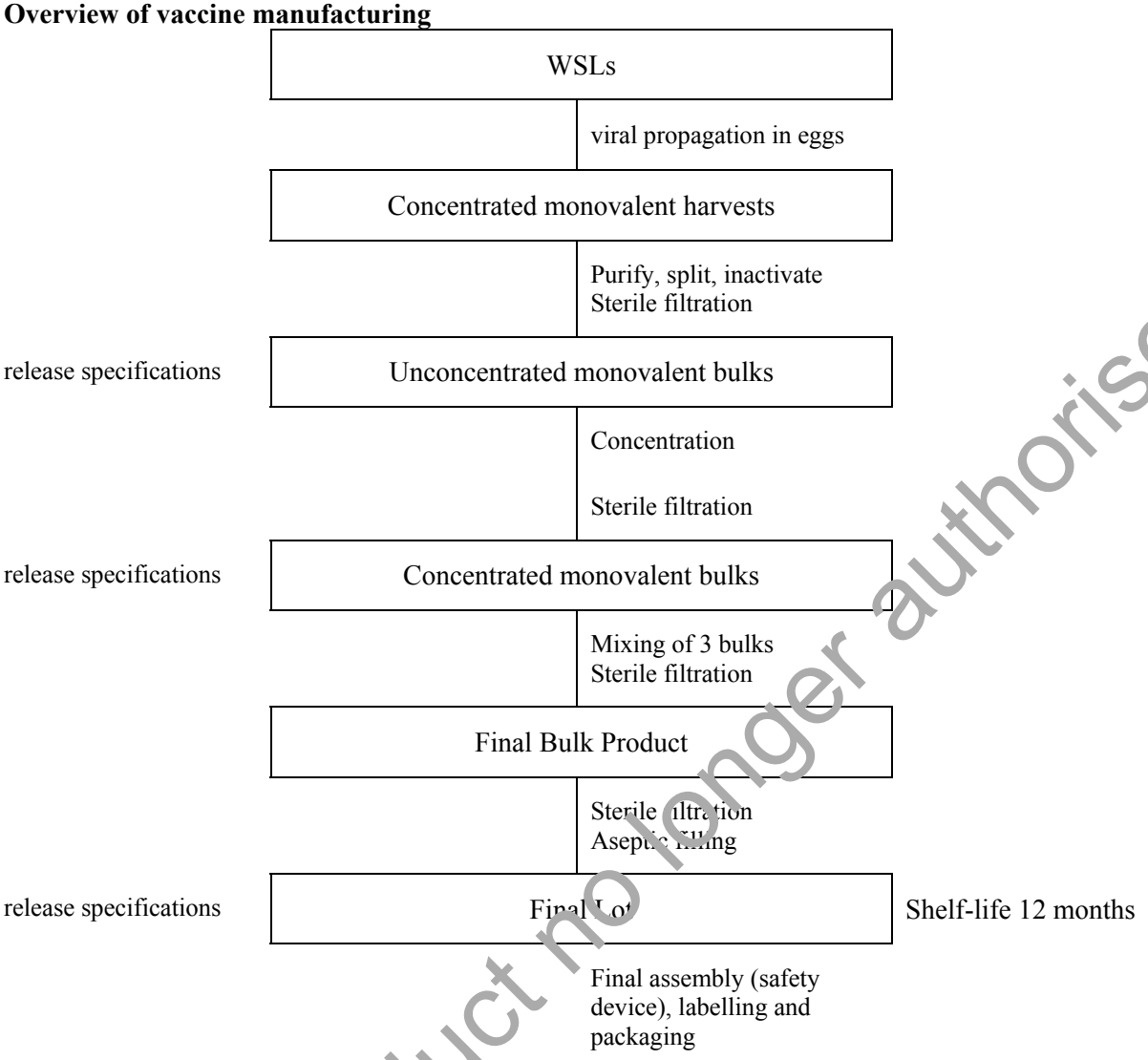
- Manufacture

The manufacturing process of the drug substance consists of two phases:

- The manufacture of the intermediate: the unconcentrated monovalent bulk
- The manufacture of the drug substance: the concentrated monovalent bulk

The manufacturing process used to develop the intradermal influenza vaccine is based on the Applicant's intramuscular seasonal influenza vaccine (Vaxigrip) process with the addition of a concentration step to obtain a lower volume vaccine for intradermal use. An overview of the manufacturing process is given below.

Overview of vaccine manufacturing



Manufacture of unconcentrated monovalent bulk
The influenza viral strains are propagated in embryonated hen's eggs. The allantoic fluid is harvested, clarified by centrifugation and filtration, and then concentrated by ultrafiltration. The concentrated monovalent harvest is then purified by zonal centrifugation. A filtration is performed. Octoxynol-9 solution (the splitting agent) is added, the split viral suspension is clarified by centrifugation and the octoxynol-9 content is reduced by diafiltration. A formaldehyde solution is added to the viral suspension for the inactivation. The inactivated and split viral suspension is filtered and diluted in PBS resulting in the unconcentrated monovalent bulk.

Manufacture of concentrated monovalent bulk
One or several batches of unconcentrated monovalent bulk are concentrated by ultrafiltration and then filtered, resulting in the concentrated monovalent bulk (drug substance). The concentrated monovalent bulk is filled into containers and stored at 5°C.

Banking system
The manufacture of the seed lot system from the inoculation of eggs to the final filling and storage, used for all three influenza strains, has been adequately described.

In compliance with Ph.Eur. monograph 0158, the total number of passages from the approved reassortant virus up to the Working Seed Lot will not exceed 15.

The tests performed on seed lots comply with Ph. Eur. monograph 0158 “Influenza vaccine (split virion, inactivated)” except for the determination of the infectious titer, which is an additional test. The applicable requirements of Ph. Eur. monograph 0153 “Vaccines for Human use” are also covered.

Controls of critical steps and intermediates
The following three manufacturing steps are considered critical for the concentrated monovalent bulk quality:

- Purification
- Splitting
- Inactivation

The in-process controls, limits and tests are considered adequate. Several in-process controls are performed on the concentrated monovalent bulk to ensure the proper control of the manufacturing process as a whole. Appearance, pH and endotoxin content are carried out in compliance with Ph. Eur. monographs. Assays for residual formaldehyde and Ovalbumin have been described and found acceptable.

The only intermediate in the drug substance manufacturing is the unconcentrated monovalent bulk. Specifications, methods and results of batch analyses for this intermediate have been provided and are considered appropriate. The specifications are in accordance with Ph.Eur. monograph 0158

Process validation and/or evaluation
Validation data was provided for the manufacturing steps considered to be critical: purification, splitting and inactivation. In addition, data on clearance of neomycin, preservation of neuraminidase activity, and elimination of impurities during the concentration step were provided.

The inactivation and splitting have been adequately validated.

A major objection was raised at day 120 regarding the validation of the avian leucosis viruses and avian cultivable mycoplasma inactivation by the splitting and inactivation process. Upon request, the Applicant provided satisfactory data demonstrating that the process is capable of inactivating these pathogens and therefore the major objection was considered resolved.

Manufacturing process development
The manufacturing process was developed starting from the manufacturing process of the influenza monovalent bulk used in the Applicant’s seasonal influenza vaccine. The main target of the manufacturing process development was to obtain a sufficiently high hemagglutinin content in a reduced volume (0.1 ml) suitable for intradermal administration.

In order to perform clinical dose ranging studies, different strengths of finished product were developed all along the process development. A concentration factor was selected and used for the manufacture of the Phase III clinical batches. The concentration step was performed by ultrafiltration which enables hemagglutinin and ovalbumin to be separated due to their different molecular weights.

Characterisation
The characterisation tests focus primarily on the characterization of the active moieties of the viruses, i.e. the hemagglutinin and neuraminidase antigens. In addition, several tests are performed to verify the purity of the unconcentrated and concentrated monovalent bulks.

HA and NA identification as well as HA content have been tested at different steps of the production (from MSL to concentrated monolvalent Bulk) in accordance to the Ph Eur.

Three impurities are routinely assayed in the unconcentrated and concentrated monovalent bulk: Ovalbumin, Octoxynol-9 and Formaldehyde. The limits for the impurities are justified. Results of three batches of unconcentrated bulk for each strain have been presented demonstrating levels below the acceptance limit in all cases.

- Specification

Control of drug substance

The drug product specifications, combined with the specifications for unconcentrated monovalent bulk, comply with the relevant Ph. Eur. monograph.

Batch analysis

Data from a sufficient number of batches of Concentrated Monovalent Bulk of A/New Caledonia (H1N1), A/Wisconsin (H3N2), and B/Malaysia were provided. The batches were used to formulate the clinical batches and to show manufacturing consistency. All batches complied with the defined acceptance criteria.

Data from corresponding batches of Unconcentrated Monovalent Bulk was presented. Also these batches fulfilled the specifications.

Container Closure System
Stainless steel vessels are used for the storage of the Unconcentrated and Concentrated Monovalent Bulks. Seals are made of silicone elastomer. Each supplier of vessels is approved by the Applicant based on a list of specifications. Before use, each vessel is qualified by the Applicant in accordance with GMP requirements.

- Stability

Stability data for the drug substance (Concentrated Monovalent Bulk) for 12 months at 5°C and 28 days at 25°C were provided for three lots of each strain. The Applicant has established suitable controls procedures to justify a shelf life of 12 months for both the 9 µg/dose the 15 µg/dose drug product.

Drug Product
The Drug Product is a trivalent, split virion, inactivated influenza vaccine to be administered by the intradermal route. It is a sterile, aqueous suspension containing a mixture of two influenza virus strains types A (H1N1 and H3N2) and one influenza virus strain type B in phosphate buffered saline (PBS) solution. The suspension is opalescent. The nominal dose of product is 0.1 ml. Two dosages were presented in this application, a 9 µg hemagglutinin/dose and a 15 µg hemagglutinin/dose.

The qualitative and quantitative composition of the Medicinal Product is provided in the following table (9 µg hemagglutinin/dose). The 15 µg hemagglutinin/dose has the same composition except for the amount of HA.

Composition of Medicinal Product – 9 µg Hemagglutinin/Dose

Component	Amount on a per unit basis	Function	Reference to quality standards
Influenza virus A (H3N2) strain, split virion, inactivated	9 µg of hemagglutinin	Active substance	Ph. Eur. monograph 0158
Influenza virus A (H1N1) strain, split virion, inactivated	9 µg of hemagglutinin	Active substance	Ph. Eur. monograph 0158
Influenza virus B strain, split virion, inactivated	9 µg of hemagglutinin	Active substance	Ph. Eur. monograph 0158
PBS solution	Buffering agent and diluent		
Water for injections	q.s. 0.1 ml	Solvent	Ph. Eur. monograph 0169

The suspension is presented in a 0.5 ml Type I glass barrel with special tip for intradermal administration. The glass barrel is fitted with a staked needle, which is covered by a needle shield. The syringe is closed by an elastomeric plunger stopper. The container closure system is assembled with a plastic pusher and a Needle Shielding System (NSS) to form a Micro-Injection System. The assembled product is packaged in a blister pack, which is then packaged in an outer cardboard box.

- Pharmaceutical Development

The pharmaceutical development was based on experience gained by the Applicant with its seasonal influenza vaccine for intramuscular administration. Different strengths of the intradermal influenza vaccine were tested to identify the strength equivalent to 15 µg hemagglutinin/strain/dose of the intramuscular influenza vaccine for each target population. The doses chosen were 9 µg for the adult population and 15 µg for the elderly population.

The development of the intradermal influenza vaccine specifically focused on the development of the Micro-Injection System. The key targets of the development of the Micro-Injection System were to facilitate the correct intradermal injection of the vaccine without any user training and to provide protection against unintentional puncturing post-injection. An intermediate version of the Micro-Injection System was used in Phase I clinical studies, however the final Micro-Injection System was used in the Phase II and Phase III clinical studies.

- Adventitious Agents

Biological materials used in the production of the drug substance and drug product are:

- *Fertilized SPF eggs* (used in the production of seed lots). The fertilized SPF eggs comply with Ph. Eur. 5.2.2. SPF eggs are sourced from validated external suppliers guaranteeing appropriate standards.
- *Embryonated eggs* (used in the production of monovalent bulks). The embryonated hen eggs from healthy flocks are used for the production of the concentrated monovalent harvest. The flocks are inspected, the animals immunized and adequately controlled.
- *Influenza strains.* Quality Control tests were performed for each of the three influenza strains by the WHO Collaborative Center supplying the strain. Compliance with the Center's specifications is certified by a certificate of analysis, accompanying each virus at delivery.

During the manufacturing process of the concentrated monovalent bulk of influenza strains, the virus in suspension is first split by octoxynol-9, and then inactivated by formaldehyde. The splitting-inactivation process was proven effective for the complete inactivation of all three influenza strains. The inactivation method has also been demonstrated to inactivate avian leucosis virus and mycoplasma.

No material derived from animals naturally susceptible to TSE is used for the preparation of the MSL, ISL, WSL or the concentrated monovalent bulk batches.

- Manufacture of the Product

  - The manufacture of the vaccine consists of the blending of the concentrated monovalent bulks of the three influenza strains with PBS solution. The mixture is homogenized and is then filtered to obtain the Final Bulk Product.
  - Aseptic filling is performed in compliance with current GMP regulations on the sterile manufacture of medicinal products.

The filled syringes are assembled with the Needle Shielding Systems, the pushers are fixed to the elastomeric stoppers and the Needle Shielding Systems are labelled. The assembled products are blistered and placed in outer cardboard boxes. After packaging, the Medicinal Product is stored at +5°C ± 3°C.

Process Validation and/or Evaluation
The manufacturing of the Finished Product has been appropriately validated. Three full-scale batches of Final Bulk Product of each strength were manufactured under worst-case conditions in terms of possible degradation of the product (i.e., maximal mixing speed and time). All six batches complied with the in-process control acceptance criteria and the FBP specifications.

Three batches of each strength of the FBP were filled .The study showed that the filling process consistently resulted in a Finished Product meeting the predefined specifications.

The aseptic conditions of the filling process were evaluated. No contaminated syringes were detected.

- Product Specification

The specifications for the vaccine comply with Ph.Eur and are found acceptable. The specifications for the 9 and 15 µg hemagglutinin/dose strength are the same apart from the specification for Hemagglutinin content.

Batch analysis
Batch release data from three batches of 9 µg HA/dose and three batches of 15 µg HA/dose were presented. All results complied with the acceptance criteria. The HA results confirm a consistency between batches of the vaccine.

Container closure
The packaging materials in contact with the product are:

1. The syringe, consisting of:
   - 0.5 ml glass barrel: Type I glass, designed with a special tip for intradermal administration and lubricated with silicone oil;
   - Needle: lubricated (silicone oil) stainless steel needle, with a length available for injection of 1.5 mm.

2. The elastomeric plunger stopper: lubricated (silicone oil) chlorobutyl stopper.

The syringe is equipped with a needle shielding device with a protective sleeve that automatically covers the needle after injection, to protect from needle stick injuries.

The justification for choosing the components of the Micro-Injection System has been provided and compatibility, integrity and technical performance has been satisfactorily described.

- Stability of the Product

Stability testing was performed on three batches of each strength of the Finished Product in real-time / real-temperature conditions (12 months at +5°C ± 3°C) and in accelerated conditions (1 month at +25°C ± 2°C). The batches used for the process validation were also used for the stability studies, i.e. the batches have been produced using the phase III process.

The stability study has shown, that after 12-month storage in real-time conditions (+5°C ± 3°C), the physico-chemical parameters are stable and conform to the acceptance criteria.

During the initial evaluation a major objection was raised in relation to the stability results of the drug product. The Applicant provided a satisfactory response , including a committment to perform a further study to address this issue The major objection is therefore considered resolved and the proposed shelf life of 12 months (at +5°C ± 3°C) is found acceptable.

2.3 Non-clinical aspects

Introduction

Two non-clinical pharmacology studies were conducted in mice. ID injections were performed with the Mantoux method as the Micro-Injection System was not adapted to the mouse skin. These studies demonstrated that ID injection results in an equal or stronger antibody response and hemagglutination inhibition (HI) when compared to IM injection.
Two repeat dose toxicity studies were performed in rabbits.

Toxicology studies were conducted according to GLP.

Pharmacology

- Primary pharmacodynamics

The studies carried out in mice were intended to evaluate the immunogenicity of a trivalent influenza vaccine when delivered by the ID route in comparison to the same vaccine administered IM. In addition, the persistence of antibodies was evaluated. The levels of antibodies were determined by ELISA against antigens of the three viral strains present in the vaccine and by the hemagglutination inhibition (HI) assay against the H1N1 virus only.

The immunogenicity of the ID trivalent influenza vaccine in mice is as good as the IM vaccine. The Applicant provided data from one study in mice in which antibodies to the three influenza viral strains (H1, H3 and B strains.) present in the vaccine were measured, and satisfactory immune response was raised against the three antigens.

No secondary pharmacodynamics studies were conducted.

The composition of the vaccine is identical to the already approved intramuscular vaccine. No studies on safety pharmacology are required.

Drug interactions have not been investigated

Pharmacokinetics

The Applicant has not included information regarding pharmacokinetics and pharmacokinetic drug interactions studies. Nevertheless the guideline for non-clinical testing of vaccines (CPMP/SWP/465/95) states “distribution studies should be considered … when alternative routes of administration are intended to be used.” Although this data probably would not provide clinically relevant information, this extent is unknown.

Toxicology

- Single dose toxicity

No single dose toxicity studies were conducted. According to the guideline "Pre-clinical pharmacological and toxicological testing of vaccines" (CPMP/SWP/465/95) single dose toxicity studies are required. Nevertheless single dose toxicity study was not considered necessary as the safety evaluation was assessed in the repeated dose toxicity studies.

- Repeat dose toxicity

The Applicant conducted two repeated dose toxicity studies in rabbits.

One repeated Dose Toxicity Study by the ID, IM or Alternate ID (at 6 and 9 µg HA/Influenza Strain/Dose) and IM (at 15 µg HA/Influenza Strain/Dose) Routes.

The objective of the study was to determine the local tolerance and systemic toxicity of the influenza vaccine in rabbits after IM injections at 15 µg HA/influenza strain or ID injections at 6 (low dose) or 9 (high dose) µg HA/influenza strain or alternate IM and ID administrations at two-week intervals. Similar high levels of serum IgG titers directed against A/H1N1 strain were observed in vaccinated animals, irrespective of dose and administration route. Toxicological findings were limited to local reactions at the injection site. Moderate to severe local reactions (erythema and oedema) were observed at the intradermal injection site following repeated intradermal treatment or alternate intramuscular and intradermal administrations. Repeated IM injection induced no macroscopic changes but a minimal to moderate interstitial inflammation at the injection site in all animals and a minimal to moderate muscle necrosis in one male and all females. Recovery after 14 days was almost complete at the ID sites and was partial at the IM sites with a low incidence of minimal interstitial inflammation seen microscopically.

Repeated Dose Toxicity Study by Alternate IM (at 15 µg HA/Influenza Strain/Dose) and ID (at 15 or 21 µg HA/Influenza Strain/Dose) Routes. The objective of the study was to evaluate the local tolerance of the intradermal influenza vaccine at 15 and 21 µg HA/influenza strain and systemic toxicity of the 21 µg HA/influenza strain after three administrations at two-week intervals via either the ID or IM route in rabbits.

Similar high levels of serum IgG titers directed against A/H1N1 strain were observed in vaccinated animals, irrespective of dose and administration route. One IM administration (at 15 µg HA/influenza strain) followed by two ID administrations of the influenza vaccine (at the dose levels of 15 or 21 µg HA/influenza strain) to rabbits induced no systemic changes except a slight decrease in white blood cell counts in females treated with repeated high dose (cumulative highest dose of HA, Group 3). Local reactions at the ID injection sites consisted of one to two-week erythema and edema, with a severity increasing with the number of injections and a dose related effect noted especially for edema after repeated ID injections. Histopathological examination revealed inflammation at the injection sites that partially recovered after fourteen days.

- Genotoxicity and Carcinogenicity

Genotoxicity and carcinogenicity have not been addressed. This is acceptable according to the relevant guidelines.

- Reproduction Toxicity

A toxicity study evaluated the effects of the intradermal influenza vaccine at 9 µg HA/influenza strain on female fertility, embryo-fetal development (including an evaluation of teratogenicity) and early post natal development in rabbits. No significant fertility and developmental toxicity effects have been shown in the data provided. The only adverse effects observed were the local reactions already reported in the repeated dose toxicity studies.

- Toxicokinetic data

No studies have been conducted.

- Local tolerance

Local tolerance was assessed in all repeat dose toxicity studies discussed above. In addition, a single dose local tolerance study in rabbit and repeat dose local tolerance study were performed.

After a single intradermal injection of the vaccine (9 µg HA/influenza strain) to rabbits, only minor local reactions were observed.

A dedicated repeat dose local tolerance study was performed where the European intradermal vaccine was evaluated with a corresponding US intradermal vaccine. Local reactions consisted of one to two-week erythema and oedema with a severity increasing after the second injection but not after the third one.

Ecotoxicity/environmental risk assessment

Ecotoxicity/environmental risk assessment studies have not been conducted. This is acceptable according to the guideline Environmental Risk Assessment of Medicinal Products for Human Use (CPMP/SWP/4447/00). No risk to the environment is expected from the use of this vaccine.

2.4 Clinical aspects

Introduction

The clinical development program has been carried out for two vaccine formulations intended for differentiated target populations:

- The ID Influenza Vaccine 9µg, which contains 9 µg hemagglutinin (HA) per influenza strain, is intended for use in adults up to 59 years. The Clinical Development Plan (CDP) was therefore designed to demonstrate non-inferiority of the humoral immune response to the vaccine with respect to the 15 µg IM standard of care, with a satisfactory safety profile in this population.
- The ID Influenza Vaccine 15µg, which contains 15 µg HA per influenza strain, is intended for use in elderly individuals aged 60 years and above. Therefore, the CDP was designed to demonstrate superiority of the humoral immune response to the vaccine with respect to the 15 µg IM standard of care, with a satisfactory safety profile in this population.

Data from eight studies are submitted in the present dossier. An overview of the studies is provided in the following Figure.

Overview of Clinical Development Program for ID Influenza Vaccine in Adults and Elderly Subjects

Study Number	Study Phase	Influenza Season	Hemisphere	Micro-Injection System used
Adults Population 18-59 years of age				
GID01	II	2002-2003	Northern	Intermediate
GID02	II	Vac1 2003-2004 Vac2 2004-2005 Vac3 2005	Northern Vac1,2 Southern Vac3	Vac1,2 Intermediate Final Vac3
GID15	II	Vac1 2005 Vac2 2006-2007	Southern Vac1 Northern Vac2,3	Intermediate

		Vac3 2007-2008		
GID23	III Key study	2006-2007	Northern	Final
Elderly Population >60 years of age				
GID07	II	2003-2004	Northern	Intermediate
GID09	II	2004-2005	Northern	Mantoux method
GID16	II – Key study	2005	Southern	Final
GID17	III Key study	Vac1 2006-2007 Vac2 2007-2008 Vac3 2008-2009	Northern	Final

Vac = Vaccination Number
All studies were controlled and randomized and used Applicant's IM seasonal Influenza Vaccine 15µg as a comparator.

Injection system

The traditional intradermal injection technique, the Mantoux method is considered difficult and requires training to perform successfully. The ID Micro-Injection System developed by Becton Dickinson was intended to make ID delivery as easy to perform as IM injection and more reproducible than vaccination using the Mantoux method. This system underwent successive modifications at Becton Dickinson. During Phase II (from 2002 to 2004), an experimental, intermediate Micro-Injection System was available. This system, identified as the "yellow limiter", was used in studies GID01, GID02 and GID07. Improvement in ergonomics led to the final Micro-Injection System, which was used from March 2005 onwards in GID02 (Vaccination 3 only) and in GID16. Differences in the intermediate and final Micro-Injection Systems had no effect on the characteristics of ID administration; the changes in the System resulted in greater consistency of ID delivery and enhanced security owing to the presence of a needle shield. Results obtained using the intermediate system can therefore be considered as being supportive to those obtained in later studies with the final Micro-Injection System as the same trends were observed in both Phase II and Phase III trials.

When using the Mantoux technique, appearance of a wheal immediately after injection is considered indicative of successful ID vaccination. Other criteria for successful ID injection are appearance of an orange peel aspect and absence of leakage at the injection site. During the clinical development program these criteria, one or more, were used to evaluate the ID injection using the Microinjection system.

GCP

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

Pharmacokinetics

No pharmacokinetic studies were conducted. As explained in the European Medicines Agency (EMEA) Note for Guidance on "the Clinical Evaluation of New Vaccines", pharmacokinetic studies are usually not required for vaccines.

Pharmacodynamics

Pharmacodynamic studies were not conducted since, as is common for vaccines, the pharmacodynamic profile for the ID Influenza Vaccine is defined by its immunogenicity profile. Since the efficacy of influenza vaccines are assessed by immunological criteria all clinical studies will be discussed under section III Clinical Efficacy.

Clinical efficacy (Immunogenicity)

Evaluation of the clinical efficacy of the vaccine was mainly based on quantification of the Hemagglutination Inhibition (HI) titers in vaccinated subjects in relation to the CHMP criteria specified in the Note for Guidance on "Harmonisation of requirements for influenza vaccines (CPMP/BEW/214/96). In each study, geometric mean of antibody titers (GMTs) were calculated both pre-and post-vaccination for each study group and for each vaccine strain. Similarly, the three CHMP parameters (increase in GMT titers, seroprotection and seroconversion rates) were also calculated.

Immunological methods

A variety of serological techniques have been developed for assessment of influenza virus vaccine responses in clinical trials or for disease detection. Methods include the hemagglutination inhibition (HI) test, single radial hemolysis (SRH), virus neutralization test (NT), and enzyme linked immunosorbent assay (ELISA). The HI assay was chosen since HI antibody titers are considered a relevant surrogate marker of protection in vaccinated populations, and the assay is simple to perform and strain-specific.

In addition, cell-mediated immunity (CMI) was evaluated before and after vaccination in some studies. The following parameters were measured:

- Frequency of IL4-secreting CD4+ and interferon (IFN)γ-secreting CD4+ and CD8+ T lymphocytes among, respectively, total CD4+ and CD8+ T lymphocytes (in GID02 and GID16),
- Number of IL2-secreting cells per 106 peripheral blood mononuclear cells (PBMCs), specific for different influenza vaccine antigens, before and 21 days after vaccination (in GID16 only),
- Secretion of a panel of T-helper (Th)1 and Th2 cytokines by PBMCs upon in vitro restimulations with different vaccine antigens (in GID16 only).

The Intracellular Cytokine Staining (ICS), Enzyme-Linked Immunospot (ELISPOT), and Cytometric Bead Array (CBA) assays were used to monitor the CD4+ and CD8+ T-cell responses induced by the ID Influenza Vaccine.

Analysis populations

Different, pre-defined analysis sets were used for evaluation of the immune response.
For the statistical assessment of non-inferiority and superiority, two analysis populations were used:

Full analysis set for immunogenicity (FASI) – included all vaccinated subjects with post-vaccination blood sample taken; used to statistically evaluate superiority.
Per protocol analysis set for immunogenicity (PPI) – excluded subjects with pre-specified protocol deviations (e.g. violation of inclusion/exclusion criteria, incorrect vaccination, blood samples taken outside of acceptable window, use of medication forbidden by the protocol) and subjects without post-vaccination immunogenicity data; used to evaluate non-inferiority.

An Other immunogenicity analysis set (OI) was also defined, including all vaccinated subjects with pre- and post-vaccination immunogenicity data. This set was specifically used for all non-comparative objectives in all individual studies, which included the evaluation of the CPMP immunogenicity requirements for influenza vaccines.

Statistical methods

Non-inferiority
For the studies evaluating ID Influenza Vaccine 9µg in adults (GID02 [Vac1], GID15, and GID23), and elderly (GID16 and 17) post-vaccination GMTs were used as the primary endpoint for the demonstration of non-inferiority of each of the groups with respect to the IM Influenza Vaccine. GMTs were considered to be a well recognized endpoint and the most informative and sensitive for the evaluation of non-inferiority. The non-inferiority margin was defined as the maximum GMT ratio (GMTR) between groups which could be considered to remain clinically acceptable, under the assumption that similar immune responses were obtained in each group. As a two-fold increase

between pre-and post-vaccination GMTs is viewed by the CPMP as a criteria of vaccine efficacy (see Note for Guidance on the "Harmonisation of Requirements for Influenza Vaccines"), a two-fold difference in GMT can justifiably be considered as clinically important. The Applicant chose to use a more conservative ratio of 1.5 to determine non-inferiority. Statistical analysis considered the confidence interval (CI) of the differences between the log_{10} GMTs, rather than the GMT ratio, to normalize antibody distribution. If the lower limit of the 95% CI of the difference was above -0.176 (-1/1.5) for each of the three strains, non-inferiority was concluded.

Superiority
In the event that non-inferiority was shown, superiority was to be tested. Superiority was concluded if the lower limit of the 95% CI of the difference between the log_{10} GMTs of each group receiving the ID Influenza Vaccine 9µg and the IM control vaccine was above 0 (i.e. lower limit of the 95% CI of the ratio of the GMTs between groups was above 1) for all vaccine strains (Phase II studies GID02 and GID15) or at least two of the strains in GID23.

In study GID16, superiority was assessed based on comparison of GMTs between groups, if the lower limit of the 95% CI of the difference between the log_{10} GMTs of each group receiving the ID Influenza Vaccine 15µg and the IM control vaccine was above 0 for all vaccine strains, superiority was concluded.

In study GID17, the Applicant chose to demonstrate superiority through comparison of the post-vaccination seroprotection rates.

- Dose response studies

Dose-response studies in adults (18-59 years)

Two phase II randomised controlled studies were performed with an intermediate Micro-Injection System to determine the dose in the adult population (GID01, GID02).

GID01 was a phase II, open (for the administration route) and double-blind (for the three dosages administered by using the investigational device) randomized study conducted in three centers in Lithuania in 2002. A total of 300 subjects aged 18 to 60 years were randomized to receive one injection of Influenza Vaccine either by the ID route with the intermediate Micro-Injection System (3, 6, or 9 µg HA per strain), by the ID route using the Mantoux method (3 µg HA per strain), or by the IM route (15 µg HA per strain).

The primary objective of GID01 was to check the compliance of the ID Influenza Vaccine, by the IM route with the CPMP criteria by evaluating the immunogenicity [18-21] days after the injection, in subjects aged between 18 and 60 years.

The mean age of the study population was 32.6 years, and the groups were similar in terms of age and gender distribution. Thirty-five percent of the study subjects had received an influenza vaccine previously.

Immunogenicity results
Pre-vaccination GMTs and the proportion of seroprotected subjects were similar between the groups for each of the three strains. Among the ID groups, the highest immune response in terms of GMTs for all strains was obtained with the ID 6 µg dose level and the ID 9 µg dose level. Overall, this response was in the range of the response obtained with the IM Influenza Vaccine. In terms of CPMP criteria, the highest response was also observed with the ID 6µg and ID 9µg dose levels.

The effectiveness of the intradermal injection was also evaluated using three criteria for successful ID injection (presence of a wheal, presence of orange peel aspect, and absence of leakage on the skin). Immunogenicity results were not different in the subset of subjects with injection meeting at least two

of these criteria for ID injection, and in the subjects with injection with no leakage on the skin after ID injection.

GID02 was a randomized phase II study conducted over a period of 3 years, from 2003 to 2005, in the Czech Republic (dose 1 and dose 2), and in Lithuania and Belgium (doses 1, 2 and 3)in 3 different EU countries . The influenza strains in the vaccine varied between each injection. The double-blind design was used for the two dose levels administered by the ID route (dose 1). Overall, two dose levels were evaluated by the ID route for dose 1 (3 and 6 µg HA per strain), and one for dose 2 and dose 3 (9 µg HA per strain). The intermediate Micro-Injection System was used for dose 1 and 2, the final one was used for dose 3. A total of 1 150 subjects aged 18 to 60 years were randomized to receive three injections of Influenza Vaccine by the ID or IM route with an interval of one year between doses. The subjects were re-randomized for each of the second and third vaccinations. The primary objective of GID02 was to compare the post-vaccination GMTs (Anti-HA antibodies) of two pharmaceutical presentations (3 µg and 6 µg of each HA) administered by the ID route with that of Vaxigrip (15 µg of each HA) administered by the IM route, 21 days after a single first vaccine injection in subjects aged 18 to 57 years. If the non-inferiority of one presentation administered by the ID route compared to the presentation administered by the IM route was demonstrated, the superiority was to be tested. At inclusion the mean age of the subjects was 39.1 years and the groups were similar in terms of age. The proportion of women was slightly higher in the two ID groups than in the IM group.

Immunogenicity results

Non-inferiority analysis first vaccination
In the PPI population, despite similar pre-vaccination GMTs in all three groups for each of the three vaccine strains, the post-vaccination GMTs observed in the ID groups were lower than those in the IM group. The non-inferiority of the immunogenicity of both ID 3 µg and ID 6 µg vaccines in respect to that of the IM 15 µg vaccine could not be demonstrated: the lowest bound of the 95% CI of the difference of log transformed post-vaccination GMTs versus IM 15 µg was lower than -0.176 in both ID groups for all strains, i.e. the GMT ratio was <1/1.5.

Therefore, given the low upper bounds of those 95% CIs for all strains, the differences observed in the ID groups versus the IM group could be considered as clinically meaningful. The results and conclusions were similar in the FASI population. This led to the decision to use a higher dose for the second and third year vaccinations, 9µg HA per dose and strain.

Additionally, a significant variability of immunogenicity results was observed across centers, with an interaction on group effect: the differences observed between groups differed significantly between centers. In particular one center showed large differences between the ID and the IM routes results, whereas this center presented the lowest percentage of leakage at injection site (8.9% of subjects). This might be due to an incorrect use of the system. The Intermediate injection system was used for the first year vaccinations.

Immunogenicity, second vaccination
The second vaccination in GID02 compared the ID Influenza Vaccine 9µg and the IM Influenza Vaccine.

In terms of GMTs, the response was similar between the ID Influenza Vaccine 9µg and the IM Influenza Vaccine. The mean differences between the ID 9µg and the IM 15µg groups in terms of log post-vaccination titers led to observed values very close to 0, with narrow 95% CIs. Indeed, the 95% CI of the GMT ratios (ID/IM) were (0.809; 1.090) for A/H1N1, (0.863; 1,120) for A/H3N2 and (0.828; 1.135) for B. The two vaccines induced a similar response in terms of CPMP criteria and high seroprotection rates for all strains.

For the second vaccination, a significant variability of immunogenicity results was observed across centers, with an interaction on group effect: the differences observed between groups differed significantly between centers. This variability seemed to be mainly due to one center (different from

the first one) results, showing large differences between results of ID vaccine and IM vaccine. This time, a large percentage of leakage at injection site (28.6% of subjects) was observed at this center. However the large differences in this center between results of ID and IM routes remain on subjects who did not present any leakage at injection site. The Intermediate injection system was used for the second year vaccinations.

Immunogenicity third vaccination
The same vaccines were evaluated for the third vaccination in GID02. It should be noted that immunogenicity was evaluated in a subset of 240 subjects only after the third dose.

Twenty-one days after dose 3, GMTs increased and remained similar between the groups for the A/H1N1 and the B strains, but they were higher for the A/H3N2 strain with the ID Influenza Vaccine 9µg (415 [1/dil]) than with the IM Influenza Vaccine (300 [1/dil]). In both groups, the three CPMP criteria were met for the A/H3N2 strain, and one criterion (seroprotection rate) was met for the A/H1N1 and B strains. The reason that the seroconversion or significant increase rate and GMT ratio criteria were not met could be due to the high pre-vaccination titres.

Presence of Leakage at the injection site
After the first injection, 18.0% in the ID 3µg group, and 20.1% in the ID 6µg group presented with product leakage at the ID injection site. Twenty-one days after the first vaccination, the immunogenicity results were higher in the subset of subjects without leakage than in the subset of subjects with leakage.

A multivariate analysis, performed on subjects vaccinated with the ID vaccine to assess the dose and leakage effects on the log-post-vaccination titers for the three strains, concluded to a significant negative effect of the presence of leakage.

After the second vaccination 10.2% of the subjects presented with leakage at the injection site. Twenty-one days after the second vaccination, the immunogenicity results were higher in groups of subjects without leakage than in the subset of subjects with leakage. Nevertheless, the post-vaccination GMTs in subjects without leakage in the ID 9µg group remained similar to those observed in the IM group.

During the third year the number of subjects presenting with a leakage was too low to allow a meaningful analysis (N=2).
The intermediate injection system as used for the first and second vaccinations, while the third vaccination was given with the final Microinjection system.

In the main/key studies only the Final Micro-Injection System was used. In these studies the applicant assessed immunogenicity. Results in subjects with leakage remained similar to those obtained on all subjects.

Cell-mediated immunity
Cellular responses against influenza were measured in 96 adults after the second vaccination and in 93 adults after the third vaccination with the 9 µg ID influenza vaccine or Vaxigrip (all subjects enrolled in a single site in Belgium). Antigenic stimulation was performed on whole blood samples before and 21 days after the second and the third immunization. Cells were in vitro stimulated with killed split vaccine strains or with MHC class I or class II restricted Flu specific peptides. The CD4 and CD8 responses were measured by intracellular IFN-γand IL-4 staining (IL-4 evaluated only after the third vaccination) by flow cytometry.

Before the second and the third vaccination, the subjects showed a Flu-specific CD4 Th1 response, as judged by a predominant IFN-γsecretion and the absence of IL-4 detection. This response was moderately increased after the second and the third vaccination. However, no significant differences were shown on CD4 responses between IM and ID route neither after the second nor the third administration. It should be noted that the third administration of the vaccine did not further increase the responses compared to the second dose.

A weak and heterogeneous CD8 response was measured before the second and the third vaccination. This response was poorly increased by the vaccination. Only 10% of the subjects from either IM or ID group presented a positive CD8 response after the second injection. No significant differences could be demonstrated between IM and ID routes on CD8 T cell activation neither after the second nor the third administration of the vaccine.

In conclusion, this study showed that the 9 µg ID influenza vaccine induced a cellular immune response comparable to that induced by Vaxigrip.

Dose response studies in elderly (≥60 years)

Two phase II randomised controlled studies were performed, one using the intermediate Micro-Injection System and one using the Mantoux method to determine the dose in the elderly population (GID07, GID09).

GID07 was a randomized study conducted in France in 2003. A total of 240 subjects aged ≥ 60 years were randomized to receive one injection on D0 of Influenza Vaccine by the ID route with the intermediate Micro-Injection System (3, 6, or 9 µg HA per strain) or by the IM route (15 µg HA per strain). The primary objective was to assess the immunogenicity of the influenza vaccine either administered by ID route (three different dosages were assessed) or by IM route (one dosage), 21 days after vaccination in subjects aged over 60 years. For each vaccine strain, the objective was to satisfy at least one of the three CPMP criteria. At inclusion the mean age was 72.3 years. All four groups were similar in terms of age and gender distribution, except for the 6 µg ID group who had a slightly higher number of females than in the other groups. A total of 226 subjects (94.2%) had received an influenza vaccine in previous years.

Immunogenicity results
Pre-vaccination GMTs were similar between the groups for each of the three strains. For the A/H3N2 strain, antibody titers were already high. Twenty-one days after vaccination, the A/H1N1 and A/H3N2 strains met at least one of the CPMP criteria in all groups except the ID 3µg group, none of these criteria was met for the B strain (including by the IM route). The response was the lowest with the ID Influenza Vaccine 3µg, regardless of the strain, and appeared to be slightly higher with the ID 9 µg dose level than with the ID 6 µg and IM Influenza Vaccine.

Due to the observed low antibody response to the B Shandong strain and in order to validate the generated data, decision was made to conduct an additional investigation and testing with the homologous B/Hong Kong strains (both native and split antigens), given WHO mandated the introduction of the B Hong Kong antigen in the Flu vaccine composition. Two additional series of results were obtained. With the native B/Hong Kong antigen, results were similar to those obtained with the B/Shandong native antigen but different from those obtained with the split B/Hong Kong antigen (pre- and post-titers were higher and pre- and postseroprotection rates were 80.5% and 93.0%, i.e. also higher). The sensitivity of the HI test was improved with the split antigen both for the pre- and post-vaccination samples, leading to a decrease of the specificity without any positive impact on the compliance with the EMEA criteria. As a result, the results generated with the B Hong Kong antigens were considered as supportive data.

Leakage at the injection site
The majority of subjects (88.5%, 81.7% and 79.7% in the ID 3, 6 and 9 µg groups respectively) did not present any product leakage of the vaccine after the ID injection. But in terms of evaluation of the effectiveness of the ID injection, no improvement of the immune response of ID injections was observed when the immunogenicity results were computed on subjects with no leakage at the injection site.

GID09 was a randomized study conducted in elderly subjects in Australia in 200 in the Southern Hemisphere 5. A total of 226 subjects aged 60 to 84 years were randomized to receive one injection of Influenza Vaccine by the ID route with the Mantoux method (9, 15, or 21 µg HA per strain) or by the

IM route (15 µg HA per strain). All subjects received the annual formulation of Influenza Vaccine by the IM route 3 months after the first vaccination to offer the subjects protection against the WHO influenza strains recommended for the 2005 Southern Hemisphere. Randomization was stratified by age group, i.e. 60 to 69 years and 70 to 85 years, within each center. The primary objective was to describe the immunogenicity of three dosages of the ID Influenza Vaccine given by Mantoux injection technique using the criteria defined in the CPMP note for guidance.

The mean age at inclusion was 69.2 years. All four groups were similar in terms of age. Regarding gender distribution, there were more males than females in the ID 9 µg, ID 15 µg, and ID 21 µg groups, while there were more females than males in the IM 15 µg group.

Previous influenza vaccination was reported in 92.9% of the subjects.

Immunogenicity results
Twenty-one days after vaccination, an immune response was observed in all groups. Overall, on the CPMP criteria point estimates observed in the ID groups, there was a trend towards a superior immunogenicity with higher ID dose levels. The three CPMP criteria were met for all strains in the ID 15µg and ID 21µg groups. In the ID 9µg group, these criteria were met for two of the three strains. In the IM 15µg group, the seroconversion or significant increase rate was met for the B strain only.

In this study the Mantoux technique, and not the Beckton Dickinson device was used for all vaccinations. The conclusion that the 15 and 21 µg ID doses induce higher immune responses than the 9µg ID dose and the 15 µg IM dose is supported by the data.

The conclusion of these studies allowed the selection of 15µg HA per strain intradermal formulation for evaluation in pivotal clinical trials with the final Micro-Injection System.

- Main studies

The pivotal studies are described together below.

The parts about adults (18-59 years) include the results of a Phase II study (GID15, first vaccination) and the data obtained in a Phase III lot-to-lot consistency study (GID23). No immunogenicity data were obtained after dose 2 and 3 in GID15. The two studies were considered as key studies because they aimed at demonstrating non inferiority of the ID Influenza Vaccine 9µg with the final Micro-Injection System to the IM Influenza Vaccine. Data from the two key studies (GID15 dose 1 and GID23) are presented and compared in this section.

The parts about elderly (≥60 years) include the results obtained within 21 days after the second vaccination in the Phase III study GID17 (data after the third vaccination were not available at the time of approval) and results of an integrated analysis combining the data of GID16 and GID17 (Vac1). The two studies were considered as key studies because they both aimed at demonstrating superiority of the ID Influenza Vaccine 15µg over the IM Influenza Vaccine, and they evaluated the final HA dose level and the final Micro-Injection System.

Methods

Study Participants

In study GID15 and GID23 the inclusion criteria included Age 18 to 57 years on the day of inclusion (GID15) and Age 18 to 60 years on the day of inclusion (GID23). For a woman of childbearing potential use of an effective method of contraception

In study GID16 and study GID17 the inclusion criteria included Age 60 to 85 years on the day of inclusion (GID16) and Age over 60 years on the day of inclusion (GID17)

The exclusion criteria are similar between the studies within each age category, with minor differences. The recruitment was not specifically targeting a population that is at higher risk of suffering complications from influenza, other than the elderly studies.

The exclusion criteria in all clinical studies (both Adults and Elderly) included:

- Self-reported allergy to any of the constituents of the vaccine
- Acute febrile disease within the 72 previous hours
- Subject with an aggravation of existing chronic illness (heart disease, respiratory disease, etc)
- Vaccination against influenza within the 6 months preceding Visit 1
- Any vaccination within the 28 days preceding Visit 1 or scheduled between Visit 1 and Visit 2
- Immunosuppressive therapy or cancer therapy within the month preceding Visit 1
- Congenital or acquired immunodeficiency,
- Immunoglobulin injection within the 3 months preceding Visit 1
- Blood or blood derived products received in the past three months
- Current abuse of alcohol or drug addiction

Treatments

In all studies described in this section (GID15, GID23, GID16 and GID17) the final Microinjection system and the final formulation of vaccine was used. Applicant's IM seasonal Influenza Vaccine 15µg was given as intramuscular comparator in all studies.

Objectives

GID15
The **primary** objective of GID15 was to demonstrate that the vaccine administered by the ID route with the final ID system (prefilled ID system allowing a better ergonomic use) is at least as immunogenic as the administration of the vaccine by the IM route after the first vaccination.

The **secondary** objectives of GID15 included to describe the safety profile after each vaccine administration, to describe the anti-HA antibody persistence after the first injection and to describe the compliance of the immunogenicity with the EMEA criteria.

Observational objectives included the assessment of the pain at the injection site, the leakage appearing at the injection site and the comfort of the vaccination assessment

GID23
The **primary** objective of GID23 was to demonstrate that three different industrial lots of the ID investigational vaccine induce an equivalent immune response.

The **secondary** objectives of GID23 included the demonstration that the ID investigational vaccine induces an immune response at least as good as the one induced by the IM control vaccine, in terms of antibody titers, to assess the immunogenicity of the ID investigational vaccine using the parameters defined by CHMP, to assess safety and the comfort of vaccination (pain).

GID16: The primary objective of GID16 was to demonstrate that at least one of the two dosages (15 µg and 21 µg of each HA per strain) of the ID Influenza Vaccine was at least as immunogenic as the IM Influenza Vaccine.

The **secondary** objectives of **GID16** included to describe the safety profile, to describe the anti-HA antibody persistence and to describe the compliance of the immunogenicity with the EMEA criteria.

GID17
The **primary** objective of GID17 was to demonstrate that the ID investigational vaccine induces a superior immune response than the IM control vaccine in terms of seroprotection rate after the first vaccination.

The **secondary** objectives included, the immunogenicity of the ID investigational vaccine after each vaccination using the CHMP criteria, the description of the antibody persistence induced by both vaccines at 3 months, 6 months, 12 months after the first vaccination in a subset of subjects and the comfort of vaccination (pain) and the assessment of safety.

Endpoints

Immunogenicity
The following immunogenicity parameters and their 95% confidence intervals were calculated

- Geometric mean of anti-HA antibody titers (GMTs) pre- and post-vaccination,
- Geometric mean of the individual titer ratios (GMTR) post-vaccination over pre-vaccination,
- Seroprotection rate defined as the proportion of subjects with a post-vaccination titer ≥ 40 [1/dil],
- Seroconversion rate defined as the proportion of subjects with pre-vaccination titers <10 [1/dil] rising to ≥ 40 [1/dil] post-vaccination
- Significant increase in titers defined as the proportion of subjects with pre-vaccination titers ≥10 [1/dil] reaching at least a 4-fold increase in pre-vaccination titers after vaccination,
- Proportion of subjects with seroconversion or with significant increase in titers.

Pain at injection site
First vaccination GID15 and GID16: The intensity of pain at the time of injection was evaluated just after vaccination using a visual analogue scale (VAS): one value (ranging between 0 mm and 100 mm) was obtained for each subject. Additionally, the answers to the acceptability questionnaire at D0 and 21 days after each vaccination were described.

GID23, GID17 and Second and third vaccinations GID15: The rating of immediate pain at the injection site obtained just after the injection (via the ID and IM routes) using a verbal rating scale (VRS).

Comfort of the vaccination assessed by the subjects 21 days after the vaccination, using the score(s) obtained to the vaccination comfort questionnaire (VCQ), a 44 items self-administered questionnaire.

Leakage at the injection site (GID15, GID23, GID16, GID17)
The presence or absence of product leakage on the skin at the injection site was considered after ID injection.

Presence of Wheal at the injection site (GID23, GID17)
For injections performed by the ID route, presence or absence of a wheal on the skin at the ID injection site was recorded. When using the Mantoux technique, appearance of a wheal immediately after injection is considered indicative of successful ID vaccination. Using the ID system, a wheal does not appear systematically after injection and one exploratory objective of this study was to confirm that presence or absence of a wheal was not related to success of the ID vaccination in terms of immune response.

Sample size

GID 15: The ID 9µg group is tested at a 2.5% alpha level (one-sided hypothesis). A maximum acceptable ratio of 1.5 in terms of post-vaccination GMT and a global power of 91% were chosen to calculate the sample size. A total of 1,000 subjects were to be enrolled in the trial.

GID 23: A total of 2 250 subjects were to be enrolled. A total of 600 subjects per lot (450 subjects per lot for immunogenicity) in the ID investigational vaccine group and 450 subjects in the IM control vaccine group gave the calculated powers for the different tests of equivalence between the three lots in terms of immunogenicity, non-inferiority of the pooled ID investigational vaccine groups versus IM control vaccine group in terms of immunogenicity and non-superiority of the pooled ID investigational vaccine groups versus IM control vaccine group in terms of safety.

GID16: The ID 15µg and 21µg groups were tested at a 2.5% alpha level (one-sided hypothesis for noninferiority). A maximum acceptable ratio of 1.5 in terms of post-vaccination GMT and a global power of 91% were chosen to calculate the sample size. Assuming for each A strain a maximal standard deviation of 0.6, and 0.5 for the B strain (from GID09 (18) trial results), 322 subjects per group were necessary to test the null hypothesis. Under the assumption that about 10% of subjects would not be evaluable, 360 subjects were needed to be included in each group. Therefore a total of 1,080 subjects were planned to be enrolled in the trial

GID17: A total of 2 580 subjects in the ID investigational vaccine group and 1 075 subjects in the IM control vaccine group gave the necessary powers for the different tests of superiority of the ID investigational vaccine group versus the IM control vaccine group in terms of seroprotection and non-superiority of the ID investigational vaccine group versus the IM reference vaccine group in terms of safety.

Randomisation
In all the 4 studies (GID15, GID23, GID16 and GID17) subjects were randomised at the time of the first vaccination. Vaccine groups were allocated using permuted block method with stratification on investigational center. For the subsequent vaccinations in GID15, a similar process has been followed to randomize the subjects to ID or IM group. For the subsequent vaccinations in GID17, only subjects having received the IM control vaccine at the previous vaccination were randomized into one of the two vaccine groups in a balanced manner; subjects having received the ID investigational vaccine at the previous vaccination were not randomized and received the ID investigational vaccine.
Blinding (masking)

All studies were double-blind for dose level and different lots of ID vaccine, but open for administration route. Study GID15 which was open, including only one dose level (9µg) of the ID vaccine.

Statistical methods

In all studies superiority was evaluated only once non-inferiority had been demonstrated.

For the studies (GID02 [Vac1], GID15, and GID23), post-vaccination GMTs were used for the demonstration of non-inferiority. The non-inferiority margin was defined as the maximum GMT ratio (GMTR) between groups. A two-fold difference in GMT can justifiably be considered as clinically important. The Applicant chose to use a more conservative ratio of 1.5 to determine non-inferiority. Statistical analysis considered the confidence interval (CI) of the differences between the $\log_{10}$ GMTs, rather than the GMT ratio, to normalize antibody distribution. If the lower limit of the 95% CI of the difference was above -0.176 (-1/1.5) for each of the three strains, non-inferiority was concluded.

Superiority was concluded if the lower limit of the 95% CI of the difference between the $\log_{10}$ GMTs of each group receiving the ID Influenza Vaccine 9µg and the IM control vaccine was above 0 (i.e. lower limit of the 95% CI of the ratio of the GMTs between groups was above 1) for all vaccine strains (Phase II studies GID02 and GID15) or at least two of the strains in GID23.

Post-vaccination GMTs were used as the primary endpoint for non-inferiority of the ID Influenza Vaccine 15µg with respect to the IM Influenza Vaccine for the studies GID16 and GID17. A ratio of 1.5 was used. Statistical analysis considered the CI of the differences between the $\log_{10}$ GMTs, rather than the GMT ratio, to normalize antibody distribution. If the lower limit of the 95% CI of the difference was above -0.176 (-1/1.5) for each of the three strains, non-inferiority was concluded.

In study GID16, superiority was assessed based on comparison of GMTs between groups; if the lower limit of the 95% CI of the difference between the $\log_{10}$ GMTs of each group receiving the ID Influenza Vaccine 15µg and the IM control vaccine was above 0 for all vaccine strains, superiority was concluded.

In study GID17, the Applicant chose to demonstrate superiority through comparison of the post-vaccination seroprotection rates. Superiority was concluded if the two-sided 95% CI of the difference in seroprotection rates was above 0 for at least two of the vaccine strains.

A supplementary analysis to evaluate superiority in GID16 using seroprotection rates was performed by the Applicant.

RESULTS

Participant flow / Numbers analysed / Conduct of the study

GID 15
A total of 978 subjects aged from 18 to 57 years were included in the study between 19 September 2005 and 28 October 2005, and randomized to one of the two study groups:

- 588 subjects were randomized in the ID 9µg group
- 390 subjects were randomized in the IM 15µg group

Enrolment stopped prior to full enrolment (1,000 subjects), because the inclusion period was shortened. However, the lower number of subjects included did not impact the primary objective of the study.

GID23
A total of 2 255 subjects aged from 18 to 60 years were included in the study between 11 September 2006 and 31 October 2006, and randomized to one of the four study groups.
The disposition of subjects in the four groups was as follows:

- 604 subjects were randomized in the ID 9µg Lot 1 group
- 596 subjects were randomized in the ID 9µg Lot 2 group
- 603 subjects were randomized in the ID 9µg Lot 3 group
- 452 subjects were randomized in the IM 15µg group

GID16
A total of 1 107 subjects aged >60 years were included in the study and randomized to one of the three study groups:

- 370 were randomized to the ID 15µg group
- 369 were randomized to the ID 21µg group
- 368 were randomized to the IM 15µg group

All subjects received the annual formulation of Influenza Vaccine by the IM route 3 months after the first vaccination to offer the subjects protection against the WHO influenza strains recommended for the 2006 Southern Hemisphere.

GID17
A total of 3 707 subjects aged >60 years were included in the study between 11 September 2006 and 31 October 2006, and randomized to one of the two study groups:

- 2 618 were randomized to the ID 15µg group
- 1 089 were randomized to the IM 15µg group

Subjects were re-randomized for the second vaccination so that the following schedules were evaluated: ID\ID (N=2 454), IM\ID (N=511), and IM\IM (N=511).

Baseline data

GID15: At inclusion, in the PPI population, subjects were aged between 18.1 and 58.0 years old and the mean age was 40.2 years (SD: 11.1 years). The male/female gender ratio was 0.6, the number of females was higher than the number of males in both groups. Both groups were similar in terms of age and gender distribution. The baseline characteristics (in terms of age, gender and previous influenza vaccination) were similar in the FASI and in the SafAS populations.

Among the 760 subjects included in the PPI population, 292 subjects (38.4%) had been vaccinated with an influenza vaccine in majority in 2004. Out of these 292 subjects, 33 (11.3%) had experienced an adverse reaction after vaccination with almost the same proportions in both groups. These reactions were nearly the same as the solicited reactions pre-listed in the subject's DC. Similar results were obtained in the FASI population.

GID23: At inclusion, in the PPI population, subjects were aged from 18.1 to 60.0 years and the mean age was 42.8 years (SD: 12.4 years). The male/female gender ratio was 0.7, the number of females being higher than the number of males in all groups. Among the 1 676 subjects included in the PPI population, 781 (46.6%) had been previously vaccinated with an influenza vaccine. Most of them had been vaccinated in 2005. Out of these 781 subjects, 56 (7.2%) had experienced an adverse reaction after vaccination (between 10 and 16 subjects per group). A total of 717 subjects (42.8%) were considered as at health risk. The most important risks were lung disease (15.2%), heart disease (13.7%) and neurological disease (13.6%). The majority of subjects had skin phototypes Type III (32.8%) or Type II (25.8%).

Baseline characteristics (in terms of age, gender, BMI, previous allergy, risk status, skin phototypes and previous influenza vaccination) were similar in the four groups, in the PPI, in the FASI, and in the SafAS populations.

GID16 At inclusion, in the PPI population, subjects were aged from 60.0 to 85.8 years and the mean age varied from 70.4 to 71.0 years (SD of 6.76 and 6.55 years, respectively). The male/female gender ratios varied from 0.8 to 1, the number of females being higher than the number of males in all three groups. All three groups were similar in terms of age and gender distribution.
Among the 1,076 subjects included in the PPI population, 978 had been previously vaccinated with an influenza vaccine.

GID17: At inclusion, the mean age of subjects in the FASI population was 70.8 years (SD: 6.8 years, range 60.6; 94.6). The male/female sex ratio was 0.8. Both groups were similar in terms of age and gender distribution. Distribution of BMI was similar amongst groups. Most of the subjects were overweight (44.2%) or obese (23.2%).

Among the 3 083 subjects included in the FASI population, a total of 2 924 subjects (79.3%) had been vaccinated with an influenza vaccine and 259 subjects (7.0%) with a pneumoccocal vaccine. Out of these subjects, 57 (1.9%) and none (0%) reported experiencing an adverse reaction after vaccination with influenza and pneumoccocal vaccines respectively.

With respect to the health risk status (65.6% in ID 15µg group and 63.6% in IM 15µg group) of the subjects included in the FASI population, heart disease was the most frequently medical condition reported (1892 subjects [51.3%]). Lung disease and diabetes were recorded for 428 subjects (11.6%) and 417 subjects (11.3%), respectively. Neurological disease was reported by 329 subjects (8.9%), and renal disease was reported by 186 subjects (5.0%). Other diseases, including hepatitis, cancer and leukemia were reported by 139 subjects (3.8%).

The baseline characteristics were equivalently distributed between groups, and were similar in the PPI and in the SafAS populations.

Outcomes and estimation

Immunogenicity results

GID15

Pre-vaccination GMTs for each strain were similar in both groups. In the PPI, non-inferiority of the immunogenicity of the ID Influenza Vaccine 9µg to the IM Influenza Vaccine was demonstrated for each of the three strains in terms of post-vaccination GMTs, with the lower bound of difference of GMTs between groups ranging from -0.003 for the B strain to 0.087 for the A/H3N2 strain (Table 1). As non-inferiority was demonstrated, superiority of the ID Influenza Vaccine 9µg over the IM Influenza Vaccine was assessed. Superiority was shown for the A/H1N1 and the A/H3N2 strains but not for the B strain.

In the FASI, superiority of the ID Influenza Vaccine 9µg over the IM Influenza Vaccine was demonstrated for the A/H1N1 and the A/H3N2 strains, with lower bounds of difference of GMTs between groups of 0.006 and 0.087, respectively, but not for the B strain, for which the lower bound was of -0.004. However, post-vaccination GMTs for the B strain were still slightly higher in the ID 9µg group than in the IM group.

Table 1: GID15 – Vac 1 - CPMP Immunogenicity Parameters, of the Three Vaccine Strains According to Injected Vaccine Group - Other Immunogenicity Analysis Set

		ID Influenza Vaccine 9µg			IM Influenza Vaccine		
Strain	**CPMP threshold**	**A/New Caledonia/20/99 (H1N1)**	**A/Wellington/1/2004 (H3N2)**	**B/Jiangsu/361/2002**	**A/New Caledonia/20/99 (H1N1)**	**A/Wellington/1/2004 (H3N2)**	**B/Jiangsu/361/2002**
N analyzed		382	383	382	385	384	385
PRE-VACCINATION							
Geometric mean (1/dil) (95% CI)		15.2 (13.2; 17.6)	29.3 (25.6; 33.5)	12.0 (10.8; 13.3)	14.4 (12.6; 16.5)	27.5 (24.3; 31.2)	11.4 (10.4; 12.6)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)		27.7 (23.3; 32.5)	43.9 (38.8; 49.0)	16.8 (13.1; 20.9)	26.2 (21.9; 30.9)	40.9 (35.9; 46.0)	16.6 (13.0; 20.7)
POST-VACCINATION							
Geometric mean (1/dil) (95% CI)		247 (215; 285)	825 (736; 924)	144 (129; 161)	198 (170; 231)	569 (501; 646)	124 (111; 139)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)	**>70%**	**92.4** (89.3; 94.9)	**99.7** (98.6; 100.0)	**90.6** (87.2; 93.3)	**88.8** (85.3; 91.8)	**98.7** (97.0; 99.6)	**85.5** (81.5; 88.8)
POST/PRE							
Ratios of Titers (95% CI)	**>2.5**	**16.2** (13.7; 19.2)	**28.2** (23.7; 33.5)	**12.1** (10.5; 13.8)	**13.8** (11.6; 16.4)	**20.7** (17.5; 24.4)	**10.84** (9.56; 12.29)
Seroconversion or significant increase							
% (95% CI)	**>40%**	**74.3** (69.7; 78.7)	**85.1** (81.2; 88.5)	**76.4** (71.9; 80.6)	**70.4** (65.6; 74.9)	**79.2** (74.8; 83.1)	**73.5** (68.8; 77.8)

N: number of subjects analyzed

Mean data fulfilling the CPMP criteria are shown in bold

The proportions of seroprotected subjects at baseline were similar between groups, ranging from 16.8% for the B strain to 43.9% for the A/H3N2 strain, and from 16.6% for the B strain to 40.9% for the A/H3N2 strain, in the ID 9µg and IM groups.

Twenty-one days after vaccination, an immune response was observed in both groups, with GMTs of 247 (1/dil), 825 (1/dil) and 144 (1/dil), for the A/H1N1, A/H3N2 and B strains, respectively in the ID 9µg group.

The three CPMP criteria were fulfilled with the ID Influenza Vaccine 9µg for the three strains, 95% CIs inclusive, with higher results in the ID 9µg group than in the IM group. As there were only 34 subjects receiving ID injection and presenting with vaccine leakage at the injection site, the assessment of immunogenicity in these subjects was not performed.

Exploratory analyses were performed to assess, on the immune response obtained after the first vaccination in both groups the influence of the baseline seroprotection, the centre and the vaccinator.
The centre and vaccinator effect were generally not significant. On the other side, the baseline seroprotection was always significant and influence the immune response in each vaccine group. Nevertheless, the vaccine effect (ID vaccine effect compared to IM vaccine effect) is independent from this covariate.

Antibody persistence
For the three strains, the antibody persistence for one year after vaccination presented a similar decrease over time of GMTs in the ID 9µg group to the IM 15µg group, despite a constant slight higher level of antibodies in the ID group versus IM group.

The decrease of GMTs between D21 and M12 were comparable between the ID and IM groups for the three strains at each time point (M3, M6 and M12). Similar observations can be performed in terms of seroprotection rates (≥40 1/dil) (Table 2). It seems to be that for the B strain the seroprotection decrease is slightly higher in ID 9µg group than in IM 15µg group.

Table 2: Antibody persistence: Seroprotection rates before first vaccination and 21 days, 3, 6 and 12 months after vaccination according to randomized vaccine group – full analysis set - first vaccination

	ID 9µg			IM 15µg		
	A/New Caledonia/ 20/99 (H1N1)	A/Wellington/ 1/2004 (H3N2)	B/Jiangsu /10/2003	A/New Caledonia/ 20/99 (H1N1)	A/Wellington/ 1/2004 (H3N2)	B/Jiangsu /10/2003
V01 (D0)						
N analyzed	383	383	383	385	385	385
Subjects with titers >=40 l/dil.						
(%)	27.7%	43.9%	16.7%	26.2%	40.8%	16.6%
95% CI	(23.3;32.4)	(38.8;49.0)	(13.1;20.8)	(21.7;30.9)	(35.8;45.9)	(13.0;20.7)
V02 (D21)						
N analyzed	382	383	382	385	384	385
Subjects with titers >=40 l/dil.						
(%)	92.4%	99.7%	90.6%	88.8%	98.7%	85.5%
95% CI	(89.3;94.9)	(98.6;100.0)	(87.2;93.3)	(85.3;91.8)	(97.0;99.6)	(81.5;88.8)
V03 (M3)						
N analyzed	377	376	377	379	378	379
Subjects with titers >=40 l/dil.						
(%)	86.7%	98.9%	77.5%	81.3%	97.1%	72.6%
95% CI	(82.9;90.0)	(97.3;99.7)	(72.9;81.6)	(77.0;85.1)	(94.9;98.5)	(67.8;77.0)
V04 (M6)						
N analyzed	372	372	370	377	377	376
Subjects with titers >=40 l/dil.						
(%)	82.0%	97.8%	61.4%	75.9%	95.8%	65.7%
95% CI	(77.7;85.8)	(95.8;99.1)	(56.2;66.3)	(71.2;80.1)	(93.2;97.6)	(60.7;70.5)

(To be continued)

	ID 9µg			IM 15µg		
	A/New Caledonia/ 20/99 (H1N1)	A/Wellington/ 1/2004 (H3N2)	B/Jiangsu /10/2003	A/New Caledonia/ 20/99 (H1N1)	A/Wellington/ 1/2004 (H3N2)	B/Jiangsu /10/2003
V05 (M12)						
N analyzed	346	346	347	350	350	350
Subjects with titers >=40 l/dil.						
(%)	68.2%	96.2%	49.9%	67.7%	89.1%	53.7%
95% CI	(63.0;73.1)	(93.7;98.0)	(44.5;55.2)	(62.5;72.6)	(85.4;92.2)	(48.3;59.0)

The ID Influenza Vaccine 9µg was at least as immunogenic as the IM Influenza Vaccine in terms of post-vaccination GMTs. The immune response induced by the ID Influenza Vaccine 9µg was superior in terms of GMTs to the one induced by the IM Influenza Vaccine for the two A strains. For each of the three strains, the three CPMP criteria were met with the ID Influenza Vaccine 9µg. The antibody persistence pattern did not differ appreciably between the ID and IM groups.

GID23

Lot-to-lot consistency

Equivalence of the immune response of the three industrial lots was demonstrated for each of the three strains, the two-sided 90% CIs of the difference between lots were between -0.176 and 0.176 for each pair of lots and for each strain. The same conclusion can be drawn with a more stringent 95% CI (Table 3). The same conclusions could be drawn when analysing the FASI population.

Comparison to the IM administration

As lot-to-lot consistency had been established, the three ID 9µg groups (one for each lot) were pooled. Immunogenicity results of the ID 9 µg investigational vaccine were compared to those of the IM control group on each strain (A/H3N2, A/H1N1, and B) in terms of GM of post-vaccination titers observed at D21. In the PPI population, GMs of pre-vaccination titers were similar in both groups and for the three strains (although those corresponding to the A/H3N2 strain were higher than those of the other strains in both groups).

Non-inferiority of the immunogenicity of the ID Influenza Vaccine 9µg (pool of the three ID groups) to the IM Influenza Vaccine was demonstrated for each of the three strains in the PPI: the lower bound of the difference of log10 transformed post-vaccination GMTs ID 9µg group versus IM group was higher than -0.176 for all strains (ranging from -0.084 for the A/H1N1 strain to -0.059 for the A/H3N2 strain) (Table 4). These results were confirmed in the FASI population.

As non-inferiority was demonstrated, superiority of the ID Influenza Vaccine 9µg over the IM Influenza Vaccine was assessed in the FASI and PPI populations. Superiority of the immunogenicity of the ID Influenza Vaccine 9µg over the IM Influenza Vaccine was not reached for any of the three strains.

Table 3: Immunogenicity Primary Criteria - Equivalence Among the Three ID Vaccine Lots - Per Protocol Analysis Set for Immunogenicity by Randomized Subjects

	ID 9µg Lot1			ID 9µg Lot2			ID 9µg Lot3		
	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004
PRE-VACCINATION									
N analyzed	418	418	417	418	418	418	414	414	412
Titers									
Geometric mean	18.8	24.1	10.9	20.0	24.9	10.4	19.7	22.4	10.4
(95% CI)	(16.4; 21.5)	(20.9; 27.8)	(10.1; 11.9)	(17.3; 23.0)	(21.4; 29.0)	(9.62; 11.3)	(17.1; 22.8)	(19.5; 25.8)	(9.56; 11.3)
POST-VACCINATION									
N analyzed	420	420	420	418	419	419	414	414	414
Titers									
Geometric mean	186	269	67.6	183	298	75.4	176	268	62.4
(95% CI)	(162; 214)	(236; 307)	(61.0; 74.9)	(159; 211)	(260; 340)	(67.4; 84.3)	(152; 204)	(234; 308)	(55.8; 69.7)
Ratio lot 1 versus lot 2									
GMT lot 1 / GMT lot 2	1.014	0.904	0.897						
(90% CI) of the ratio	(0.861;1.197)	(0.771;1.059)	(0.791;1.019)						
Log difference lot 1 versus lot 2									
log10(GMT lot 1)-log10(GMT lot 2)	**0.006**	**-0.044**	**-0.047**						
(90% CI) of the difference	(-0.065; 0.078)	(-0.113; 0.025)	(-0.102; 0.008)						

(to be continued)

	ID 9µg Lot1			ID 9µg Lot2			ID 9µg Lot3		
	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004
Equivalence lot 1 & 2*	Yes	Yes	Yes						
(95% CI) of the difference	(-0.079; 0.092)	(-0.126; 0.038)	(-0.113; 0.018)						
Ratio lot 1 versus lot 3									
GMT lot 1 / GMT lot 3	1.054	1.003	1.084						
(90% CI) of the ratio	(0.889;1.247)	(0.855;1.175)	(0.955;1.230)						
Log difference lot 1 versus lot 3									
log10(GMT lot 1)-log10(GMT lot 3)	**0.023**	**0.001**	**0.035**						
(90% CI) of the difference	(-0.051; 0.096)	(-0.068; 0.070)	(-0.020; 0.090)						
Equivalence lot 1 & 3*	Yes	Yes	Yes						
(95% CI) of the difference	(-0.065; 0.110)	(-0.082; 0.084)	(-0.031; 0.100)						
Ratio lot 2 versus lot 3									
GMT lot 2 / GMT lot 3				1.032	1.109	1.208			
(90% CI) of the ratio				(0.877;1.230)	(0.944;1.303)	(1.059;1.377)			

* Equivalence among the three lots if for each pair of lots and for each strain, the two-sided 90% CI of the log difference of the geometric mean titers lies between -0.176 and 0.176.

(to be continued)

	ID 9µg Lot1			ID 9µg Lot2			ID 9µg Lot3		
	A/New Caledonia /20/99(H1N1)	**A/Wisconsin 67/2005(H3N2)**	**B/Malaysia 25/06/2004**	**A/New Caledonia /20/99(H1N1)**	**A/Wisconsin 67/2005(H3N2)**	**B/Malaysia 25/06/2004**	**A/New Caledonia /20/99(H1N1)**	**A/Wisconsin 67/2005(H3N2)**	**B/Malaysia 25/06/2004**
Log difference lot 2 versus lot 3									
log10(GMT lot 2)-log10(GMT lot 3)				**0.017**	**0.045**	**0.082**			
(90% CI) of the difference				(-0.057; 0.090)	(-0.025; 0.115)	(0.025; 0.139)			
Equivalence lot 2 & 3*				Yes	Yes	Yes			
(95% CI) of the difference				(-0.071; 0.104)	(-0.038; 0.128)	(0.014; 0.150)			

Table 4: Immunogenicity Secondary Criteria . Non-inferiority of ID 9µg versus IM 15µg Randomized Vaccine Group - Per Protocol Analysis Set for Immunogenicity

	ID 9µg Pooled Lots			IM 15µg		
	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia 25/06/2004
PRE-VACCINATION						
N analyzed	1250	1250	1247	421	421	421
Titers						
Geometric mean	19.5	23.8	10.6	19.2	24.1	10.4
(95% CI)	(18.0; 21.1)	(21.9; 25.8)	(10.1; 11.1)	(16.6; 22.3)	(20.9; 27.9)	(9.65; 11.3)
POST-VACCINATION						
N analyzed	1252	1253	1253	421	421	421
Titers						
Geometric mean	182	278	68.3	187	274	69.8
(95% CI)	(168; 197)	(257; 301)	(64.1; 72.7)	(162; 216)	(244; 309)	(62.7; 77.8)
Ratio versus IM 15µg						
GMT ID / GMT IM	0.971	1.015	0.978			
(95% CI) of the ratio	(0.824;1.146)	(0.873;1.180)	(0.863;1.107)			

(to be continued)

	ID 9µg Pooled Lots			IM 15µg		
	A/New Caledonia /20/99(H1N1)	**A/Wisconsin 67/2005(H3N2)**	**B/Malaysia 25/06/2004**	**A/New Caledonia /20/99(H1N1)**	**A/Wisconsin 67/2005(H3N2)**	**B/Malaysia 25/06/2004**
Log difference versus IM 15µg						
log10(GMT ID)-log10(GMT IM)	-0.013	0.006	-0.01			
(95% CI) of the difference	(-0.084; 0.059)	(-0.059; 0.072)	(-0.064; 0.044)			
Non-inferiority*	Yes	Yes	Yes			
Superiority†	No	No	No			

* Non-inferiority if for each strain, the two-sided 95% CI of the log difference of the geometric mean titers ID-IM lies above -0.176.

† Superiority if for at least two strains, the two-sided 95% CI of the log difference of the geometric mean titers ID-IM lies above 0.

Table 5: GID23 - CPMP Immunogenicity Criteria of the Three Vaccine Strains According to Injected Vaccine Group - Other Immunogenicity Analysis Set

Injected Vaccine Group		ID Influenza Vaccine 9µg			IM Influenza Vaccine		
	CPMP criteria	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia /2506/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia /2506/2004
N analyzed		1296	1297	1294	436	436	436
PRE-VACCINATION							
Geometric mean of titer (1/dil) (95%CI)		19.8 (18.3; 21.4)	24.1 (22.2; 26.2)	10.6 (10.1; 11.1)	19.1 (16.6; 22.1)	24.2 (21.0; 27.9)	10.4 (9.64; 11.2)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)		32.4 (29.9; 35.0)	37.7 (35.1; 40.4)	10.4 (8.7; 12.1)	31.2 (26.9; 35.8)	38.1 (33.5; 42.8)	8.5 (6.0; 11.5)
POST-VACCINATION							
Geometric mean of titer (1/dil) (95%CI)		181 (168;197)	277 (257;299)	67.7 (63.7;72.0)	186 (161;214)	271 (241;306)	68.9 (61.9;76.8)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)	**>70%**	**87.2** (85.2; 89.0)	**93.5** (92.0; 94.8)	**72.9** (70.4; 75.3)	**86.2** (82.6; 89.3)	**95.4** (93.0; 97.2)	**74.8** (70.4; 78.8)
POST/PRE							
Ratios of Titers							
Geometric mean (1/dil) (95% CI)	**>2.5**	**9.17** (8.33; 10.1)	**11.5** (10.4; 12.7)	**6.39** (5.96; 6.84)	**9.71** (8.19; 11.5)	**11.2** (9.58; 13.1)	**6.63** (5.90; 7.46)
Seroconversion or significant increase rate							
% (95% CI)	**>40%**	**57.5** (54.7; 60.2)	**66.5** (63.8; 69.0)	**56.**7 (54.0; 59.4)	**56.4** (51.6; 61.1)	**69.3** (64.7; 73.6)	**60.8** (56.0; 65.4)

N: number of subjects analyzed

Mean data fulfilling the CPMP criteria are shown in bold

Table 5 presents the assessment of CPMP criteria and GMTs. Baseline seroprotection rates were similar between groups for each strain, and were slightly higher for the A/H3N2 strain than for the A/H1N1 and B strains.

After vaccination, an immune response was observed in the ID and IM groups, with GMTs of 181 (1/dil), 277 (1/dil) and 67.7 (1/dil), for the A/H1N1, A/H3N2 and B strains, respectively in the ID 9µg group. These GMTs were similar to those observed in the IM group. The three CPMP criteria were fulfilled with the ID Influenza Vaccine 9µg for each of the three strains, 95% CIs inclusive.

Effect of presence of wheal after injection
In the SafAS population, 46.7% of the subjects receiving the ID 9 µg vaccine presented a wheal at injection site. Geometric mean of titers ratios (GMTRs) and seroconversion or significant increase rates results were very similar in subjects presenting a wheal with respect to those without a wheal at injection site, for each of the three strains, as well as post-vaccination GMTs and seroprotection rates for the A/H3N2 and B strains. For the A/H1N1 strain, post-vaccination GMTs and seroprotection rates were slightly higher in subjects presenting a wheal at injection site. All three CPMP criteria were met in both groups and for each of the three strains.

Effect of presence of leakage at the injection site
In the FASI population, only 80 subjects (4.5%) vaccinated by ID route presented a leakage at the injection site. In the OI population, 59 subjects were assessed for the A/H1N1 and A/H3N2 strains, and 58 subjects for the B strain. Subjects presenting a leakage obtained slightly lower immunogenicity results than those without leakage. However for these subjects the three EMEA criteria were fulfilled for each strain, except seroprotection rate for the B strain: 57.9% [41.2; 81.5].

Influence of co-variates on post-vaccination titers
The influence of several covariates was explored separately on log10-transformed post-vaccination titers in the FASI population. Seroprotection status at baseline (categorized as <40 and ≥40 [1/dil]), previous influenza vaccination status, age (<40 years or >40 years), country, BMI and risk status (defined as any lung, heart, renal, neurological diseases, any diabetes, or any other significant history (such as HIV, cancers, Hepatitis [A, B, C], epilepsy, auto-immune diseases, blood disorders) were found to have a statistically significant effect on log10-transformed post-vaccination titers in each group. However, whatever the studied covariate, the differences between vaccine groups were not significantly different across the covariate categories and the same trends were observed in both vaccine groups.

Influence of risk status on immune responses
The immune responses in subjects at risk were consistently lower than in subjects not at risk, however, there are no consistent differences between the ID and IM administration routes in this study.

Elderly
GID16
Non inferiority of the ID 15 µg vaccine versus the IM15 µg vaccine
Results of this primary analysis are summarized in Table 6 for the PPI population. The immunogenicity results observed in the ID 15µg group were first compared to those in the IM 15µg group using a non-inferiority testing approach on each strain. For each strain, the primary parameter for non-inferiority was the difference of the log10 transformation of post-vaccination GMTs between the compared vaccine groups: $\log_{10}(GMT_{ID})$- $\log_{10}(GMT_{IM})$. The non-inferiority criteria was that $\log_{10}(GMT_{ID})$- $\log_{10}(GMT_{IM})$>-0.176, (equivalent to GMT_{IM}/GMT_{ID}<1.5).

Table 6: Immunogenicity Primary Criteria. Non-inferiority of ID 15µg versus IM 15µg Injected Vaccine Groups - Per Protocol Analysis Set for Immunogenicity

	ID 15µg			IM 15µg		
	A/New Caledonia/20/99	A/Wellington/1/2004	B/Jiangsu/10/2003	A/New Caledonia/20/99	A/Wellington/1/2004	B/Jiangsu/10/2003
PRE-VACCINATION (D0)						
N analyzed	357	356	358	357	357	358
Titers						
Geometric mean	23.2	96.5	27.4	24.1	87.1	25.1
(95% CI)	(20.8; 26.0)	(83.5; 112)	(24.4; 30.7)	(21.6; 26.8)	(75.1; 101)	(22.5; 28.1)
POST-VACCINATION (D21)						
N analyzed	358	358	359	357	358	358
Titers						
Geometric mean	86.6	402	101	57.1	236	67.9
(95% CI)	(76.5; 98.1)	(355; 455)	(90.8; 113)	(51.2; 63.7)	(206; 271)	(60.7; 76.0)
Log titers difference vs 15µg IM						
log10(GMT ID)-log10(GMT IM)	0.181	0.231	0.174			
95% CI	(0.109; 0.252)	(0.152; 0.311)	(0.106; 0.242)			
Non-inferiority*	Yes	Yes	Yes			
Superiority†	Yes	Yes	Yes			
Adjusted p-value‡	<.0001	<.0001	<.0001			

*Non-inferiority if the left limit of the 95% CI >-0.176
†Superiority if the left limit of the 95% CI >0
‡Dunnett adjustment for multiple (2) group comparisons for each strain

In the PPI population, the non-inferiority of the immunogenicity of the ID 15 µg vaccine versus that of the IM 15 µg vaccine was demonstrated for each of the three strains: the lower bound of the difference of log10-transformed post-vaccination GMTs was higher than -0.176 for all strains. The following ratios of GMTs (95%CI) versus the IM 15µg group were observed: 1.52 (1.29; 1.79) for the A/H1N1 strain, 1.70 (1.42; 2.05) for the A/H3N2 strain and 1.49 (1.28; 1.74) for the B strain. As non-inferiority was demonstrated, superiority of the ID 15 µg vaccine over the IM 15 µg vaccine was assessed.

The superiority of the immunogenicity of the ID 15 µg vaccine versus that of the IM 15 µg vaccine was demonstrated in the FASI population for the three strains as the lower bound of the difference of log10-transformed post-vaccination GMTs was greater than 0 (lower bounds of 0.102 for the B strain, 0.112 for the A/H1N1 strain, and 0.153 for the A/H3N2 strain, with adjusted p values <0.0001). The observed GMTs were significantly higher in the ID 15µg group than in the IM 15µg group. The following ratios of GMTs (95%CI) versus the IM 15µg group were observed: 1.52 (1.29; 1.79) for the A/H1N1 strain, 1.70 (1.42; 2.04) for the A/H3N2 strain and 1.48 (1.26; 1.73) for the B strain.

Results obtained in the FASI and in the PPI populations led to the same conclusions, i.e. non-inferiority and superiority of the ID 15 µg vaccine for the three strains.

Comparison between each of the two ID vaccines and the IM vaccine
The comparison between the immune responses of the ID 15 µg and ID 21 µg vaccines versus that of the IM 15 µg vaccine, demonstrated a significant superiority of the two ID dose levels over the IM 15 µg dose level on at least two strains for each CPMP criterion. The comparison between the two ID dose levels both in terms of CPMP criteria and GMTs did not show the superiority of the ID Influenza Vaccine 21µg over the ID Influenza Vaccine 15µg.

Leakage at the injection site
Twenty-four subjects (6.5%) presented a leakage in the ID 15µg group and 21 subjects (5.7%) presented a leakage in the ID 21µg group. Theoretically, leakage of vaccine from the injection site may result in lower dose of vaccine being delivered and subsequently a lower immunogenicity response could be seen in individuals with leakage. As leakage was observed in less than 15% of subjects, the potential effect of the presence of leakage at the injection site on the immunogenicity results was not statistically assessed. However, the immunogenicity of the two ID groups was compared again to the IM 15µg group, in subjects with no leakage on the skin after ID injection, and the results remain similar to those obtained on all subjects.

Cell-mediated immunity
The cellular responses against influenza were measured in 90 elderly subjects after one injection of ID influenza vaccine (either 15 or 21 µg HA/strain per 0.1 ml dose) or the Vaxigrip Flu IM vaccine (15 µg HA/strain per 0.5 ml dose). Antigenic *in vitro* re-stimulations were performed on purified frozen PBMC before and 21 days after vaccination with either killed split of live homologous or heterologous influenza viruses. Both CD4 and CD8 responses were monitored by 3 different techniques; 1) intracellular IFN-γ and IL-4 staining by flow cytometry, 2) IL-2 release by ELISPOT and 3) Th1/Th2 cytokine profile /IL-4, IL-5, IL-10, IFN-γ, TNF-α, IL-2) by Cytometric Bead Array.

Before vaccination, an influenza-specific CD4 Th1 response was observed in all subjects, as judged by a predominant IFN-γ and IL-2 secretion and the absence of IL-4, IL-5 and IL-10 detection. This response detected before vaccination was only moderately increased by the vaccination and no significant difference was demonstrated between IM and ID routes on DC4 T-cell activation. A CD4 response against heterologous strains probably due to recognition of conserved CD4 epitope was observed pre and post vaccination, but once again, no significant difference was observed between ID and IM immunization routes.

A weak and heterogenous CD8 response was measured by ICS before and after vaccination. This response was not increased by the vaccination whatever the virus strain used for the in vitro re-stimulation. No significant differences could be demonstrated between IM and ID routes on CD8 T cell activation.

In conclusion, this study showed that, in elderly population, 21 days after vaccination, the ID influenza vaccine, with a dosage equivalent or superior to that of Vaxigrip, induced a cellular response of comparable profile and intensity that the Vaxigrip administered by the IM route.

GID17
First vaccination

Superiority analysis
Pre-vaccination GMTs and seroprotection rates for each strain were similar between groups. The primary objective was to demonstrate that the ID investigational vaccine induces a better immunogenicity than the IM control vaccine in terms of seroprotection rate after the first vaccination. A two-step approach was adopted. First, the non-inferiority of the ID investigational vaccine was assessed based on the analysis performed on the PPI population. As a second step, superiority of the ID investigational vaccine was assessed, using the FASI population. These analyses are summarised in Table 7.

Table 7: Immunogenicity Primary Criteria . Superiority of ID 15µg versus IM 15µg Injected Vaccine Groups. First Vaccination

Injected Vaccine Group	ID 15µg			IM 15µg		
Strain	A/New Caledonia/20/99 (H1N1)	A/Wisconsin /67/2005 (H3N2)	B/Malaysia/2506/2004	A/New Caledonia/20/99 (H1N1)	A/Wisconsin /67/2005 (H3N2)	B/Malaysia/2506/2004
PRE-VACCINATION*						
N analyzed*	2600	2600	2597	1077	1076	1077
Titers (1/dil)*						
Geometric mean	20.6	36.3	11.0	21.7	33.8	11.5
95% CI	(19.7 ; 21.5)	(34.2 ; 38.6)	(10.7 ; 11.4)	(20.2 ; 23.3)	(30.8 ; 37.2)	(10.9 ; 12.1)
POST-VACCINATION*						
N analyzed*	2595	2595	2592	1077	1078	1078
Titers (1/dil)*						
Geometric mean	81.9	298	39.9	69.1	181	34.9
95% CI	(78.2 ; 85.8)	(282 ; 315)	(38.2 ; 41.6)	(64.1 ; 74.4)	(167 ; 197)	(32.7 ; 37.3)
Ratio vs. IM 15µg†						
GMT_{ID}/GMT_{IM}	1.190	1.641	1.143			
(95% CI) of the ratio	(1.091 ;1.300)	(1.483 ;1.816)	(1.062 ;1.242)			
Log difference vs. IM 15µg†						
$log10(GMT_{ID})-log10(GMT_{IM})$	0.076	0.215	0.06			
(95% CI) of the difference	(0.038 ; 0.114)	(0.171 ; 0.259)	(0.026 ; 0.094)			
Non-inferiority	Yes	Yes	Yes			
Seroprotection (>= 40 l/dil)‡						
n/N	1998/2595	2422/2595	1443/2592	767/1077	947/1078	529/1078
%	77.0	93.3	55.7	71.2	87.8	49.1
Difference vs. IM 15µg	5.78	5.49	6.60	-	-	-
(95%CI) of the difference	(2.67 ; 8.97)	(3.40 ; 7.76)	(3.05 ; 10.1)	-	-	-
Superiority*	Yes	Yes	Yes	-	-	-

* FASI population results

† PPI results: Non-inferiority if for each strain, the two-sided 95% CI of the log difference of the geometric mean titers ID-IM lies above -0.176.

‡ FASI results: Superiority if for at least two strains, the two-sided 95% CI of the difference of the seroprotection rate ID-IM lies above 0.

As non-inferiority was demonstrated, superiority of the ID 15 µg vaccine over the IM 15 µg vaccine was assessed in the FASI population. The superiority of the immunogenicity of the ID 15 µg vaccine versus that of the IM 15 µg vaccine was demonstrated in the FASI population for the three strains as the lower bound of the 95% CI of the difference of the seroprotection rates (ID - IM) was above zero (lower bound of 2.67 for the A/H1N1 strain, 3.40 for the A/H3N2 strain and 3.05 for the B strain). The point estimates for the differences of seroprotection rates between the two groups (ID 15 µg . IM 15 µg) were 5.78 for the A/H1N1 strain, 5.49 for the A/H3N2 strain and 6.60 for the B strain.

Results obtained in the FASI and in the PPI populations led to the same conclusions; respectively, non-inferiority of the ID 15 µg vaccine versus the IM 15 µg vaccine in terms of GMTs for the three strains, and superiority of the ID 15 µg vaccine over the IM 15 µg vaccine in terms of seroprotection rates for the three strains.

CPMP criteria
Overall, seroprotection rates obtained met the CPMP requirements, in both groups, for the A/H1N1 and A/H3N2 strains, values obtained for these two strains being >60%, 95% CIs inclusive. In terms of GMTRs, this CPMP criteria is met for all strains in both groups, 95% CIs inclusive. Seroconversion rates or significant increase in titers obtained meet the CPMP requirements for all strains in the ID 15µg group (95% Cis inclusive), and for the A/H3N2 and B strains in the IM 15µg group.

Table 8 presents the assessment of the CPMP criteria and GMTs.

Table 8: GID17 Vac1 - CPMP Immunogenicity Parameters of the Three Vaccine Strains According to Injected Vaccine Group - Subjects with Pre- and Post-vaccination Titers – Other Immunogenicity Analysis Set

		ID Influenza Vaccine 15µg			**IM Influenza Vaccine**		
Strain	**CPMP threshold**	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia /2506/2004	A/New Caledonia /20/99(H1N1)	A/Wisconsin 67/2005(H3N2)	B/Malaysia /2506/2004
N analyzed		2585	2586	2582	1076	1075	1077
PRE-VACCINATION							
Geometric mean (1/dil) (95% CI)		20.6 (19.7; 21.5)	36.3 (34.2; 38.6)	11.0 (10.7; 11.4)	21.6 (20.1; 23.2)	33.9 (30.8; 37.2)	11.5 *(10.9; 12.1)*
Seroprotection (≥ 40 [1/dil])							
% (95% CI)		32.5 (30.7; 34.3)	48.9 (47.0; 50.9)	12.0 (10.7; 13.3)	33.8 (31.0; 36.7)	47.0 (44.0; 50.0)	12.4 *(10.5; 14.6)*
POST-VACCINATION							
N analyzed		2585	2586	2582	1076	1075	1077
Geometric mean (1/dil) (95% CI)		81.7 (78.0;85.6)	298 (282;315)	39.9 (38.3;41.6)	68.8 (63.8; 74.2)	181 (167;197)	34.8 *(32.6;37.2)*
Seroprotection (≥ 40 [1/dil])							
% (95% CI)	**>60%**	**77.0** (75.3; 78.6)	**93.3** (92.3; 94.3)	55.7 (53.8; 57.6)	**71.1** (68.3; 73.8)	**87.9** (85.8; 89.8)	48.9 *(45.9; 52.0)*
POST/PRE							
Ratios of Titers (95% CI)	**>2**	**3.97** (3.77; 4.18)	**8.19** (7.68; 8.74)	**3.61** (3.47; 3.76)	**3.19** (2.94; 3.45)	**5.35** (4.87; 5.88)	**3.04** *(2.85; 3.24)*
Seroconversion or significant increase							
% (95% CI)	**>30%**	**38.7** (36.8; 40.6)	**61.3** (59.3; 63.1)	**36.4** (34.5; 38.3)	30.0 (27.3; 32.9)	**46.9** (43.9; 49.9)	**30.7** *(28.0; 33.6)*

N: number of subjects analyzed

Mean data fulfilling the CPMP criteria are shown in bold

Table 9: GID17 Vac2 - EMEA Immunogenicity Parameters of the Three Vaccine Strains According to Injected Vaccine Group - Subjects with Pre- and Post-vaccination Titers - Other Immunogenicity Analysis Set

		ID Influenza Vaccine 15µg			IM Influenza Vaccine		
Strain	**EMEA threshold**	**A/Solomon Islands/3/2006 (H1N1)**	**A/Wisconsin /67/2005 (H3N2)**	**B/Malaysia/2506/2004**	**A/ Solomon Islands/3/2006 (H1N1)**	**A/Wisconsin /67/2005 (H3N2)**	**B/Malaysia/2506/2004**
N analyzed		261	259	262	143	142	143
PRE-VACCINATION							
Geometric mean (1/dil) (95% CI)		20.8 (18.2; 23.7)	112 (94.4; 132)	24.3 (21.6; 27.3)	19.0 (15.6; 23.0)	102 (81.8; 127)	22.4 (19.3; 25.9)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)		29.1 (23.7; 35.0)	80.3 (74.9; 85.0)	34.4 (28.6; 40.4)	25.9 (18.9; 33.9)	80.3 (72.8; 86.5)	35.0 (27.2; 43.4)
POST-VACCINATION							
N analyzed		261	259	262	143	142	143
Geometric mean (1/dil) (95% CI)		204 (175; 239)	382 (334; 438)	46.2 (41.4; 51.6)	137 (108; 175)	293 (240; 357)	37.4 (32.0; 43.7)
Seroprotection (≥ 40 [1/dil])							
% (95% CI)	**>60%**	**93.1** (89.3; 95.9)	**98.1** (95.6; 99.4)	59.9 (53.7; 65.9)	**81.8** (74.5; 87.8)	**95.8** (91.0; 98.4)	53.1 (44.6; 61.5)
POST/PRE							
Ratios of Titers (95% CI)	**>2**	**9.84** (8.43; 11.5)	**3.42** (2.99; 3.91)	1.90 (1.75; 2.07)	**7.24** (5.82; 9.02)	**2.88** (2.43; 3.41)	1.67 (1.50; 1.86)
Seroconversion or significant increase							
% (95% CI)	**>30%**	**76.2** (70.9; 81.3)	**45.9** (39.8; 52.2)	17.2 (12.8; 22.3)	**63.6** (55.2; 71.5)	**40.1** (32.0; 48.7)	9.8 (5.5; 15.9)

N: number of subjects analyzed

Second vaccination

Immunogenicity results after Vac2 are presented (Table 9) in the Other Immunogenicity Analysis Set population. Prevaccination GMTs for each strain were similar between groups. The proportions of seroprotected subjects at baseline were similar between the ID 15µg and IM groups.

Data are not yet available for Vac3. The interim iCSR including results obtained up to 21 days after Vac3 will be available in May 2009.

Based on the results after Vac2, it is expected that the same trend will be obtained after Vac3, confirming acceptable repeatability of ID vaccination.

Antibody persistence
In GID17, 12 months after Vac1, the seroprotection rates decreased over time with a similar trend observed for the ID Influenza Vaccine 15µg and the IM Influenza Vaccine for the three strains. At D180 and D365, seroprotection rates remained slightly higher to similar in the ID 15µg and IM 15µg groups for the A/H1N1 and the A/H3N2 strains. For the B strain, at D180 and D365, the seroprotection rates were slightly lower to similar in the ID 15µg and IM 15µg groups (30.5% at D180 and D365 in the ID 15µg group versus 34.0% and 38.3% at D180 and D365, respectively, in the IM 15µg group).

In both groups, antibody titers remained higher than pre-vaccination titers 12 months after Vac1. At D90 and D180, the seroprotection rate remained ≥ 60% in the ID 15µg and IM 15µg group for the A strains, except for the A/H1N1 strain in the IM 15µg group at D180. At D365, the seroprotection rate remained ≥ 60% in both groups for the A/H3N2 strain, but not for the A/H1N1 and the B strains.

As regards GMTs, they remained slightly higher in the ID 15µg group than in the IM 15µg group for the A/H3N2 strain until D365. For the A/H1N1 strain, anti-HA antibodies remained higher in the ID 15µg group than in the IM 15µg group until D90, and were similar at D180 and D365.

For the B strain, the antibody persistence curve was similar to the A strains although titers were lower.

There were no major differences between the ID Influenza Vaccine 15µg and the IM Influenza Vaccine regarding the drop in GMTs, i.e. ratios of GMTs V03/V02, V04/V02, and V05/V02 for any strain.

Effect of presence of wheal or after injection or presence of leakage at the injection site
Among the subjects vaccinated with the ID 15 µg vaccine, 1 149 (44.1%) presented a wheal at the injection site. In the OI analysis set, no difference was observed in the immune response between subjects presenting a wheal or not at injection. The immune response in these subsets of subjects was similar to the one observed in the whole population

Analysis of immunogenicity parameters (post-vaccination GMTs and CPMP criteria) was conducted on subgroups of subjects of the ID 15µg group (OI population) presenting (or not) a leakage of the vaccine product after injection. Among the subjects vaccinated with the ID 15 µg vaccine, 65 (2.5%) presented a leakage, and 2 539 (97.4%) had no leakage. All parameters presented showed no relevant differences between subjects presenting a leakage and those without leakage

Baseline seroprotection status
The post-vaccination GMTs and GMTRs were described in the FASI population in the subjects who were not seroprotected at baseline (titer <40 [1/dil]). A large number of subjects were not seroprotected before vaccination: 1 749 (66.3%) and 711 subjects (57.0%), (A/H1N1 strain), in the ID 15µg and IM 15µg groups, respectively, 1 325 (87.0%) and 570 (77.4%) (A/H3N2 strain), and 2 280 (49.9%) and 942 (42.6%) (B strain).

Among these subjects, post-vaccination seroprotection rates were higher for subjects vaccinated with the ID 15 µg vaccine than for those vaccinated with the IM 15 µg vaccine. Indeed, the differences in seroprotection rates (ID-IM) and 95% CI were of 9.36% (5.12; 13.6) for the A/H1N1 strain, 9.65% (5.88; 13.6) for the A/H3N2 strain and 7.34% (3.56; 11.1) for the B strain. The responses were consistently lower in subjects with a baseline titer<40 [1/dil], than in subjects who were seroprotected at baseline.

Influence of potentially important covariates on seroprotection rates at D21
Additionally, the influence of several covariates (previous influenza vaccination status, gender, age group, country, BMI and risk status) on seroprotection rates observed in the ID 15µg and IM 15µg groups were explored separately in the FASI population. Whatever the studied covariate, the odds ratio between vaccine groups was not significantly different across the covariate categories and the same trends were observed in both vaccine groups.

- Clinical studies in special populations

No studies in special populations were performed.

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

An integrated analysis of study GID15 and GID23 was performed. The analysis provides a descriptive comparison between the ID 9µg and IM 15 µg vaccines. The results of the analysis did not change the conclusions from each individual study.

An integrated analysis of studies GID16 and GID17 was performed. The analysis provides a descriptive comparison of between the ID 15µg and IM 15 µg vaccines. The results of the analysis did not change the conclusions from each individual study.

Clinical safety

- Patient exposure

The overall safety analysis set across all the studies of the clinical development program, regardless of the delivery system used, included 3 994 vaccinations for the ID Influenza Vaccine 9µg and 3 031 vaccinations for the ID Influenza Vaccine 15µg.

Pooled data from the four key trials represent a total of 2384 adult subjects administered ID Influenza Vaccine 9µg and 2974 elderly subjects administered ID Influenza Vaccine 15µg. Comparison is made with 843 and 1458 subjects, respectively, having received IM Influenza Vaccine as a comparator.

The demographic characteristics at baseline were homogeneous between the key studies and between the groups of each individual study. In the adult indication fewer males than females were included in these studies: 40.9% versus 59.1% in the ID 9µg group and 39.1% versus 60.9% in the IM 15µg group. Baseline parameters such as skin type, body mass index (BMI), and risk status were measured in GID23 and were homogeneous between the ID 9µg and IM 15µg groups.

Fewer males than females were included in the key elderly studies: 45.3% versus 54.7% in the ID 15µg group and 46.3% versus 53.7% in the IM 15µg group. Baseline parameters such as skin type, BMI, and risk status were measured in GID17 and were homogeneous between the ID 15µg and IM 15µg groups.

- Adverse events

Table 10 presents an overall summary of solicited and unsolicited reactions and events and SAEs 21 days post-vaccination in the key studies in adults and in the elderly. As shown in this overview table, the frequency of injection site reactions was expectedly higher in subjects vaccinated by the ID route than by the IM route. Moreover, no difference emerged between the ID and the IM group in terms of

solicited systemic AEs and unsolicited AEs. In terms of age group, AEs and reactions tended to be more frequent in adults than in the elderly overall.

Table 10: Key Studies – Adults and Elderly – Summary of Adverse Events and Reactions within 21 Days after Vaccination (Safety Analysis Set)

	ADULTS						ELDERLY					
	Overall ID 9µg (N=2384)			Overall IM 15µg (N=843)			Overall ID 15µg (N=2974)			Overall IM 15µg (N=1458)		
	n/M	%	95% CI	n/M	%	95% CI	n/M	%	95% CI	n/M	%	95% CI
SUBJECTS WITH AT LEAST ONE:												
- Solicited injection site reaction	2185/2356	92.7	(91.6; 93.8)	485/829	58.5	(55.1; 61.9)	2353/2965	79.4	(77.9; 80.8)	491/1451	33.8	(31.4; 36.3)
- Severe solicited injection site reaction	452/2356	19.2	(17.6; 20.8)	34/829	4.1	(2.9; 5.7)	469/2965	15.8	(14.5; 17.2)	42/1451	2.9	(2.1; 3.9)
- Solicited systemic reaction	1050/2356	44.6	(42.5; 46.6)	404/829	48.7	(45.3; 52.2)	726/2965	24.5	(22.9; 26.1)	351/1451	24.2	(22.0; 26.5)
- Moderate or severe solicited systemic reaction	320/2356	13.6	(12.2; 15.0)	108/829	13.0	(10.8; 15.5)	142/2965	4.8	(4.0; 5.6)	79/1451	5.4	(4.3; 6.7)
- Severe solicited systemic reaction	65/2356	2.8	(2.1; 3.5)	25/829	3.0	(2.0; 4.4)	40/2965	1.3	(1.0; 1.8)	22/1451	1.5	(1.0; 2.3)
- Unsolicited event	617/2357	26.2	(24.4; 28.0)	225/830	27.1	(24.1; 30.3)	338/2966	11.4	(10.3; 12.6)	150/1451	10.3	(8.8; 12.0)
- Severe unsolicited event	52/2357	2.2	(1.7; 2.9)	22/830	2.7	(1.7; 4.0)	28/2966	0.9	(0.6; 1.4)	7/1451	0.5	(0.2; 1.0)
- Unsolicited systemic event	594/2357	25.2	(23.5; 27.0)	218/830	26.3	(23.3; 29.4)	329/2966	11.1	(10.0; 12.3)	146/1451	10.1	(8.6; 11.7)

	ADULTS						ELDERLY					
	Overall ID 9µg (N=2384)			Overall IM 15µg (N=843)			Overall ID 15µg (N=2974)			Overall IM 15µg (N=1458)		
	n/M	%	95% CI	n/M	%	95% CI	n/M	%	95% CI	n/M	%	95% CI
- Moderate or severe unsolicited systemic event	240/2357	10.2	(9.0; 11.5)	84/830	10.1	(8.2; 12.4)	138/2966	4.7	(3.9; 5.5)	65/1451	4.5	(3.5; 5.7)
- Severe unsolicited systemic event	48/2357	2.0	(1.5; 2.7)	22/830	2.7	(1.7; 4.0)	28/2966	0.9	(0.6; 1.4)	7/1451	0.5	(0.2; 1.0)
- Unsolicited reaction	161/2357	6.8	(5.8; 7.9)	57/830	6.9	(5.2; 8.8)	60/2966	2.0	(1.5; 2.6)	28/1451	1.9	(1.3; 2.8)
- Severe unsolicited reaction	15/2357	0.6	(0.4; 1.0)	5/830	0.6	(0.2; 1.4)	4/2966	0.1	(0.0; 0.3)	2/1451	0.1	(0.0; 0.5)
- Unsolicited injection site reaction	41/2357	1.7	(1.3; 2.4)	12/830	1.4	(0.7; 2.5)	13/2966	0.4	(0.2; 0.7)	5/1451	0.3	(0.1; 0.8)
- Severe unsolicited injection site reaction	4/2357	0.2	(0.0; 0.4)	0/830	0.0	(0.0; 0.4)	0/2966	0.0	(0.0; 0.1)	0/1451	0.0	(0.0; 0.3)
- Unsolicited systemic reaction	124/2357	5.3	(4.4; 6.2)	46/830	5.5	(4.1; 7.3)	50/2966	1.7	(1.3; 2.2)	23/1451	1.6	(1.0; 2.4)
- Moderate or severe unsolicited systemic reaction	42/2357	1.8	(1.3; 2.4)	16/830	1.9	(1.1; 3.1)	26/2966	0.9	(0.6; 1.3)	8/1451	0.6	(0.2; 1.1)
- Severe unsolicited systemic reaction	11/2357	0.5	(0.2; 0.8)	5/830	0.6	(0.2; 1.4)	4/2966	0.1	(0.0; 0.3)	2/1451	0.1	(0.0; 0.5)

	ADULTS						ELDERLY					
	Overall ID 9µg (N=2384)			**Overall IM 15µg (N=843)**			**Overall ID 15µg (N=2974)**			**Overall IM 15µg (N=1458)**		
	n/M	**%**	**95% CI**	**n/M**	**%**	**95% CI**	**n/M**	**%**	**95% CI**	**n/M**	**%**	**95% CI**
- Any SAE within 21 days	11/2384	0.5	(0.2; 0.8)	1/843	0.1	(0.0; 0.7)	20/2974	0.7	(0.4; 1.0)	9/1458	0.6	(0.3; 1.2)
- Any non-fatal SAE within 21 days	11/2384	0.5	(0.2; 0.8)	1/843	0.1	(0.0; 0.7)	19/2974	0.6	(0.4; 1.0)	9/1458	0.6	(0.3; 1.2)

Notes:

For solicited reactions, the denominator for percentages is the number of vaccinated subjects with at least one safety record available for solicited reactions. For unsolicited events, the denominator for percentages is the number of vaccinated subjects with at least one safety record available. For Serious Adverse Events, the denominator is the number of vaccinated subjects.

n: number of subjects

By convention for the integrated analysis, solicited AEs (at injection site or systemic) were considered as related to the vaccination and are called solicited injection site reactions or solicited systemic reactions.

Results on key studies correspond only to the data of GID23 in adults and GID17 in the elderly.

Immediate reactions
In the key studies, few immediate reactions were reported overall and most were reported in adults. These reactions tended to occur in a similar proportion in the ID and IM group.

In the ID group, 11 adult subjects out of 1796 (0.6%) had 16 immediate reactions and three elderly subjects out of 2612 (0.1%) had four immediate reactions. In the IM group, two adult subjects out of 453 (0.4%) had two immediate reactions; none occurred in the elderly. None of the subjects with at least one immediate reaction had a SAE.

In the adult and in the elderly population, immediate reactions in the ID group occurred mostly in the System Organ Class (SOC) of Nervous System Disorders (five reactions in five subjects), Gastro-Intestinal Disorders (three reactions in three subjects), Infections and Infestations (two reactions in two subjects), General Disorders (two reactions in one subject), and Musculo-Skeletal Disorders (two reactions in two subjects).

All immediate reactions in the elderly were mild. In adults, most were mild. Four adult subjects experienced eight moderate or severe immediate reactions.

In the overall adult and elderly populations combined, 17 subjects out of 6537 vaccinations (0.3%) had 23 immediate reactions in the ID group and two subjects out of 3001 vaccinations (0.1%) had two immediate reactions in the IM group.

Solicited Reactions

By convention for the integrated analysis, solicited AEs, at injection site or systemic, were considered as related to the vaccination and are called solicited injection site reactions or solicited systemic reactions

Injection site reactions

In both the adult and elderly population, injection site reactions following ID vaccination with respect to IM injection were more frequent, as seen in the key trials. This was expected, and confirmed results obtained in the earlier trials. In the pool of all studies, the frequency of solicited injection site reactions was similar to what was observed in the key studies.
All solicited injection site reactions, with the exception of echymosis, were observed with incidences ≥ 10% in the ID Influenza Vaccine groups (both adult and elderly population). The injection site reactions erythema, swelling, induration were more frequent and more extensive in subjects vaccinated with the ID Influenza Vaccine with respect to the IM Influenza Vaccine. Pruritus was also more frequently reported following ID vaccination. The majority of the injection site reactions initially occurred the day following vaccination. Importantly, the majority lasted only 3 days and resolved spontaneously.

In terms of severity, a marked difference was observed for erythema, swelling and induration in favour of the IM group, especially for erythema, and tended to occur longer than in the subjects vaccinated by the IM route. In both the adult and the elderly population, solicited injection site reactions in the subjects vaccinated by the ID route were more frequent (especially erythema, induration, swelling and pruritus)

In both adults and elderly injection site pain, as well as injection site ecchymosis, whether severe or not, occurred in similar proportions in the IM and ID group

Table 11: Incidences of Injection Site and Systemic Solicited Reactions after Vaccination with either ID Influenza Vaccine 9µg, ID Influenza Vaccine 15µg or IM Influenza Vaccine (Key studies)

Symptom	Grade	Adults				Elderly			
		9µg ID N = 2384		15µg IM N = 843		15µg ID N = 2974		15µg IM N = 1458	
		n	%	n	%	N	%	N	%
Injection site reactions (evaluated from Day 0 to Day 7 after vaccination)									
Injection site pain	Any	985	41.9	364	44.0	657	22.2	248	17.1
	Severe	3	0.1	1	0.1	5	0.2	0	0.0
Injection site erythema	Any	2002	85.0	157	19.0	2132	71.9	23[illegible]	16.1
	Severe	401	17.0	24	2.9	392	13.2	30	2.1
Injection site swelling	Any	1474	62.7	123	14.9	1157	39.0	140	9.7
	Severe	147	6.3	13	1.6	117	3.9	16	1.1
Injection site induration	Any	1445	61.5	165	19.9	1214	40.9	183	12.6
	Severe	104	4.4	9	1.1	66	2.2	13	0.9
Injection site ecchymosis	Any	195	8.3	54	6.5	128	4.3	61	4.2
	Severe	12	0.5	3	0.4	12	0.4	3	0.2
Injection site pruritus	Any	1005	42.7	75	9.1	867	29.2	98	6.8
	Severe	9	0.4	1	0.1	10	0.3	1	0.1
Systemic reactions (evaluated from Day 0 to Day 21 after vaccination)									
Fever	Any	89	3.8	29	3.5	72	2.4	51	3.5
	Moderate/severe	18	0.8	6	0.7	14	0.5	8	0.6
Headache	Any	709	30.2	249	30.1	405	13.7	202	13.9
	Moderate/Severe	191	8.1	70	8.5	69	2.3	32	2.2
Malaise	*Any*	*407*	*17.3*	*152*	*18.4*	*268*	*9.0*	*122*	*8.4*
	Moderate/Severe	*127*	*5.4*	*50*	*6.0*	*59*	*2.0*	*33*	*2.3*
Myalgia	*Any*	*531*	*22.6*	*244*	*29.5*	*321*	*10.8*	*163*	*11.2*
	Moderate/Severe	*110*	*4.7*	*41*	*5.0*	*64*	*2.2*	*40*	*2.8*
Shivering	*Any*	*205*	*8.7*	*66*	*8.0*	*122*	*4.1*	*69*	*4.8*
	Moderate/Severe	*47*	*2.0*	*14*	*1.7*	*22*	*0.7*	*9*	*0.6*

Systemic reactions

In adults and in the elderly, *headache, malaise*, and *myalgia* were the most commonly reported solicited reactions.In both the adult and the elderly population, solicited systemic reactions were found to occur with the same frequency in the subjects vaccinated by the ID route or by the IM route.

Solicited systemic reactions were not found either to be more severe or to occur longer in the ID group than in the subjects vaccinated by the IM route. Except for three reactions that were not solicited in the key studies, i.e. *asthenia, arthralgia*, and *sweating*, no difference emerged from the analysis of solicited systemic reactions in the pool of all studies compared to the key studies. There was no safety signal as regards the solicited systemic reactions that occurred within 7 days after vaccination, whatever the dose level of ID Influenza Vaccine and the delivery route

Overall the systemic solicited reactions were more frequent in the adult than in the elderly population. Data from the key studies confirm that the incidences of systemic reactions were similar following ID administration with respect to IM administration in both the adults and the elderly population (Table 11).

CHMP immunogenicity criteria for influenza vaccines

In both the adults and the elderly population, EMEA-defined reactions occurred at similar frequencies following ID or IM administration in the key studies. The most frequently reported reactions in both groups were malaise, shivering, and injection site ecchymosis.

Unsolicited adverse events

Unsolicited events reported for approximately 21 days after vaccination were analyzed across key studies, first by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC), then by primary Preferred Term (PT). Unsolicited AEs occurring at injection site are considered as reactions.

Approximately 75% of the events reported were considered as unrelated to vaccination by the study investigators. The frequencies of all reactions by SOC were <3% for adults administered ID Influenza Vaccine 9µg and < 1% for elderly subjects administered ID Influenza Vaccine 15µg. Analysis of SOCs corresponding to reported reactions showed no clinically relevant differences between the ID Influenza Vaccine and the IM vaccine in both adults and elderly. Each individual reaction was reported at a frequency below 1%.

Adult Studies

In the **key studies**, the most common unsolicited AEs and reactions occurred in the same SOCs in the ID and the IM group, although not in the same order of frequency. Overall frequencies were similar between the ID and the IM group, for each SOC and in terms of severity and relation to vaccination.

In the ID group, the SOCs with the highest frequencies of events and reactions were Infections and Infestations (9.3% - mostly nasopharyngitis and rhinitis), Nervous System Disorders (5.2% - mostly headache and migraine), General Disorders (4.2% - mostly fatigue, influenza-like illness, asthenia, and injection site warmth), Respiratory, Thoracic and Mediastinal Disorders (4.2% - mostly pharyngolaryngeal pain), Gastrointestinal Disorders (3.6% - mostly diarrhea and nausea), and Musculoskeletal and Connective Tissue Disorders (3.6% - mostly back pain, myalgia, arthralgia, pain in extremity). Similar results were found in the overall IM group.

The *AEs* categorized as common (i.e. with a frequency >1%) in the ID group were:

- Nasopharyngitis (3.9%)
- Headache (3.4%)
- Pharyngolaryngeal pain (2.6%)
- Rhinitis (1.4%)
- Back pain (1.3%)
- Cough (1.1%)
- Dysmenorrhea (1.1%)

In terms of severity, the highest proportion of subjects with unsolicited moderate or severe *AEs* occurred, in both the ID and the IM group, in the SOC of Infections and Infestations (3.1% of ID subjects).

No unsolicited adverse *reaction* was found to be common at the PT level in the key adult studies. Some systemic reactions appeared to be common at the SOC level (General Disorders and Administration Site Conditions, and Infections and Infestations). Those were never moderate or severe.

In both the ID and the IM group, the highest proportion of subjects with moderate or severe unsolicited *reactions* was found in the SOC of General Disorders and Administration Site Conditions, with 0.5% to 0.6% (and in the SOC of Infections and Infestations in the IM group with 0.5%), including five cases of severe influenza-like illness (0.2%), three cases of severe asthenia (0.1%), and three cases of severe fatigue (0.1%). As for severe *injection site* reactions, they included one severe injection site discoloration, one severe injection site reaction, and one severe injection site warmth.

In the pool of **all studies**, the most frequent unsolicited events and reactions occurred in the same SOCs as in the key studies. Overall frequencies were similar between the ID and the IM group, for each SOC and in terms of relation to vaccination.

Elderly Studies

In the key studies, the most common unsolicited events and reactions occurred in the same SOCs in the ID and the IM group, although not in the same order of frequency. Overall frequencies were similar between the ID and the IM group, for each SOC and in terms of severity and relation to vaccination. In GID16, however, a higher proportion of subjects had unsolicited systemic AEs in the ID group (20.6%) than in the IM group (14.4%) overall, although similar frequencies were found at the level of each SOC individually.

In the ID group, the SOCs with the highest frequencies of events and reactions were Infections and Infestations (4.2%, mostly nasopharyngitis), Musculoskeletal and Connective Tissue Disorders (2.4%, mostly back pain), General Disorders and Administration Site Conditions (1.4%, mostly fatigue, pyrexia, and asthenia), Gastrointestinal Disorders (1.2%, mostly diarrhea), Respiratory, Thoracic and Mediastinal Disorders (1.2%, mostly pharyngolaryngeal pain and cough), Nervous System Disorders (0.9%, mostly headache and dizziness). Similar results were found in the overall IM group.

Nasopharyngitis was the only *AE* considered as common at the PT level (1.0% in the IM group, and 1.2% in the ID group). The SOCs with an overall ID frequency >1% were similar in the IM group. In the ID group, no moderate or severe systemic AE appeared to be common at the level of the PT, only at the level of the SOC: Infections and Infestations (1.6%) and Musculoskeletal and Connective Tissue Disorders (1.1%). No systemic or injection site reaction was found to be common at the PT level. Similar results were found in the overall IM group.

In terms of severity, the highest proportion of subjects with unsolicited moderate or severe *AEs* occurred, in the ID and the IM group, in the SOC of Infections and Infestations (1.6% of ID subjects). In the ID group, the most frequent events in this SOC included nasopharyngitis, bronchitis, and rhinitis (those had a frequency >0.1%).

No unsolicited adverse *reaction* was found to be common either at the PT or at the SOC level in the key elderly studies.

The highest proportion of subjects with moderate or severe unsolicited *reactions* occurred in the SOC of Infections and Infestations in the ID group (0.4%) and the IM group (0.3%). In this SOC, in the ID group, severe reactions included three severe cases of influenza (0.1%), three severe cases of rhinitis (0.1%), two severe cases of bronchitis (0.1%), one severe case of herpes simplex, and one severe case of laryngitis, one severe pharyngitis, one severe respiratory tract infection, and one severe pneumonia. There was no severe injection site reaction.

In the pool of **all studies**, the most frequent unsolicited events and reactions occurred in the same SOCs as in the pool of key studies. Overall frequencies were similar between the ID and the IM group, for each SOC and in terms of relation to vaccination.

In addition to the AEs categorized as common in the pool of key studies, two *AEs* had a frequency >1% in the ID group when all studies are taken into account: headache (1.0%) and injection site pruritus, with a frequency of 1.4% in the ID group, being the only adverse *reaction* categorized as

common in the pool of all studies in the elderly population. No SOC had a markedly higher frequency in all elderly studies altogether compared to the key studies only.

During the procedure the Applicant has provided new safety data for the 3rd ID vaccination in adults (study GID15) (494 subjects, including 71 for the first time), and for the 2nd vaccination in elderly in GID17 (2 974, including 511 for the first time). Overall the adult safety database has been increased to 3 825 doses of ID Influenza Vaccine 9µg (with the final Micro-Injection System) in 3 049 adults. The elderly database provides safety data after administration of 5 939 doses of ID Influenza Vaccine 15µg in 3 485 elderly subjects.

Results of GID15 (adults) for the third vaccination provided no indication in either the adult or the elderly populations that there is an increase in frequency or severity of adverse reactions following repeated vaccinations, and the ID and IM vaccinations appear to be interchangeable from a safety perspective.

Additional data of a 6-month follow up after a 2nd vaccination in adults and elderly show that the frequency of SAEs and Deaths, including AESI.s, did not increase after revaccination with the ID or the IM route. The Applicant commits to provide remaining data from 2nd and 3rd vaccination in elderly (GID17).

- **Serious adverse events/deaths/other significant events**

GID15
After the second vaccination, 25 subjects (4.7%) in the ID 9µg group had 25 SAEs and 14 subjects (4.0%) had 16 SAEs in the IM 15µg group. In each group, SAEs occurred mostly in the SOC of Injury, Poisoning, and Procedural Complications (mainly fractures) with 6 subjects (1.1%) in the ID group and 5 subjects (1.4%) in the IM group. The next most frequent SOCs in the ID group were Benign, Malignant, and Unspecified Neoplasms (1.1%) and Psychiatric Disorders (0.8%). In the IM group, the next most frequent SOCs were Musculoskeletal and Connective Tissue Disorders in the IM group (0.9%), Nervous System Disorders (0.6%), and Psychiatric Disorders (0.6%).
No deaths occurred.

GID23
Over the whole study a total of 47 subjects experienced 49 SAEsa including three deaths, 39 subjects (2.2%) in the ID 9µg group experienced 41 SAEs and 8 subjects (1.8%) in the IM 15µg group experienced 8 SAEs.

All SAEs were considered to be unrelated to the vaccine or experiment according to both the Investigator and the Sponsor. The time to onset and heterogeneous distribution of these cases across SOCs did not raise any specific area of concern regarding the safety profile of the vaccine.

Three deaths were reported during the 6-month follow-up period, two in the ID 9µg group and one in the IM 15µg. None of these deaths were assessed as related to vaccination according to both the Investigator and the Sponsor.

GID17
VAC1

Overall, in the 6-month period after the first vaccination, 138 subjects (5.3%) in the ID 15µg group and 53 subjects (4.9%) in the IM 15µg group had at least one serious adverse event (SAE). There were no related SAEs in the IM group.

One subject in the ID group had a serious episode of myopericarditis. According to the Investigator, the event could have been related to the study vaccine as it is known that the influenza virus can cause myopericarditis.

The outcome was fatal for 19 subjects (0.7%) in the ID group and 4 (0.4%) in the IM group, life-threatening for 8 subjects (0.3%) in the ID group and 5 subjects (0.5%) in the IM group.

VAC2
Overall, more than 21 days after the second vaccination, 29 subjects (1.2%) in the ID\ID group, 6 subjects (1.2%) in the IM\ID group, and 6 subjects (1.2%) in the IM\IM group had at least one SAE. No SAEs were considered by the Investigator to be related to the vaccine.

In the ID\ID group, 5 subjects died, 17 recovered, 5 recovered with sequelae, and for 3 subjects the SAE was still ongoing at the end of the follow-up period. In the IM\ID group, 1 subject died and 5 recovered. In the IM\IM group, 1 subject died, 1 recovered, 1 recovered with sequelae, and for 3 subjects the SAE was still ongoing at the end of the follow-up period.

- Laboratory findings

As the ID Influenza Vaccine is manufactured according to a process derived from the Applicant's IM Influenza Vaccine, no clinical laboratory evaluations have been performed during this clinical development program.

- Safety in special populations

Analysis of the influence of gender or risk status on the safety of the Influenza Vaccine revealed similar trends between the ID and the IM group. Overall, there were more vaccinations followed by reactions and events in female than male subjects, and more SAEs were reported in the male population. In Phase III studies, especially in the elderly, the subjects with a risk status had more SAEs in the ID and in the IM groups.

No clinical data on exposed pregnancies are available. A follow-up of pregnancies conducted in GID02, GID15, and GID23 did not reveal any safety signal in the outcome of pregnancies.

- Safety related to drug-drug interactions and other interactions

No drug interaction studies have been performed for the investigational product, although in all studies subjects were not included if vaccination had been performed in the 4 weeks prior to vaccination with the investigational product – or was planned in the 4 weeks following vaccination. This was in order to minimize possible vaccine-vaccine interactions.

- Discontinuation due to adverse events

In the key studies, in both the adult and elderly populations, the proportion of subjects who discontinued due to an AE or SAE was similar between the ID and IM groups within each individual study.

- Post marketing experience

There is no safety data from post-marketing experience with the ID Influenza Vaccine.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan

Table Summary of the risk management plan

• Safety Concern	• Proposed Pharmacovigilance Activities (routine and additional)	• Proposed Risk Minimization Activities (routine and additional)
Important Identified Risks: none		
	▪ Not applicable	▪ Not applicable
Important Potential Risks:		
neuritis, encephalomyelitis, Guillain Barre syndrome, convulsion, vasculitis, thrombocytopenia, severe allergic reactions*	▪ Routine Pharmacovigilance activities ▪ The PSURs will provide a cumulative overview on these AESIs and will be delivered during the first two years of post-marketing experience. ▪ A six-monthly evaluation of the incidence of the above potential risks using a large medical record database (THIN) as well as the calculation of reporting rates for other EU countries not included in THIN (a UK only database) will be done. These analyses will examine trends over time and can be provided to EMEA. These analyses will allow for the measurement of the incidence of potential risks or changes in their reporting rates in larger and different populations than those studied during clinical development	Statements in Section 4.8 of the SPC: *Blood and lymphatic system disorders* Transient thrombocytopenia, transient lymphadenopathy *Immune system disorders* Allergic reactions, in rare cases leading to shock, angioedema *Nervous system disorders* Neuralgia, febrile convulsions, neurological disorders, such as encephalomyelitis and Guillain Barré syndrome *Vascular disorders* Vasculitis associated in very rare cases with transient renal involvement *Skin and subcutaneous tissue disorders* Generalised skin reactions including urticaria
Important Missing Information:		
1 - Clinical trials may have identified AEs with a frequency lower 0.04% (about 4 per 10 000). Very rare AEs could not be identified during the clinical development.	▪ Routine Pharmacovigilance activities	▪ Not applicable
2 - Repeated use data in the elderly are not currently available	▪ Topic under investigation in the GID17 clinical trial (2280 subjects); results will be available in 2009.	▪ Not applicable

* This list of Adverse Events of Special Interest (AESI) has been identified for pandemic influenza vaccines by the European Vaccine Manufacturers working group in collaboration with the EMEA considering the annual flu vaccines safety profile as a reference

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.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

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

During the evaluation, two major objections and a number of other concerns related to quality were identified. These have been appropriately addressed by the Applicant and are considered resolved. Two minor unresolved quality issues, having no impact on the Risk-benefit balance of the product, will be resolved as post-approval commitments

Non-clinical pharmacology and toxicology

The safety of the intradermal vaccine was studied in two repeat-dose toxicity studies in rabbits. There was no evidence for systemic toxicity. ID vaccination caused inflammatory reactions at the injection sites at all doses tested, characterized by reversible erythema and edema. Similar observations have been made in the clinic, and the importance of local reactions for the benefit-risk of this product should be based on clinical data.

A developmental toxicity study was conducted in rabbit addressing female fertility, embryo-fetal development (including an evaluation of teratogenicity) and early postnatal development. There were no adverse effects on any of these parameters. Antibodies to the vaccine were observed in both the dams and the foetuses.

Efficacy

The Applicant has provided evidence that the ID route of immunization is at least as immunogenic as the IM route. The immune responses as determined by HI after ID vaccination with 9 µg in the adult population (18-59 years) are non-inferior to the responses to the Applicant's licensed IM influenza vaccine (15 µg/dose) (Vaxigrip). Likewise, the immune responses after ID vaccination with 15 µg/dose in an elderly population (>60 years) were shown to be non-inferior. In addition the immune response in elderly was also shown to be statistically superior to that after IM vaccination with 15 µg/dose. Although the difference between the ID and IM administration routes in elderly was statistically significant, the clinical relevance of the difference is questionable.

Safety

Overall the adult safety database includes 3 825 doses of ID Influenza Vaccine 9µg (with the final Micro-Injection System) administered in 3 049 adults. The elderly database provides safety data after administration of 5 939 doses of ID Influenza Vaccine 15µg in 3 485 elderly subjects. This is considered to be sufficient to describe adverse reactions that occur uncommonly and to give an indication of any rare events.

The ID vaccine is very commonly associated with a range of local and systemic adverse reactions. These adverse events are not often of severe intensity and the safety profile would not preclude the use in adults 18 to 59 years and elderly aged > 60 years.

Although injection site reactions were as expected higher in subjects vaccinated by the ID route than by the IM route, no other data indicate that the safety of this vaccine is different from other authorised IM influenza vaccines.

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

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

Based on the justification stated by the MAH regarding the testing of one version only of the PIL, it is acceptable that only the 15 µg strength has been tested.

The legibility test report provided by the applicant is considered acceptable.

Risk-benefit assessment

Context

The active substances present in the vaccine are known and are produced in a manner that is identical to that of the Applicant's IM seasonal Influenza Vaccine, with all excipients present in the ID Influenza Vaccine being also present in the Applicant's IM seasonal Influenza Vaccine. Thus, from the composition point of view it is not anticipated any specific new risk associated with this vaccine.

Benefits

IDflu induces an adequate immune response in adults between 18 to 60 years and in the older than 60 years of age that was general comparable to that induced by a comparator IM vaccine containing 15 µg of antigen.

The vaccine uses a system that delivers the antigens into the dermis. The final Micro-injection system features a pre-filled, ready-to-use syringe with an integral micro-needle that protrudes 1.5 mm from the proximal end of the glass syringe. A benefit of this system compared to the classical intra dermal injection (Mantoux method) is that it overcomes the difficulties associated with the Mantoux method. The short length of the needle minimizes the risk of mechanical damage to nerves and blood vessels during ID administration.

Risk

IDflu is very commonly associated with a range of local and systemic adverse reactions. These adverse events are not often of severe intensity and the safety profile would not preclude the use in adults 18 to 59 years and elderly aged > 60 years.

The current safety database is considered to be sufficient to describe adverse reactions that occur uncommonly and to give an indication of any rare events. All adverse events of special interest will be continuously followed- up and be cumulatively presented in the PSURs as well as be addressed in the RMP.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- routine pharmacovigilance was adequate to monitor the safety of the product.
- no additional risk minimisation activities were required beyond those included in the product information.

Balance

The overall B/R of IDflu is positive.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of IDflu in the following indication:

9 microgram strength:
Prophylaxis of influenza in adults up to 59 years of age, especially in those who run an increased risk of associated complications.

The use of IDflu should be based on official recommendations.

15 microgram strength:
Prophylaxis of influenza in individuals 60 years of age and over, especially in those who run an increased risk of associated complications.

The use of IDflu should be based on official recommendations.

was favourable and therefore recommended the granting of the marketing authorisation.