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<b>Sponsor/ Company:</b>	Sanofi Pasteur	<b>Study Code:</b> GID02
		<b>Study Identifier:</b> NCT00703651
<b>Proprietary Vaccine Name:</b>	Inactivated, split-virion influenza vaccine for intradermal route	

**Title of the Study:** Immunogenicity of Two Dosages of Inactivated, Split-Virion Influenza Vaccine Administered by Intradermal Route in Comparison with Intramuscular Vaccination with Vaxigrip® in Adults

**Study centres:** 1 site in Czech Republic, 3 sites in Lithuania, and 5 sites in Belgium

**Publications:** None at the time of report writing.

**Study period:** First Visit of First Subject (first vaccination): 01 September 2003  
Last Visit of Last Subject (last V08): 17 November 2005  
6-Month safety follow-up: 19 May 2006

**Development phase:** Phase II

**Methodology / Trial Design:** Multicenter, randomized, open (for the administration route), double-blind (for the two dosages administered with the investigational system on Year 0) trial with three pharmaceutical presentations (two ID and one IM) used on Year 0 and two pharmaceutical presentations (one ID and one IM) on the second and third years:

Inclusion of subjects – Allocation of subjects number												
<b>V01 (Y0)</b>	<b>1<sup>st</sup> randomization</b>											
	<b>Group A</b>			<b>Group B</b>				<b>Group C</b>				
	ID 3 µg			ID 6 µg				IM 15 µg				
	<b>2<sup>nd</sup> randomization</b>											
<b>V05 (Y1)</b>	<b>A1</b>	<b>A2</b>	<b>B1</b>	<b>B2</b>	<b>C1</b>	<b>C2</b>						
	ID <sup>1</sup> 9 µg	IM 15 µg	ID 9 µg	IM 15 µg	ID 9 µg	IM 15 µg						
<b>V07 (Y2)</b>	<b>3<sup>rd</sup> randomization</b>											
	<b>A1a</b>	<b>A1b</b>	<b>A2a</b>	<b>A2b</b>	<b>B1a</b>	<b>B1b</b>	<b>B2a</b>	<b>B2b</b>	<b>C1a</b>	<b>C1b</b>	<b>C2a</b>	<b>C2b</b>
	ID <sup>2</sup>	IM	ID	IM	ID	IM	ID	IM	ID	IM	ID	IM

<sup>1</sup> A statistical analysis was performed on results obtained 21 days after the first vaccination, allowing the choice of the dosage to be administered for the second and third vaccinations.

<sup>2</sup> Same dosage as for Year 1.

Year 0:

- Two pharmaceutical presentations injected by the ID route with the BD ID system: 3 µg and 6 µg of each HA per dose of 0.1 mL.
- One pharmaceutical presentation injected by the IM route, i.e. control influenza vaccine: 15 µg of each HA per dose of 0.5 mL.

Year 1 and Year 2 (specified in Amendment 1 dated 20 April 2004):

- One pharmaceutical presentation injected by the ID route with the BD ID system: 9 µg of each HA per dose of 0.1 mL.
- One pharmaceutical presentation injected by the IM route, i.e. control influenza vaccine: 15 µg of each HA per dose of 0.5 mL.

**Objectives:****Primary objective:**

To compare the immunogenicity in terms of post-vaccination geometric mean titers (GMT) (anti-hemagglutinin [HA] antibodies) of two pharmaceutical presentations (3 µg and 6 µg of each HA) administered by the intradermal (ID) route with the Becton Dickinson (BD) ID system, with that of the current pharmaceutical presentation (15 µg of each HA) administered by the intramuscular (IM) route, 21 days after a single first vaccine injection in subjects aged 18 to 57 years.

*Hypothesis: For at least one of the two pharmaceutical presentations administered via the ID route, the GMTs of anti-HA antibodies against all three strains observed 21 days after a single vaccination are non-inferior to those observed 21 days after a single first vaccination of the pharmaceutical presentation administered by the IM route. 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.*

**Primary endpoint:**

Anti-HA antibody titers for the three strains obtained 21 days after the first vaccination were used to derive the post-vaccination geometric mean of titers (GMTs) in the ID 3 µg, ID 6 µg, and IM 15 µg groups.

**Secondary objective(s):**

- To evaluate the safety profile during the 21-day period following each vaccination (D0, D365/Y1, and D730/Y2) in each study group.
- To assess the effect of the repetition of ID/ID injections and of the interchangeability of ID/IM and IM/ID injections on the safety of vaccination.
- To evaluate the anti-HA antibody persistence 3 months, 6 months, and 1 year after the first injection.
- To assess the compliance of the pharmaceutical presentations administered by the ID route (two dosages) and by the IM route (one dosage) with the European Medicines Agency (EMA) Note for Guidance, by evaluating the immunogenicity, 21 days after each injection (Y0, Y1, and Y2 [Y2: for an immunogenicity subset of 240 subjects overall as modified in protocol Amendment 2, dated 20 May 2005]), in each study group.

For each vaccine strain, the recommendations are to meet at least one of the three following criteria:

Immunogenicity criteria defined by EMA	Age: 18 to 60 years
Seroconversion rate <sup>1</sup> or significant increase of titer <sup>2</sup> on D21	> 40%
Mean geometric increase <sup>3</sup> between D0 and D21	> 2.5
Percentage of seroprotected subjects <sup>4</sup> on D21	> 70%

<sup>1</sup> Proportion of subjects with a pre-vaccination titer <10 (1/dil) to a post-vaccination titer ≥40 (1/dil)

<sup>2</sup> Proportion of subjects with titers ≥10 before vaccination and ≥4-fold increase of the titer.

<sup>3</sup> Geometric mean of individual ratios (post-/pre-vaccination titers)

<sup>4</sup> Proportion of subjects achieving a post-vaccination titer ≥40 (1/dil)

**Secondary Objective added in Amendment 1, dated 20 April 2004:**

- To evaluate the immune response (anti-HA antibodies) induced by the pharmaceutical presentation (9 µg of each HA) administered by the ID route, in comparison to that of the current pharmaceutical presentation (15 µg of each HA) administered by the IM route, 21 days after the vaccine injection performed on Year 1 and on Year 2.

## **Secondary endpoints:**

### Safety

For each vaccination, the analysis of safety in all study groups addressed the occurrences of AEs within 30 minutes of injection, local or systemic AEs for 21 days following injection (solicited AEs within seven days after vaccination and unsolicited AEs within 21 days after vaccination), and SAEs at any time during the follow-up period.

For the second and third vaccinations, the effects of repetition of ID injections and interchangeability between ID and IM injections on safety were assessed.

### Immunogenicity

Anti-HA antibody titers for the three strains obtained before and 21 days after each vaccination were used to derive the evaluation criteria including:

- GMT
- Geometric mean of the individual titers ratio (GMTR) post-vaccination over pre-vaccination
- Proportion of subjects with seroconversion (defined as the conversion from a pre-vaccination titer of rising from  $< 10$ , initially seronegative subject, up to a post-vaccination titer of  $\geq 40$  after vaccination) or with a significant increase post-vaccination (defined as at least a 4-fold increase from a pre-vaccination titer of  $\geq 10$  initially seropositive subject, with at least a 4-fold increase in post- vaccination titer)
- Seroprotection rate (defined as the proportion of subjects with a titer  $\geq 40$ ) pre- and post-vaccination

In addition, anti-HA antibody titers for the three strains were to be obtained 3 months, 6 months and 1 year after the first vaccination and were to be summarized using descriptive statistics (such as GMT in all study groups).

For the first and second vaccinations (Year 0 and Year 1), the immunogenicity was evaluated in all subjects. For the third vaccination (Year 2), the immunogenicity evaluation was to be performed in the immunogenicity subset of 240 subjects, as specified in Amendment 2, dated 20 May 2005.

Moreover, given that subjects were re-randomized and re-vaccinated at Year 1 with a different dosage as the one previously used, it was decided to evaluate the antibody response at 3 and 6 months only in subjects who had received the 6  $\mu\text{g}$  dosage by the ID route and those who had received the IM formulation. Therefore, for subjects vaccinated with the ID 3  $\mu\text{g}$  dosage at D0, antibody titers at M3 and M6 were not measured. The same decision was applied to antibody titers of the ID 3  $\mu\text{g}$  group at M12, for the strains that differed from that contained in the formulation of the second vaccination.

The difference between the ID 9  $\mu\text{g}$  and IM 15  $\mu\text{g}$  groups was explored using the log- difference between the GMTs.

Antibody persistence after the first vaccination was described as mentioned in terms of GMTs, seroprotection rates and 95% confidence interval (CI) before, 21 days, 3 months, 6 months, and 1 year after the first vaccination and according to vaccine group.

### **Exploratory Objectives:**

- To evaluate the pain at the injection site with a visual analog scale (VAS) in each group and the acceptability of the ID injection with a questionnaire in ID groups.
- To evaluate the leakage appearing at the injection site immediately after injection, for both ID groups.

*Exploratory Objectives added in Amendment 1, dated 20 April 2004:*

- To evaluate the safety profile of suspected atopic subjects during the 21-day period following the second (Y1) and third (Y2) vaccinations.
- To evaluate the cellular mediated immune (CMI) response in a subset of subjects after the second (Y1) and third (Y2) vaccinations

*Exploratory Objective added in Amendment 2, dated 20 May 2005:*

- To evaluate the acceptability of the ID injection with a questionnaire in all groups 21 days after the third vaccination.

**Exploratory Endpoints:**

- The intensity of pain at the time of injection was evaluated after vaccination by each subject using a VAS: one measure was obtained, ranging between 0 mm and 100 mm. The mean of these values was presented for each study group. As a complementary analysis, the frequencies of the answers to the acceptability questionnaire after the second and the third vaccinations were presented by vaccine schedule.
- The presence of leakage was summarized for each ID group after each vaccination.
- The evaluation of the safety profile of suspected atopic subjects from Year 0.
- The percentage of  $\gamma$ IFN secreting T lymphocytes from Year 1.

**Sample size (Number of Subjects):**

	<b>Year 0 V01-V04</b>	<b>Year 1 V05-V06</b>	<b>Year 2 V07-V08</b>	<b>Safety follow Up</b>
<b>Planned sample size</b>	1 146 (382 subjects per group)			
<b>Number of subjects included at V01, V05 and V07</b>	1 150	1 095	832	
<b>Number of vaccinated subjects</b>	1 147	1 091	827	
<b>Number of subjects having completed the safety follow-up</b>				1 029
<b>Number of subjects randomized</b>	1 150	1 091	828	
<b>Number of subjects who completed the studied period (until V04, V06, and V08, respectively)</b>	1 143	1 086	826	
<b>Number of discontinued subjects by studied period</b>	7	9	6	
<b>Number of discontinued subjects between two studied periods</b>	NA*	48 (V04-V05)	254 (V06-V07)	
<b>Sample size for analysis</b>				
<b>Per protocol set population</b>		NA*	NA*	
A/H1N1 strain	1 128			
A/H3N2 strain	1 129			
B strain	1 117			
<b>Full analysis set population</b>		NA*	NA*	
A/H1N1 strain	1 148			
A/H3N2 strain	1 149			
B strain	1 137			
<b>Safety analysis set population</b>	1 149	1 091	828	
<b>Other immunogenicity population</b>				
A/H1N1 strain	1 147	1 082	212	
A/H3N2 strain	1 146	1 070	212	
B strain	1 125	1 080	210	

\*Not applicable

**Schedules of Vaccination and Specimen Collection:**

Three vaccinations: V01 (Y0), V05 (D365/Y1), and V07 (D730/Y2).

Blood samples for serology assessment at each visit of the study (8 blood samplings of 7 mL): D0 (V01), D21 (V02), month (M) 3 (V03), M6 (V04), Year 1 (V05), Year 1 + 21 days (V06), Year 2 (V07)\*, and Year 2 + 21 days (V08)\*.

*Blood samples added in Amendment 1 dated 20 April 2004:*

Blood sample (2 mL) for the specific IgE multi-allergen screening test at Year 1 (V05).

Blood samples (10 mL) for CMI assessment in a subset of subjects at Year 1 (V05), Year 1 + 21 days (V06), Year 2 (V07), and Year 2 + 21 days (V08).

\* Planned to be performed only in a subset of 240 subjects as specified in Amendment 2, dated 20 May 2005.

**Duration of Participation in the Trial:** 2.5 years per subject

**Product Under Investigation:**

*For the first vaccination (D0/Y0), two different dosages of the inactivated, split virion (with octoxinol 9) influenza vaccine (2003-2004 Northern Hemisphere formulation) administered with the intermediate BD ID system.*

*For the second vaccination (D365/Y1), one dosage of the inactivated, split virion (with octoxinol 9) influenza vaccine (2004-2005 Northern Hemisphere formulation) administered with the intermediate BD ID system.*

*For the third vaccination (D730/Y2), one dosage of the inactivated, split virion (with octoxinol 9) influenza vaccine (-2005 Southern Hemisphere formulation) administered with the final BD ID system.*

**Form/Dose/Route:**

Suspension in a pre-filled BD ID system/0.1 mL/ID injection into the deltoid area

**Batch number:**

First vaccination at D0:

ID 3 µg: S3906

ID 6 µg: S3909

Second vaccination at D365: ID 9µg: S3944

Third vaccination at D730: ID 9µg S3983

**System:**

For the first and the second vaccinations: intermediate BD ID system

For the third vaccination: final BD ID system

**Control Product:**

Inactivated, split-virion (with octoxinol 9) influenza vaccine administered by the IM route.

**Form/Dose/Route:**

0.5 mL/IM injection into the deltoid area

**Batch number:**

First vaccination at D0: X0635

Second vaccination at D365: Y0588

Third vaccination at D730: Z5718

**Other Product(s):** Not applicable

**Assessment methods****Immunogenicity:**

Antibody titers against HA for each strain of influenza were measured in sera using the hemagglutination inhibition (HI) technique.

Titration (expressed as reciprocal of dilution) was performed by the Sponsor's laboratories.

**Safety:**

The safety assessment was the same for all subjects (including suspected atopic subjects) (*added in Amendment 1 dated 20 April 2004*).

**EMEA criteria within 3 days following vaccinations:**

Number and percentage of subjects with at least one of the following reactions occurring in the three days following vaccination (based on the EMEA Note for Guidance): injection site induration > 5 cm observed for more than 3 days, injection site ecchymosis (bruising); fever characterized by axillary body temperature increased > 37.5°C and lasting 24 hours or more, malaise, shivering (characterized by the occurrence of rigors).

**Immediate reactions**

Immediate safety was assessed on the occurrence of any local and/or systemic reactions within 30 minutes after each vaccination. The subject was kept under medical supervision for 30 minutes. The Investigator recorded these reactions in the source documents and in the Case Report Form (CRF).

**Local and systemic safety**

Local and systemic reactions/events were recorded in the evening following each vaccination and daily for 14 days after vaccination by the subject in a diary card. The occurrence of any other adverse event between 15 days and 21 days after each vaccination was also recorded on the diary card. All safety information was transcribed in the source documents and the CRF by the Investigator following an interview with the subject at the following visit. For systemic events, the causal relationship to the vaccine was evaluated by the Investigator according to the following scale: unrelated, possible, probable and definite.

**Serious adverse events (SAE)**

Information on SAEs was collected from inclusion until 6 months after the last vaccination. They were reported in source documents, in the CRF, and specific forms (SAE Initial Reporting and SAE Follow-up Reporting forms). SAEs were followed until their resolution or until they were considered as stabilized by the investigator

**Pain at the injection site:**

Pain induced by the injection was assessed using a VAS for all subjects and with an "acceptability questionnaire" for subjects vaccinated by the ID route.

The acceptability questionnaire was to be completed in all groups just after each vaccination. After the first (Y0) and the second (Y1) vaccinations, the acceptability questionnaire was to be completed 21 days after vaccination only by subjects having received an ID injection. Twenty-one days after the third injection (Y2), the acceptability questionnaire was to be completed by all included subjects.

**Leakage at the injection site:**

For the injections performed with the BD ID system, the presence of leakage of the product on the skin was recorded.

**Criteria added in Amendment 1 dated 20 April 2004 for the second vaccination (Year 1):****Identification of suspected atopic subjects**

A clinical questionnaire upon atopic diseases was performed at V05 and a specific IgE multi-allergen screening test was used as a qualitative biological indicator of allergy to various common inhalant allergens in all subjects.

**Cellular mediated immune response**

The cellular response of T lymphocytes was analyzed by detection of  $\gamma$  interferon ( $\gamma$ IFN) secreting cells using the intracellular cytokine cytometry (ICC) named also as intracellular cytokine staining (ICS) method on whole blood in a subset of subjects. The percentage of  $\gamma$ IFN secreting CD8+ and CD4+ T lymphocytes for each stimulation (against peptides or Flu vaccine) for each subject of the subset was considered before and 21 days after the second vaccination.

The ICS method was performed by the Sponsor's laboratory.

**Statistical methods**

A first statistical analysis was performed on all data collected from the first vaccination (D0/Y0) to 21 days after the first vaccination (D21/Y0). A second analysis was performed on all data collected until 21 days after the second vaccination (D386/Y1), except for CMI data. A third analysis was performed on all data collected until 21 days after the second vaccination (D386/Y1), to include the CMI data. A fourth analysis was performed on all data

days after the third vaccination (D751/Y2) and the final 6-month safety follow-up. The final statistical analysis, including CMI results for the third vaccination\*, was performed in September 2005. In addition, to these analyses, two unplanned analyses (one during Y0 and one during Y1) were performed for project strategy reasons.

### **Primary analysis**

Each ID group was compared to the IM group using a non-inferiority testing approach on each strain (A/H3N2, A/H1N1, and B).

For each strain, the primary parameter was the difference of the  $\log_{10}$  transformation of post-vaccination GMTs between the compared vaccine groups. For each strain, the hypotheses tested were as follows:

$$H_0: \log_{10}(\text{GMT}_{\text{ID}}) - \log_{10}(\text{GMT}_{\text{IM}}) \leq -0.176 \Leftrightarrow \text{GMT}_{\text{IM}} / \text{GMT}_{\text{ID}} \geq 1.5$$

$$H_1: \log_{10}(\text{GMT}_{\text{ID}}) - \log_{10}(\text{GMT}_{\text{IM}}) > -0.176 \Leftrightarrow \text{GMT}_{\text{IM}} / \text{GMT}_{\text{ID}} < 1.5$$

The tested ID group was considered as non-inferior to the IM group if the hypothesis  $H_0$  was rejected on each strain.

If non-inferiority was demonstrated for a tested ID group, the superiority of this ID group was then to be tested. The corresponding hypotheses were stated as follows for each strain:

$$H_0: \log_{10}(\text{GMT}_{\text{ID}}) - \log_{10}(\text{GMT}_{\text{IM}}) \leq 0 \Leftrightarrow \text{GMT}_{\text{ID}} \leq \text{GMT}_{\text{IM}}$$

$$H_1: \log_{10}(\text{GMT}_{\text{ID}}) - \log_{10}(\text{GMT}_{\text{IM}}) > 0 \Leftrightarrow \text{GMT}_{\text{ID}} > \text{GMT}_{\text{IM}}$$

The tested ID group was considered as superior to the IM group if the hypothesis  $H_0$  was rejected on each strain.

For both non-inferiority and superiority, the statistical methodology was based on the use of the 95% two-sided CI of the difference of  $\log_{10}$  of the post-vaccination GMT.

Both per-protocol (as the main analysis for non-inferiority) and full analysis sets (as the main analysis for superiority) were used on the primary analysis.

### **Secondary analyses**

All secondary analyses were descriptive.

### **Exploratory analyses**

All exploratory analyses were descriptive.

All variables were described by vaccine group, using usual descriptive statistical analyses. The 95% CIs were calculated using:

- The normal approximate method for CIs of geometric means (GMTs and GMTRs)
- The exact binomial distribution (Clopper-Pearson method) for CIs of proportions

### **Sample Size**

Each ID group was tested at a 2.5% alpha level (one-sided hypothesis), which provided a global alpha level of 5%. A global alpha level of 5% (2.5% one-sided hypothesis for the test of each ID group), a maximum acceptable ratio of 1.5 in terms of post-vaccination GMT (i.e. a difference of 0.176 in terms of  $\log_{10}$  (GMT)) and a global power of 80.4% (93% for the individual test of each strain) were chosen to calculate the sample size. Assuming a maximal standard deviation of 0.67, 344 subjects per group were necessary to test the null hypothesis. Under the assumption that 10% of subjects would not be evaluable as per protocol, 382 subjects were needed in each group, therefore a total of 1,146 subjects were enrolled.

\*CMI results after the second and the third vaccinations are presented in an additional study report.

## **Results summary:**

### **Disposition of subjects**

A total of 1,150 subjects aged 18 to 57 years were included in the study: 383 were included and randomized in the ID 3  $\mu\text{g}$  group (group A), 382 were included and randomized in the ID 6  $\mu\text{g}$  group (group B) and 385 were included and randomized in the IM group (group C).

All subjects respected all inclusion and exclusion criteria. At inclusion, in the per protocol analysis set, the mean age was 39.1 years ( $\pm$  11.6 years) overall and similar in all treatment groups. The male/female sex ratio was 0.7 with a proportion of women slightly higher in the two ID groups than in the IM group.

One year later, for the second vaccination, a total of 1,091 subjects were randomized to one of the two study groups: 544 were included in the ID 9  $\mu\text{g}$  group and 547 were included in the IM group.

One year later, for the second vaccination, a total of 1,091 subjects were randomized to one of the two study groups: 544 were included in the ID 9 µg group and 547 were included in the IM group. All the subjects respected the V05 inclusion criterion. For the second part of the study, in the safety analysis set, the two groups were similar in terms of age and gender distribution.

For the third part of the trial, the Czech Authorities did not accept the use of a 2005 Southern Hemisphere influenza formulation and rejected the protocol Amendment 2 dated 20 May 2005. Therefore Czech subjects could not participate in Y2, leading to an important decrease in the number of subjects included at V07. Indeed, for the third vaccination, a total of 828 subjects (418 in the ID 9 µg group and 410 in the IM 15 µg group) received the third vaccination. All these subjects respected the V07 additional inclusion criterion (addendum 3 to the ICF signed). In the safety analysis set, the two groups were similar in terms of age (mean age of  $40.1 \pm 11.29$  years) and gender distribution (male/female sex ratio of 0.7).

### **Primary Objective: Immunogenicity**

The primary objective was to demonstrate that post-vaccination GMTs (anti-HA antibodies for the three strains) induced by at least one pharmaceutical presentation administered by the ID route were non-inferior to those induced by the pharmaceutical presentation administered by the IM route.

The non-inferiority of the immune response of the ID 3 µg and ID 6 µg vaccines in respect to that of the IM 15 µg vaccine could not be demonstrated in the per protocol set, as the lower bound of the 95% CIs of the difference of log transformed post-vaccination GMTs of the two ID groups versus the IM group was lower than -0.176 in both ID groups for all strains. Additionally, given the low upper bounds of these 95% CIs for all strains, these differences observed in the ID groups versus the IM group could be considered as clinically meaningful. Conclusions were similar using the full analysis set.

### **Secondary Objectives:**

#### Immunogenicity results:

Immunogenicity results obtained to assess the secondary objective are described in the other immunogenicity population.

#### *First part of the study*

In the OI population, in terms of EMEA criteria in the IM group, all three criteria were met for the three strains (except for the seroprotection rate for the B strain).

In the ID 6 µg group all three criteria were met for the strain A/H1N1 and A/H3N2 and only the GMTR criterion (mean geometric increase) was met for the B strain.

In the ID 3 µg group all three criteria were met for the A/H1N1 strain, two criteria (seroprotection rate and mean geometric increase) were met for the A/H3N2 strain and none for the B strain.

Both the ID 6µg and the IM 15 µg groups complied with the EMEA recommendations.

A significant variability of immunogenicity results was observed across centers, with an interaction on group effect. This might be due to an incorrect use of the system.

In the FASI population, the antibody persistence until M12 after the first vaccination against the three strains presented a similar shape and the decrease over time of GMTs in the ID 6 µg group did not seem to be different from that in the IM group, despite a constant higher level of antibodies in the IM group versus ID groups.

Moreover, 12 months after vaccination with either the ID 6 µg or IM 15 µg vaccine, GMTs remained higher than those observed before vaccination, for all strains. Similar observations can be performed in terms of seroprotection rates.

Following these immunological results, it was decided to use a higher ID dosage for the second vaccination, i.e. 9 µg of each HA/mL.

#### *Second part of the study*

Before the second vaccination, GMTs were similar between the ID 9µg and IM 15µg groups for the three strains.

In the OI population, in terms of EMEA criteria, in the IM 15µg group, all three criteria were met for all three strains including the 95% CIs. For the ID 9µg group, most of EMEA criteria were met: the seroconversion for A/H1N1 strain and seroprotection for B strain did not include the 95% CIs, but were close to the EMEA threshold.

Additionally, when considering the immunogenicity results of this second vaccination according to the first vaccine received at Year 0: no major difference could be observed between the IM 15µg group and the ID 9µg group results among subjects having received the same vaccine at Year 0, whatever this vaccine was.

#### *Third part of the study*

For the third vaccination, immunogenicity was evaluated in a subset of subjects randomized to blood sample by the IVRS.

In the OI population, in terms of EMEA criteria, each of the three parameters reached their corresponding thresholds in both groups for the A/H3N2 strain. One criterion (seroprotection rate) was met in both groups for the A/H1N1 and the B strains, 21 days after vaccination.

After the third vaccination, immunogenicity results were similar among the various vaccine schedules received over the 3-year period.

#### **Safety results:**

Safety results are presented in the safety analysis set.

- Serious Adverse Events:

Overall the trial, four deaths, not related to vaccination according to both the Investigator and the Sponsor were reported to Health Authorities (three deaths were reported after the first vaccination, and the fourth was reported after the second vaccination): one subject (ID 3µg group) due to hepatocellular carcinoma, one subject (ID 9µg group) due to thyroid carcinoma, myosarcoma, and pulmonary metastasis, two subjects (IM 15µg group) due to myocardial infarction or cerebral hemorrhage.

- EMEA criteria within 3 days following vaccinations:

Overall the trial and after each of the three vaccinations, the most frequently reported reactions were shivering and malaise in the ID and IM groups. No induration >5 cm for more than 3 days was observed in any group during the trial.

The safety profile according to the EMEA Note for Guidance was similar between the ID and IM groups and over the 3 years of the trial.

- Immediate reactions:

Similar safety profiles in terms of immediate reactions were observed after each of the three yearly vaccinations. In the ID and IM groups, the most frequently reported immediate reactions were injection site erythema, induration and edema in the ID groups and injection site pain in the IM group. After each vaccination, immediate reactions were more frequently reported in the ID than in the IM groups and this frequency was low.

- Solicited reactions within 7 days after vaccination:

After each vaccination, the proportion of subjects with at least one solicited local reaction was approximately twice higher than the proportion of subjects with at least one solicited systemic reaction in the ID groups. After each vaccination, there were more solicited local reactions in the ID groups than in the IM groups, whereas solicited systemic reactions were reported at similar frequencies in each study group. Most frequently reported solicited local reactions in the ID groups were injection site erythema (after each injection), injection site induration (after the first and the second vaccinations), injection site pain and injection site induration (after the third vaccination). In the IM groups, the most frequently reported solicited local reaction was injection site pain (after each vaccination). In both ID and IM groups and after each vaccination, asthenia and headache were the most frequently reported solicited systemic reactions.

Solicited reactions mainly occurred within 3 days after vaccination, were mild or moderate in severity and resolved within 3 days, whatever the group and the vaccination.

Globally after the third vaccination, the proportions of subjects with at least one solicited reaction slightly decreased in ID and IM groups in comparison with the second vaccination and were similar to those observed after the first vaccination.

- Unsolicited reactions/adverse events appearing within 7 days after vaccination:

Unsolicited reactions were reported by low proportion of subjects in each group and following each vaccination (after each vaccination, less than 10% of subjects had at least one unsolicited reaction). Unsolicited local reactions were mainly mild in severity, occurred within 3 days after vaccination and resolved within 3 days. In all groups and after each vaccination, they mostly concerned general disorders and administration site condition. Unsolicited systemic reactions, mainly mild and moderate in severity, also occurred within 3 days of vaccination for a duration of 3 days. They mainly concerned respiratory, thoracic and mediastinal disorders.

The occurrence and the severity of unsolicited local reactions within 7 days after vaccination were similar after each of the three vaccinations. Concerning unsolicited systemic reactions, a slight decrease of their occurrences was observed in the ID group between the first and the second vaccination.

- Unsolicited reactions/adverse events appearing from day 8 after vaccination:

The majority of unsolicited reactions that occurred from day 8 after vaccination, whatever the vaccination considered, were unsolicited systemic reactions, mainly mild in severity. Overall the trial in the ID and IM groups, the most frequently reported unsolicited reactions were asthenia and headache, of similar severity.

- Pregnancy:

Overall the trial, 18 pregnancies were reported. In the ID 3 µg group, among the five cases, four pregnancies were considered as normal, and one pregnancy ended with delivery by caesarean because of alteration of child's heart sounds. In the ID 6 µg group, among the four pregnancies reported, three were considered as normal and there was a voluntary termination. In the IM 15 µg group, two pregnancies were reported during Y0 and were considered as normal.

During Y1, two pregnancies were reported in each of the ID 9µg and IM 15 µg groups (all considered as normal). During the third part of the trial (Y2), three pregnancies were reported in the ID 9 µg group, two considered as normal and one with a premature delivery.

- Interchangeability results / Repetitiveness:

The safety profile observed after the second vaccination seems independent of the first vaccination route, whatever the safety endpoint considered.

The repetition of ID injections does not induce an increase of reactogenicity, as well as a previous vaccination via the ID route does not have an impact on reactogenicity of an IM vaccination.

After the third vaccination, the safety profile was similar to the one observed after the second vaccination, showing that the occurrence of solicited local reactions reaches a plateau after two repetitive ID injections.

The higher rate of solicited local reactions after three ID vaccinations in comparison with three IM vaccinations is not due to the repetition of ID vaccinations, but to the difference between the last route administration (ID or IM). Further, the local safety profile of the vaccination, whatever the route of administration, is independent of the previous vaccination route.

### **Exploratory Objectives:**

Results presented to describe the exploratory objectives are described in the safety analysis set.

Pain immediately after the injection was reported using a VAS. The median of the values given by the subjects on the VAS over on a 100 mm scale after the first vaccination varied between 5 mm and 6 mm for ID groups and was 4 mm for the IM group. After the second vaccination, the VAS median was 8.3% for the ID 9 µg group and 3.7% for the IM group. After the third vaccination, the VAS median was of 16 mm in the ID 9 µg group, and 5 mm in the IM 15 µg group.

- Acceptability questionnaire

After each vaccination, the results obtained with the acceptability questionnaire were different from the ones obtained with the assessment of pain induced by injection on a VAS. Indeed, the subjects vaccinated with the ID vaccine, who globally estimated the ID route as less painful than the IM route, had rated the pain with very high VAS scores.

Overall the trial, the ID route of vaccination remained more chosen than IM injection in the ID groups, but these proportions decreased after each vaccination.

As regards results of the acceptability questionnaire by schedule, subjects having received only ID injections felt ID injection less painful than subjects having received both ID and IM injections and therefore would more frequently

choose an ID rather than an IM injection for the next vaccination than subjects having received ID and IM injections.

- Leakage at the injection site

After the first injection, a leakage of vaccine at the ID injection site was reported for around 20% of subjects, whatever the dose administered. Even when the immunogenicity results were slightly higher in the subset of subjects without leakage, they were still lower than for the IM group. After the second vaccination of subjects, product leakage was reported in around 10%. After the third vaccination, product leakage was reported in 1.4% of subjects. This decrease of leakage may be due to the use of the final BD ID system.

- Safety profile of suspected atopic subjects

To describe the safety profile of suspected atopic subjects, three subsets of suspected atopic subjects were defined for the second part of the study according to the presence of at least one atopic clinical symptom, urticaria or allergy to drugs (subset 1: 417 subjects), according to a positive specific IgE multi-allergen screening test (subset 2: 320 subjects) and according to both (subset 3: 179 subjects).

For the first and the third vaccinations, only the safety profile of the subset 3 of atopic subjects, considered as the more relevant, was described and compared to the other subjects, i.e. subjects with negative result to the specific IgE multi-allergen screening test and/or without any atopic clinical symptom, urticaria, or allergy to drugs. Over the 3 years, between 55 and 86 subjects were included in the subset 3 of subjects, and 296 to 457 subjects were included in the other subjects subset in the ID groups. In the IM groups, between 57 and 93 subjects were included in the subset 3 of subjects, and 307 to 454 subjects were included in the other subjects subset over the 3 years.

In both groups and after each vaccination, the safety profiles in terms of immediate reactions and solicited injection site reactions were similar between the subset 3 of suspected atopic subjects in the subset of other subjects.

However, the proportions of subjects with at least one systemic event/reaction, solicited or not, were slightly higher in the subset 3 of suspected atopic subjects than in the other subjects, especially in the IM 15 µg groups. Further, solicited reactions were slightly more severe in subset 3 than in other subjects.

Although some differences between the overall safety profiles, both the ID and the IM vaccines are well tolerated in suspected atopic subjects.

- Cellular mediated immune response

CMI response results demonstrated that in an adult population the cellular immune response induced by either the ID or the IM vaccines was not significantly different. The level and the profile of the response induced by ID route with lower vaccine doses were indeed quite comparable to those induced by IM route with full standard dose. This is consistent with the quite potent humoral responses induced by the ID route that were equal or superior to those induced by a higher dose of influenza vaccine administered via the IM route.

### **Conclusions:**

- In this Phase II trial conducted in adults, after a first influenza vaccination using the ID 3 µg and 6 µg vaccines and the IM 15 µg vaccine as control group, the primary objective was not reached. Both ID 3 µg and 6 µg vaccines were inferior to the IM 15 µg vaccine in terms of post-vaccination GMTs for all three strains, even if the ID 6 µg vaccine fulfilled the EMEA requirements.
- The ID 9 µg vaccine, chosen for the second and the third vaccinations, and the IM 15 µg vaccine fulfilled the EMEA criteria 21 days after the vaccination during two consecutive seasons in the OI population.
- The ID 9 µg vaccine induced higher rates of local reactions, and of higher severity than the IM 15 µg vaccine following the second and the third vaccinations. No increase in terms of frequency and severity of local reactions was observed between the second and third vaccinations.
- Systemic safety profiles were similar in both the ID and IM groups following each of the vaccination.
- A previous vaccination via the ID route does not induce an increase of the reactogenicity of IM vaccination. The local safety profile was due to the last route of administration.
- In suspected atopic subjects, the number of systemic reactions was slightly increased compared to the general population. Furthermore, this slight increase in systemic reactions was observed in both the ID and the IM groups and slightly higher in IM than in ID groups.

According to the EMEA Note for Guidance, the benefit-risk ratio is similar between the ID 9 µg and the IM 15 µg vaccines in terms of immunogenicity and safety, which is why the ID 9 µg vaccine can be further developed in the adult population through Phase III trials in a larger sample size. Further, the administration of trivalent influenza vaccine is safe in subjects previously immunized by the vaccine administered by an alternate route.

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