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Sponsor/ Company:	Sanofi Pasteur	**Study Code:** GID16 **Study Identifier:** NCT00296829
Proprietary Vaccine Name:	Inactivated, split-virion influenza vaccine for intradermal route	

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

Study centres: 8 sites in Australia and 3 sites in New Zealand

Publications: None at the time of report writing.

Study period:	First visit of first subject: 16 January 2006 Last visit V04 of last subject : 04 July 2006 Last 6-months follow-up contact: 5 October 2006
Development phase:	Phase II

Methodology / Trial Design: Multicenter, randomized, open (for the administration route), double-blind (only for the two dosages administered by the ID route with the final system), controlled trial with three dosages (two ID and one IM).

There were three groups:

- ID 15 µg group: vaccine injected by the ID route with the final micro delivery system: 15 µg of each HA per 0.1 mL dose (2005 Southern Hemisphere formulation).
- ID 21 µg group: vaccine injected by the ID route with the final micro delivery system: 21 µg of each HA per 0.1 mL dose (2005 Southern Hemisphere formulation).
- IM 15 µg group: vaccine injected by the IM route, i.e. control influenza vaccine: 15 µg of each HA per 0.5 mL dose (2005 Southern Hemisphere formulation).

Subjects of the three groups were revaccinated with a 2006 Southern Hemisphere IM formulation of the influenza vaccine registered in Australia and in New Zealand, to ensure the protection of the subjects against the World Health Organization (WHO) recommended influenza strains.

Objectives:

Primary objective:

To demonstrate that at least one of the two dosages (15 µg and 21 µg of each hemagglutinin [HA]per strain) of the vaccine administered by the intradermal (ID) route with the final micro delivery system manufactured by Becton Dickinson (BD), is at least as immunogenic as the intramuscular (IM) administration of the vaccine.

Primary endpoint:

Anti-HA antibody titers for the three strains obtained on D21 after vaccination.

Secondary objective(s):

- To compare the immune response 21 days after vaccination between each ID group versus the IM group for each strain in terms of:
  1) post-vaccination seroprotection rate,
  2) seroconversion and/or significant increase rate,
  3) geometric mean of individual titer ratio (GMTR) (individual post- /pre-vaccination antibody titers).

- To describe the safety profile after the vaccination in each trial group.
- To describe the compliance of the two dosages of the vaccine administered by the ID route with the Committee for Proprietary Medicinal Products (CHMP)

  Note for Guidance specific for elderly subjects (CPMP/BWP/214/96).

Secondary endpoints:

Immunogenicity:

For each vaccine strain, anti-HA antibody titers were expressed as HI titers obtained in duplicate, summarized at the subject level by individual geometric mean of duplicates.

The derived endpoints were:

- Individual titer on D0 and D21
- Individual titers ratios: D21/D0
- Seroprotection status: titer ≥40 (1/dil) on D0 and D21
- Seroconversion for subjects with a pre-vaccination titer <10 (1/dil) on D0: post- vaccination titer ≥40 (1/dil) on D21, or significant increase for subjects with a pre-vaccination titer ≥10 (1/dil): ≥four-fold increase from pre- to post- vaccination titer on D21.

Safety/Reactogenicity:

- The occurrence of the following reactions (Medical Dictionary for Regulatory Activities [MedDRA] preferred terms) in the 3 days following vaccination was more especially reported (as defined by the CHMP Note for Guidance):
  - Injection site induration >5 cm observed for more than 3 days
  - Injection site ecchymosis (injection site bruising)
  - Fever (oral temperature >37.5°C) for 24 hours or more (pyrexia)
  - Malaise
  - Shivering (rigors)
- Occurrence, time to onset, number of days of occurrence, and severity of solicited (prelisted in the subject's DC and CRF) injection site reactions and systemic reactions occurring up to 7 days after the vaccination.
- Occurrence, nature (MedDRA preferred term), time to onset, duration, severity, and relationship to vaccination (only for systemic events) of unsolicited (spontaneously reported) AEs up to 21 days after the vaccination.
- SAEs were to be reported up to 6 months after the vaccination. SAEs related to the vaccination and all (even unrelated) fatal or life-threatening SAEs were collected throughout the trial.

Observational objective:

- To describe the safety profile during the 21-day period following an IM re-vaccination in each group and the possibility of any reaction at the first injection site (reactivation).
- To describe the pain at the injection site with a visual analog scale (VAS) and the acceptability of all the injections using an acceptability questionnaire in each group after each vaccination
- To describe the leakage appearing at the injection site immediately after the ID injection and to explore the relationship with immunogenicity.
- To evaluate the cellular mediated immune (CMI) response in a subset of 90 subjects (thus approximately 30 subjects from each group).

Observational endpoint:

Safety after the IM re-vaccination:

The endpoints were the same as above. Additionally, any injection site reactions, which were observed at the site of the previous vaccination, were assessed (the two vaccinations were not performed on the same side [one in the left deltoid and one in the right deltoid]).

Reactivation of the former site before IM re-vaccination:

The presence of a reaction on the first injection site was checked before the re-vaccination.

Pain at injection and acceptability of the vaccination:

The intensity of pain at the time of injection was evaluated just after each vaccination using a VAS: one value (ranging between 0 mm and 100 mm) was obtained for each subject. Additionally, the answers to the "acceptability questionnaire" (used on the day of vaccination and 21 days after the vaccination in all subjects) were described.

Leakage at the injection site:

The presence of product leakage on the skin at the ID injection site was considered just after the injection.

CMI response:

The frequency of γ -IFN (Th1 cytokine) or IL-4 (Th2 cytokine)-secreting CD8+ and CD4+ T lymphocytes, specific for different vaccine antigens, among respectively total CD8+ and CD4+ T lymphocytes was determined, before and 21 days after the vaccination.

The number of IL-2 (Th1 cytokine)-secreting cells per 10^6 PBMCs, specific for different vaccine antigens, was also assessed, before and 21 days after the vaccination.

Finally, the secretion of a panel of Th1 and Th2 cytokines by PBMCs, upon *in vitro* restimulations with different vaccine antigens, was quantified, before and 21 days after the vaccination.

Sample size (Number of Subjects):

- Planned sample size:

Total: 1,080 subjects, 360 per group

- Number of subjects included at D0: 1,107
- Number of subjects completed: 1,068
- Number of discontinued subjects: 39
- Sample size for primary analysis:

  Per protocol immunogenicity (PPI) population: 1,076 subjects

  Full analysis set for immunogenicity (FASI) population: 1,100 subjects

Schedules of Vaccination and Specimen Collection:

Vaccination: one ID or IM injection on Day 0 (D0).

Re-vaccination: one IM injection 3 months after the vaccination.

Blood samples were drawn in each group: immediately before the vaccination on D0 (V01) and 21 days after (V02) as follows:

- 7 mL drawn in each group for immunogenicity evaluation (antibody titers).
- 40 mL drawn in a subset of 90 subjects (30 subjects from each of the three groups) for the CMI assessment. For this subset of subjects, only one puncture was performed at both visits. A total of 94 mL were drawn per subject.

Duration of Participation in the Trial:

The total duration of follow-up for each subject was 6 months (6 months safety follow-up after the initial vaccination).

Product Under Investigation:

The inactivated, split-virion (with octoxinol 9) influenza vaccine (2005 Southern Hemisphere formulation) was administered by the ID route with the final micro delivery system:

Form/Dose/Route:

Suspension contained in a pre-filled micro delivery system/0.1 mL (dosage 1 and 2) /ID into the upper arm (deltoid area)

Batch number: S4006 (15 µg) / S4005 (21 µg)

System: Final micro delivery system allowing a better ergonomic use

Control Product:

The inactivated, split-virion (with octoxinol 9) influenza vaccine (2005 Southern Hemisphere formulation) administered by the IM route.

Form/Dose/Route:

Suspension in a pre-filled syringe/0.5 mL/ IM into the upper arm (deltoid area)

Batch number: Z5718

Other Product(s):

The inactivated, split-virion (with octoxinol 9) influenza vaccine (2006 Southern Hemisphere formulation) administered by the IM route was used for the re- vaccination (marketed batches).

Form/Dose/Route:

Suspension in a pre-filled syringe/0.5 mL/IM into the upper arm (deltoid area)

Batch number: A0053 (Australia); A5045 (New - Zealand)

Assessment Methods

Immunogenicity

Immunogenicity was evaluated using the hemagglutination inhibition (HI) technique in all subjects for the vaccination. Titration (expressed as reciprocal of dilution [1/dil]) was performed by the Sponsor s laboratory.

Safety

- 30-minute observation period

Subjects were kept under medical supervision for 30 minutes after each vaccination. The onset of any injection site and/or systemic reaction within this period was recorded by trial personnel.

- Solicited reactions within 7 days of vaccination

Subjects recorded any of the solicited reactions in the safety Diary Card (DC) after each vaccination on the day of vaccination and daily for the 7 following days with the following information:

- Maximum daily oral temperature
- Maximum daily measurement or maximum severity grade of all other solicited injection site or systemic reactions
- Date of last day of presence of the reaction if it lasted beyond D7
- Action taken for each event, if any (medication, etc.).
- Adverse events (AEs) within 21 days after each vaccination

Any AE occurring between D0 and 21 days after each vaccination was also recorded in the DC. All safety information was transcribed in the Case Report Form (CRF) by the Investigator following an interview with the subject at the following visit. For unsolicited systemic events, the causal relationship to the vaccine was evaluated by the Investigator as either: not related / no relationship or related.

- Serious adverse events (SAEs)

Information on SAEs was collected from inclusion until 6 months after the vaccination.

Pain at injection site:

Pain induced by the ID and IM injections was assessed using a VAS and with an acceptability questionnaire on the day of vaccination and 21 days after each vaccination in all subjects.

Leakage at the injection site:

The presence or absence of product leakage on the skin at the injection site was recorded in the ID groups.

CMI response:

For a subset of subjects, the cellular response mediated by T lymphocytes was first analyzed by detection of γ-interferon (γ-IFN, T helper [Th] 1 cytokine) and interleukin-4 (IL-4) (Th2 cytokine)-secreting CD4+ and CD8+ T cells using the intracellular cytokine staining (ICS) method.

Note: At the time of the protocol writing the number of IL-5-secreting CD8+ and CD4+ T lymphocytes was planned to be measured. At the time of the implementation of this exploratory method, it was decided that the measurement of IL-4-secreting CD8+ and CD4+ T lymphocytes would be performed.

The frequency of IL-2 (Th1 cytokine)-secreting cells was also assessed using the enzyme-linked immunospot (ELISPOT) technique.

Finally, the secretion of a panel of Th1 and Th2 cytokines by peripheral blood mononuclear cells (PBMCs) was quantified using the cytometric bead array (CBA).

All of these CMI response assays were performed by the Sponsor laboratory at Marcy L'Etoile, France.

Statistical methods

The statistical analysis was performed in several steps:

The primary statistical analysis was performed on all data collected for 21 days after the vaccination (D21, [V02]). The blind was broken for this primary analysis.

The analysis of safety results obtained until 21 days after the re-vaccination (M3 + 21 days [V04]) and after the 6-month safety follow-up after the ID or IM initial vaccination (M6) was performed in a second step.

The analysis of the CMI was performed in a third step.

All variables were described by vaccine group, using usual descriptive statistical analyses. The 95% confidence intervals (CIs) were calculated.

Primary analysis

The immunogenicity observed in each ID group was compared to that of the IM group using a non-inferiority testing approach on each strain (A/H1N1, A/H3N2 and B).

For each strain, the primary criterion was the difference of the $\log_{10}$ transformation of post-vaccination geometric mean of titers (GMT) between paired vaccine groups. For each strain, the tested hypotheses were as follows:

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

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

Each ID (test) group was considered as non-inferior to the IM group if the hypothesis H_0 was rejected for each strain.

If non-inferiority was demonstrated for at least one ID (test) group, the superiority of this (these) ID group(s) was then tested. The following hypotheses were stated for each strain:

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

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

Each ID group was considered as superior to the IM group if the hypothesis H_0 was rejected for at least two strains.

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

Secondary analyses

The post-vaccination seroprotection rates were tested between groups (titer ≥40 [1/dil] on D21).

The seroconversion and/or significant increase rates were tested between groups. The GMTRs (individual post-/pre-vaccination titer ratios) were tested between groups.

The compliance of the immunogenicity with the CHMP Note for Guidance was assessed as follows: for each vaccine strain and in each group, the immunogenicity recommendations were to meet at least one of the three European Medicines Agency (EMEA) criteria:

Immunogenicity criteria defined by EMEA	over 60 years
Seroconversion rate* or significant increase of titer† on D21	>30%
Mean geometric increase‡ between D0 and D21	>2
Percentage of seroprotected subjects§ on D21	>60%

*Proportion of subjects with a pre-vaccination titer <10 (1/dil) to a post-vaccination titer ≥40 (1/dil)
†Proportion of subjects with titers ≥10 (1/dil) before vaccination and ≥four-fold increase of the titer
‡Geometric mean of individual ratios (post-/pre-vaccination titers)
§Proportion of subjects achieving a post-vaccination titer ≥40 (1/dil)

All other secondary analyses were descriptive.

Observational analyses

All observational analyses were descriptive.

Sample size

The ID 15 µg and ID 21 µg groups were tested at a 2.5% alpha level (one-sided hypothesis for non-inferiority). 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 91% were chosen to calculate the sample size. Assuming for the two A strains a maximal standard deviation of 0.6 and of 0.5 for the B strain, 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 to be enrolled in the trial.

Results summary:
Disposition of subjects

A total of 1,107 subjects aged from 60.0 to 85.8 years were included in the study. Altogether, 370 subjects were randomized in the ID 15 µg group, 369 in the ID 21 µg group and 368 in the IM 15 µg group. Over the D0-D90 period, 39 subjects discontinued the trial. At inclusion, in the PPI population, the number of females was higher than the number of males in all the groups that were all similar in terms of age (mean age varying from 70.4 to 71.0 years [standard deviation of 6.76 and 6.55 years, respectively]) and gender distribution.

A total of 31 subjects (2.8%), balanced across vaccine groups, had at least one protocol deviation over the D0-D21 period. The most frequently observed protocol deviations were post-vaccination anti-HA antibody titers not available, time interval between the vaccination and the second blood sample over the planned period, early termination before V02, and prohibited therapy taken until V02. All inclusion and exclusion criteria were respected by all the subjects.

Primary Objective: Non-Inferiority and Superiority Results

The primary objective was to demonstrate that the ID vaccines administered with the final micro delivery system were at least as immunogenic as the IM vaccine after the first vaccination.

Non-inferiority of the ID 15 µg vaccine versus the IM 15 µg vaccine

In the PPI population, GMTs of pre-vaccination titers were similar in both groups and for the three strains (although the titer corresponding to the A/H3N2 strain was higher than that of the other strains in both groups).

The non-inferiority of the immunogenicity of the ID 15 µg vaccine in respect to that of the IM 15 µg vaccine was demonstrated for each of the three strains: the lower bound of the difference of $\log_{10}$-transformed post-vaccination GMTs versus the IM 15 µg group was higher than -0.176 for all strains (lower bound of 0.106 for the B strain, 0.109

for the A/H1N1 strain and 0.152 for the A/H3N2 strain) in the PPI population. 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 log_{10}-transformed post-vaccination GMTs versus the IM 15 µg group 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.

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.

Non-inferiority of the ID 21 µg vaccine versus the IM 15 µg vaccine

In the PPI population, pre-vaccination GMTs were similar in both groups and for the three strains (although the titer for the A/H3N2 strain was higher than those of the other two strains in both groups).

The non-inferiority of the immunogenicity of the ID 21 µg vaccine in respect to that of the IM 15 µg reference vaccine was demonstrated for each of the three strains: the lower bound of the difference of log-transformed post- vaccination GMTs versus the IM 15µg group was higher than -0.176 for all three strains (lower bounds of 0.081 for the B strain, 0.136 for the A/H1N1 strain and 0.155 for the A/H3N2 strain) in the PPI population. As non-inferiority was demonstrated, superiority of the ID 21 µg vaccine over the IM vaccine was assessed. The superiority of the immunogenicity of the ID 21 µg vaccine versus that of the IM vaccine was demonstrated in the FASI population for the three strains (lower bounds of 0.085 for the B strain, 0.136 for the A/H1N1 strain and 0.156 for the A/H3N2 strain), with adjusted p-values <0.0001.

Results obtained in the FASI and in the PPI populations led to the same conclusions, i.e. non-inferiority and superiority of the ID 21 µg vaccine for the three strains.

Although a significant center effect was observed on the post-vaccination titers for the B strain in the FASI population, no significant interaction between vaccine group and center effects was detected, that is the differences between vaccine groups were not significantly different across centers.

Secondary Objectives:

EMEA Criteria After the First Vaccination - FASI population

Before vaccination, the distribution of titers was similar between the three vaccine groups for each strain, except for the ID 15 µg group for the B strain, whose seroprotection rate was statistically significantly higher than for the other two groups (from 38.7% in the IM 15 µg group to 47.4% in the ID 15 µg group).

- ID 15 µg vaccine versus IM 15 µg vaccine:

For all three strains, after the vaccination, the seroprotection rate was higher in the ID 15 µg group. For the A/H1N1 strain, this superiority was not statistically significant (lower bound of the 95% CI of -0.948%). For the other two strains, the seroprotection rates were significantly higher for the ID 15 µg group (the observed differences versus the IM 15µg group were of 4.68% [lower bound of 1.77% for the A/H3N2 strain and 10.8% [lower bound of 4.93%] for the B strain). The superiority was detected for these two strains.

As regards the log transformation of the GMTRs and seroconversion or significant increase rates, the observed differences versus the IM 15 µg group were significant for all three strains. The observed differences varied from

13.3% (B strain) to 19.2% (A/H1N1 strain) for the seroconversion or significant increase rates, and the log_{10} difference of the GMTRs varied from 0.133 (B strain) to 0.198 (A/H1N1 strain). The ID 15 µg dosage may therefore be considered as superior to the IM 15 µg vaccine in terms of EMEA immunogenicity parameters. As no adjustment was made to control the type I error for this multiple testing, these results remain exploratory and would have to be confirmed in further assessment of the vaccine.

- ID 21 µg vaccine versus IM 15 µg vaccine

For the three strains, after the vaccination, all the EMEA immunogenicity parameters were higher in the ID 21µg group. The observed difference of the seroprotection rates varied from 5.77% (lower bound of 3.12%) for the A/H3N2 strain to 13.8% (lower bound of 7.93%) for the A/H1N1 strain. The log10 difference of the GMTRs versus the IM 15 µg group varied from 0.202 (lower bound of 0.137) for the B strain to 0.255 (lower bound of 0.184) for the A/H1N1 strain. For the seroconversion or significant increase rates the observed differences versus the IM 15 µg vaccine varied from 19.8% (lower bound of 12.7%) for the B strain to 23.3% (lower bound of 16.5%) for the A/H1N1 strain.

As a statistical superiority versus the IM 15 µg vaccine was observed for each parameter in all three strains, the immunogenicity of the ID 21 µg dosage may be therefore considered as superior to that of the IM 15 µg vaccine in terms of EMEA immunogenicity parameters. As no adjustment was made to control the type I error for this multiple testing, these results remain exploratory and would have to be confirmed in further assessment of the vaccine.
As the superiority of each ID dosage versus the IM 15 µg vaccine was demonstrated, the comparison between the two ID dosages was performed.

- ID 15 µg vaccine versus ID 21 µg vaccine

No superiority of the seroprotection rate of the ID 21 µg vaccine versus that of the ID 15 µg vaccine was observed for the A/H3N2 and B strains. However, a statistically significant difference was observed for the A/H1N1 strain (observed difference of 8.45%) in favor of the superiority of the ID 21 µg dosage.

Compliance of the Two ID Dosage Vaccines with the CHMP Note for Guidance

In the OI population, the pre-vaccination titers (GMTs and seroprotection rates) were similar in all the groups for each strain, except for the ID 15 µg group, whose seroprotection rate against the B strain was higher than in the other two groups (47.3% versus 34.9% and 38.6% in the ID 21 µg group and IM 15 µg group, respectively). Twenty-one days after vaccination, an immune response was observed in all three groups.

This response was higher in the ID 15 µg and ID 21 µg groups than in the IM 15 µg group for each of the three EMEA parameters and for each strain.

In terms of EMEA parameters, each of the three criteria was met in the two ID groups for the three strains. In the IM group, two of the three EMEA criteria (i.e. seroprotection rates and GMTRs) were met for the three strains. These conclusions remain the same when considering the lower bound of each associated 95% CI.

Safety results:

All results were obtained on the safety analysis set.

- Reactions listed in the CHMP Note for Guidance

The safety profile corresponding to the EMEA criteria was similar in all the groups.

A total of 67 subjects (18.4%) in the ID 15 µg group, 51 subjects (13.9%) in the ID 21 µg group as well as in the IM 15 µg group experienced at least one of the reactions listed in the CHMP Note for Guidance. The most frequently reported ones were malaise (experienced by 10.2% and 7.9% of subjects in the ID 15 µg and ID 21 µg groups, respectively and 7.6% in the IM 15 µg group) and injection site ecchymosis (reported by 8.5%, 3.8% and 4.9% of subjects in the ID 15 µg, ID 21 µg, and IM 15 µg groups, respectively). Fever was similarly reported in the ID 21 µg and IM 15 µg groups (2.4% of subjects in each) and in the ID 15 µg group (1.9% of subjects). Shivering was slightly more frequently reported in the ID 21 µg group (1.6%) than in the ID 15 µg and IM 15 µg groups (0.3%). No subjects reported injection site induration >5cm for more than 3 days in any of the three groups. A total of 21 subjects reported at least one severe reaction listed in the CHMP Note for Guidance, which mostly resolved spontaneously within 7 days.

- Solicited Reactions within 7 days after vaccination

A total of 326 subjects (89.6%) in the ID 15 µg group, 324 subjects (88.0%) in the ID 21 µg group and 146 subjects (39.7%) in the IM 15 µg group reported at least one solicited injection site reaction within 7 days after vaccination. In both ID groups, the most frequently reported injection site reactions were injection site erythema (reported by 78.8% of subjects in the ID 15 µg group and by 77.7% of subjects in the ID 21 µg group), injection site induration (64.6% and 65.2% of subjects, respectively) and injection site swelling (62.3% and 58.2% of subjects, respectively). In the IM 15 µg group, the most frequently reported injection site reactions were injection site erythema (reported by 19.1% of subjects) as for the ID vaccines, injection site pain (16.8% of subjects) and injection site induration (16.7% of subjects).

Eighty-three subjects (22.8%) in the ID 15 µg group, 88 subjects (23.9%) in the ID 21 µg group and 24 subjects (6.5%) in the IM 15 µg group reported at least one severe solicited injection site reaction. These reactions were mainly severe for 3 days or less, and mainly resolved spontaneously (medication was taken for a few reactions).
A total of 110 subjects (30.2%) in the ID 15 µg group, 107 subjects (29.1%) in the ID 21 µg group and 101 subjects (27.4%) in the IM 15 µg group reported at least one solicited systemic reaction within 7 days after vaccination. In both ID groups the most frequently reported systemic reactions were headache (18.1% of subjects in the ID 15 µg group and 16.0% in the ID 21 µg group), malaise (13.2% and 10.1% respectively) and myalgia (12.4% and 12.2%

respectively). In the IM 15 µg group the most frequently reported systemic reactions were headache (17.4% of subjects), myalgia (12.2% of subjects) and malaise (10.1%). Systemic reactions mostly occurred within 3 days, for 3 days or less and were mainly mild in severity.

A total of 14 subjects, five subjects (1.4%) in the ID 15 µg group, three (0.8%) in the ID 21 µg group and six (1.6%) in the IM 15 µg group reported at least one severe solicited systemic reaction. These reactions were mainly severe for 1 day, except for an episode of headache and an episode of malaise which lasted 2 days and were reported by a subject in the ID 15 µg group.

- Unsolicited Reactions within 21 days after vaccination

A total of 13 subjects reported at least one unsolicited injection site reaction within 21 days after vaccination, with quite similar frequencies in each of the three groups (1.4% of subjects in the ID 15 µg group, 1.6% of subjects in the ID 21 µg group and 0.5% in the IM 15 µg group). Among these subjects, four experienced at least one unsolicited injection site reaction within 7 days after vaccination (two subjects in the ID 15 µg group, one subject in the ID 21 µg group and one in the IM 15 µg group). These reactions were mainly mild in severity (except for an injection site induration, an injection site pain, a discoloration, and injection site warmth that were moderate). Mostly, these reactions lasted for less than 3 days and resolved spontaneously (except for an injection site pain in the IM 15 µg group that needed medication). One severe injection site discoloration which appeared 1 day after vaccination and resolved spontaneously the same day was reported.

Unsolicited systemic reactions occurring within 21 days after vaccination were reported by 36 subjects (16 subjects [4.4%] in the ID 15 µg group, 14 subjects [3.8%] in the ID 21 µg group and 6 subjects [1.6%] in the IM 15 µg group). Among them, 27 subjects (13 subjects [36%] in the ID 15 µg group, 9 subjects [2.4%] in the ID 21 µg group and 5 subjects [1.4%] in the IM 15 µg group) reported at least one unsolicited systemic reaction within 7 days following vaccination. These reactions were mainly mild and moderate and resolved within 3 days spontaneously or medication taken. Four subjects (one subject in the ID 15 µg group as well as in the IM 15 µg group and two subjects in the ID 21 µg group) experienced at least one severe unsolicited systemic reaction.

Observational Objectives

Safety after the IM re-vaccination

The time interval between the first vaccination and the IM re-vaccination was generally respected: 18 subjects (1.6%) received the IM re-vaccination before the planned period (D90-D105) and 29 subjects (2.6%) received the IM re-vaccination after the planned period. A total of 1,063 subjects received the IM re-vaccination.

- Reactions listed in the CHMP Note for Guidance

A total of 57 subjects (16.1%) in the ID 15 µg group, 57 subjects (16.0%) in the ID 21 µg group and 42 subjects (12.0%) in the IM 15 µg group experienced at least one of the reactions listed in the CHMP Note for Guidance within 3 days after the IM re-vaccination. The most frequently reported reactions were malaise and injection site ecchymosis. No subject reported injection site induration >5 cm for more than 3 days in any of the three groups. A total of 21 subjects experienced at least one severe reaction listed in the CHMP Note for Guidance. These reactions resolved within 7 days spontaneously or following medication intake. The safety profile corresponding to the EMEA criteria was similar in all the groups after the IM re-vaccination. The reactogenicity profile was similar after the first vaccination and after the IM re-vaccination for all the groups.

- Solicited Reactions within 7 days after IM re-vaccination

A total of 165 subjects (46.6%) in the ID 15 µg group, 166 subjects (46.6%) in the ID 21 µg group and 134 subjects (38.3%) in the IM 15 µg group reported at least one solicited injection site reaction within 7 days after the IM re-vaccination. The incidence of solicited injection site reactions was higher in the ID groups than in the IM groups. Whatever the vaccine received, the most frequently reported injection site reactions were erythema, induration, swelling and pain. Injection site reactions mostly occurred within 3 days and for 3 days or less. These reactions were mostly mild in severity (except for erythema which was mostly mild and moderate in the IM 15 µg group). Thirty subjects (8.5%) in the ID 15 µg group, 20 (5.6%) in the ID 21 µg group and 21 (6.0%) in the IM 15 µg group reported at least one severe injection site reaction.

A total of 84 subjects (23.7%) in the ID 15 µg group, 85 subjects (23.9%) in the ID 21 µg group, 71 subjects (20.3%) in the IM 15 µg group experienced at least one solicited systemic reaction within 7 days after the IM re-vaccination. Whatever the vaccine received, headache, malaise and myalgia were the most frequently reported reactions. Systemic reactions mostly occurred within 3 days (except for an episode of fever), for 3 days or less and were mostly mild in severity. Eight subjects (2.3%) in the ID 15 µg group, nine subjects (2.5%) in the ID 21 µg

group and eight subjects (2.3%) in the IM 15 µg group reported at least one severe solicited systemic reaction. Severe fever was not reported in any groups.

- Unsolicited reactions within 21 days after IM re-vaccination

Two subjects in the ID 15 µg group and one subject in the ID 21 µg group experienced at least one unsolicited injection site reaction between 7 and 21 days after the IM re-vaccination. No severe unsolicited reaction was reported.

Four subjects (1.1%) in the ID 15 µg group, seven subjects (2.0%) in the ID 21 µg group and four subjects (1.1%) in the IM 15 µg group reported an unsolicited systemic reaction within 21 days after vaccination. They all experienced at least one reaction within the 7 days after vaccination except for two subjects in the ID 21 µg group. Two subjects (0.6%) in the ID 21 µg group and one subject (0.3%) in the IM 15 µg group experienced a severe unsolicited systemic reaction. These reactions (severe tinnitus and severe neuralgia in the ID 21 µg group and severe dizziness in the IM 15 µg group) occurred within 7 days following the IM re-vaccination and lasted less than 3 days.

Reactivation of the former site before re-vaccination

Eleven subjects (3.1%) in the ID 15 µg group and four subjects (1.1%) in the ID 21 µg group experienced an unsolicited injection site reaction between V02 and V03. These reactions were mostly mild erythema that lasted more than 8 days.

Safety on the first injection site after IM re-vaccination

A total of 17 subjects (5.3%) in the ID 15 µg group, 23 subjects (7.1%) in the ID 21 µg group and 20 subjects (6.3%) in the IM 15 µg group experienced at least one solicited injection site reaction on the site of the first vaccination that was not present before the IM re-vaccination. Among them, one subject in each group experienced a severe injection site reaction. The most frequently reported reactions were erythema, induration, pain and pruritus. These reactions were mostly mild in severity, occurred within 3 days (except for an episode of swelling in the ID 21 µg group) and for 3 days or less (except for an episode of erythema in the ID 15 µg group which lasted more than 8 days). A severe erythema was reported in each trial group and a severe swelling was reported in the ID 15 µg group. These reactions lasted less than 3 days.

No unsolicited injection site reaction was reported.

Pain at the time of injection

The median of the values given by the subjects on the VAS was 2.17 mm for the three groups. On the day of vaccination (D0), 69.6% of subjects in the ID 15 µg group and 70.2% of subjects in the ID 21 µg group declared that the ID injection was less painful than an IM injection (these subjects had not received an IM injection at this step). Among subjects included in the ID 15 µg group, 20.9% declared that the ID injection was as painful as an IM injection, and 4.9% that the ID injection was more painful than an IM injection. Among subjects included in the ID 21 µg group, 18.7% declared that the ID injection was as painful as an IM injection, and 6.5% that the ID injection was more painful than an IM injection.

Generally, in each of the three groups, injection site reactions were judged acceptable by most of the subjects. Twenty-one days after vaccination, 74.8% of subjects in the ID 15 µg group and 77.7% in the ID 21 µg group declared they would prefer to have an ID injection rather than an IM injection for the next influenza vaccination.

Leakage at the injection site

After the ID injection, twenty-four subjects (6.5%) and 21 subjects (5.7%) presented a leakage in the ID 15 µg group and in the ID 21 µg group, respectively.

CMI

The cellular mediated immune response against influenza was measured in 90 subjects, 30 subjects in each group. The ID vaccine, with a dosage equivalent or superior to that of the IM vaccine, induced a cellular response of comparable profile and intensity to that of the vaccine administered by the IM route, 21 days after vaccination. However the frequencies of influenza-specific CD4 T cells measured 21 days after the vaccination in this population were very low. Based on the literature, the peak of the cellular response for influenza vaccines may be observed at earlier time points (D7 or D10).

Deaths, Other SAEs and Other Significant AEs:

Four deaths were reported during the trial, these deaths were assessed as not related to the study vaccine by both the Investigator and the Sponsor. Two occurred in the ID 21 µg group, one during the study period and one after the subject discontinued. Two occurred in the IM 15 µg group, one during the study period and one after the subject discontinued. In the ID 21 µg group, a 63-year-old woman died following a multiform glioblastoma, the event was diagnosed during the study period and the fatal outcome was reported after the subject discontinued. The second death in the ID 21 µg group involved an 84-year old woman who died following coronary artery disease. In the IM group, an 82-year-old woman died following an acute myeloid leukemia, the fatal outcome was reported after the subject discontinued. The second fatal event in the IM 15 µg group was an acute myocardial infarction experienced by a 78-year-old man.

Including these deaths, a total of 83 SAEs were reported, 27 in the ID 15 µg group, 27 in the ID 21 µg group and 29 in the IM group. One SAE (ID 15 µg group) (case of brachial neuritis) was rated as related to the vaccination by both the Investigator and the Sponsor.

Conclusions:

- In this Phase II study conducted in an elderly population, after a first vaccination with the ID 15 µg or the ID 21 µg vaccines using the final micro delivery system, the primary objective was reached, demonstrating the non- inferiority in terms of post-vaccination GMTs of the two ID investigational vaccines in comparison to the IM 15 µg reference vaccine for each of the three strains. Moreover, superiority in terms of post-vaccination GMTs of the ID 15 µg and ID 21 µg investigational vaccines over the IM 15 µg reference vaccine was demonstrated for the three strains. This conclusion is based on results obtained in the PPI and in the FASI populations.
- The superiority of the two ID investigational vaccines to the IM reference vaccine was also observed in terms of EMEA criteria (except for the seroprotection rate with the A/H1N1 strain in the ID 15 µg group) in the FASI population. However these conclusions need to be confirmed in further studies. Superiority of one of the two ID dosages was thus assessed and it appeared that the ID 21 µg vaccine was not superior to the ID 15 µg vaccine, except for the A/H1N1 strain.
- In all the groups, each of the three EMEA criteria was fulfilled for each of the three strains considering the mean GMTs, seroconversion and seroprotection rates, but also for the lower bound of each associated 95%CI, in the OI analysis set.
- The ID vaccines induced a cellular response of comparable profile and intensity to that observed with the IM vaccine, 21 days after vaccination.
- When considering the safety profile, in the SafAS, the ID 15 µg and the ID 21 µg vaccines induced a higher frequency of injection site reactions. More severe solicited injection site reactions were observed in the ID 15 µg group and in the ID 21 µg group than in the IM 15 µg group. Systemic reactions occurred at a similar frequency in all the groups. These reactions were mainly mild in severity and occurred within 3 days. On the other hand, a total of about 75% of subjects declared that they would prefer an ID vaccination for a subsequent influenza vaccination.
- Whatever the first vaccine received, the safety profile after the IM re-vaccination was quite similar to the safety profile observed after the first vaccination with the IM 15 µg vaccine.
- The presence of reactions at the first site of injection after the IM re-vaccination as well as the reactivation of the site of the first injection before the IM re-vaccination were observed at a low but similar frequency in the three groups.
- As the overall benefit / risk ratio, the results obtained in this trial showed that the two ID investigational vaccines were more immunogenic than the IM 15 µg reference vaccine, and that their safety profiles were satisfactory. As no immunological difference was observed between the 15 µg ID and the 21 µg ID groups, the ID 15 µg dosage vaccine was selected for further investigation at Phase III.

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