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Sponsor/ Company:	Sanofi Pasteur	**Study Code:** GPF01 **Study Identifier:** NCT00457509
Proprietary Vaccine Name:	H5N1 inactivated, split-virion pandemic flu vaccine made in embryonated eggs, adjuvanted with AF03	

Title of the Study: Safety and Immunogenicity of Different Formulations of an Intramuscular H5N1 Adjuvanted, Inactivated, Split-Virion Pandemic Influenza Vaccine in Healthy Adult Subjects

Study centres: 3 sites in Belgium

Publication: Levie K, Leroux-Roels I, Hoppenbrouwers K, Kervyn AD, Vandermeulen C, Forgus S et al. An adjuvanted, low-dose, pandemic influenza A (H5N1) vaccine candidate is safe, immunogenic, and induces cross-reactive immune responses in healthy adults. J Infect Dis. 2008 Sep 1; 198(5):642-9.

Study period:	Date of first visit of the first subject (Screening Step 1): 02 January 2007 End date of the entire trial (last visit of the last subject for Step 2): 13 November 2009
Development phase:	Phase I

Methodology / Trial Design:

A Phase I trial in two steps:

Step 1: open, monocenter (Center 1) step in 15 subjects who received two injections 21 days apart of a pandemic Flu H5N1 15µgHA+AF03 vaccine for immunogenicity and clinical and biological safety evaluation.

Step 2: blind-observer, multicenter, controlled, randomized step. Once the results of the clinical and biological safety of Step 1 were available and supported initiation of the step 2, 250 subjects were included and randomized in five groups (50 subjects per group) to receive two injections 21 days apart of a pandemic Flu H5N1 vaccine.

Primary Series (A/Vietnam strain)

The formulations per vaccine dose used in the five groups were defined as follows:

Group 1: 1.9µgHA+AF03

Group 2: 3.75µgHA+AF03

Group 3: 7.5µg HA+AF03

Group 4: 15µgHA+AF03

Group 5: 7.5µgHA control vaccine

12-Month Booster vaccination (A/Indonesia strain

Groups 1 to 4 (as defined in step 2 primary series) received the 3.75µgHA+AF03 as booster

Group 5 (as defined in step 2 primary series) received the 7.5µgHA control vaccine

6-, 12- and 18-Month Safety Follow-up and Antibody (Ab) Persistence

Groups 1 to 5: all subjects who received the booster vaccination were followed for safety (SAEs) and Ab persistence post-booster was only evaluated.

Main Objectives:

- To describe the safety profile following each injection
- To describe the humoral immune response after each injection
- To describe the antibody (Ab) persistence

Endpoints:

Safety:

- For step 1 subjects only: occurrence of out-of-normal-range biochemical or hematological test results at any time of laboratory testing within two and eight days after the first and second vaccinations
- Occurrence, time to onset, number of days of occurrence, and severity of solicited (prelisted in the subject's diary and case report form [CRF]) injection site reactions and systemic reactions within 7 days following each injection
- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] version
- 9.0 preferred term), time to onset, duration, severity, and relationship to vaccination of unsolicited (spontaneously reported) adverse events within 21 days following each injection
- Serious adverse events (SAEs) throughout the trial period, .i.e. any SAEs from the beginning of the study up to 6 months after the booster vaccination and related SAEs only, up to 18 months after the booster vaccination.
- Occurrence of the following reactions (MedDRA preferred terms given in parentheses) in the 3 days following each injection was more especially reported (as defined by the EMEA Note for Guidance [NfG] for interpandemic influenza vaccines for adults [CPMP/BWP/214/96]):
  - Injection site induration ≥5 cm observed for more than 3 days
  - Injection site ecchymosis (injection site bruising)
  - Temperature >38°C for 24 hours or more (pyrexia)
  - Malaise
  - Shivering (rigors)

Immunogenicity (primary series):

Immune Response After Two-Dose Schedule

- Anti-Hemagglutinin (anti-HA) Ab titers against the A/Vietnam/H5N1 strain were expressed as described below: hemagglutination inhibition (HI) titers obtained on D0, D21, and D42. The HI assay was performed using horse erythrocytes (HIH) for all subjects. To compare the immunogenicity response according to EMEA
- requirements from the NfG CPMP/BWP/214/96, the following endpoints were derived:
  - Individual titers ratios D21/D0, D42/D0, and D42/D21
  - Titer ≥32* (1/dil) on D0, D21, and D42
  - Significant increase of titer (titer ≥8 [1/dil] on D0 and ≥4-fold increase of titer after injection) or a seroconversion rate (titer <8 [1/dil] on D0 and post- injection titer ≥32 [1/dil]) on D21 and D42
  - Detectable Ab (titer ≥8 [1/dil]) on D0, D21, and D42

**due to the starting dilution of 8, the percentage of subjects with a titer ≥32 (1/dil) was considered*

- Seroneutralization (SN) titers obtained on D0, D21, and D42. The following endpoints were derived:
- Individual titers ratios D21/D0, D42/D0, and D42/D21
- 2-fold increase of titers from D0 to D21 and to D42
- 4-fold increase of titers from D0 to D21 and to D42
- Detectable Ab (titer ≥10 [1/dil]) on D0, D21, and D42
- Titer ≥20 (1/dil), ≥40 (1/dil) and ≥80 (1/dil) on D0, D21 and D42

Ab Persistence Against A/Vietnam strain (Step 2 only):

Anti-HA titers (HIH method) and seroneutralizing antibody titers (SN method) obtained on V04 (M3), V05 (M6) and prior to booster vaccination on V06 (M12):

- Detectable Abs (titer ≥8 [1/dil] for HIH method and titer ≥10 [1/dil] for SN method) on V04 (M3), V05 (M6) and V06 (M12)
- Titer ≥32 (1/dil) (HIH method) and titer ≥40 (1/dil) (SN method) on V04 (M3), V05 (M6) and V06
- Proportion of subjects with neutralizing Ab titer ≥20 (1/dil) and ≥40 (1/dil) on V04 (M3) and V05 (M6)

Observational objective:

- To describe the serostatus of subjects for influenza A/New Caledonia/20/99 (H1N1) strain
- To describe the cell-mediated immune (CMI) response

Observational endpoints:

Observational Immunogenicity for Primary Series:

Immunogenicity Serostatus Against A/ New Caledonia/20/99 (H1N1) Strain:

Anti-HA Ab titers against A/New Caledonia/20/99 (H1N1) strain were expressed as described below using the HI method. The assay was performed using chicken erythrocytes for all subjects.

HI titers were obtained on D0 and D42. The following endpoints were derived:

- Individual titers ratios D42/D0
- Titer ≥40 (1/dil) on D0 and D42
- Significant increase of titer (titer ≥10 [1/dil] on D0 and ≥4-fold increase of the titer after injection) or a seroconversion rate (titer <10 [1/dil] on D0 and post- injection titer ≥40 [1/dil]) on D42.

Cross-Reactive Immunogenicity Against A/*Indonesia/02/2005_RG2 Strain*

Observational endpoints were the same as immunogenicity endpoints for the description of immunogenicity against A/Vietnam (only for D42).

Immunogenicity (Booster):

Humoral Immune Response Against A/Vietnam and A/Indonesia Strains:

Anti-HA Ab titers against the A/H5N1 strain after booster injection on V06 (D365) were expressed as described below:

- HI Ab titers (HIH method) at each timepoint obtained on V06 (D365), V07 (D372) and V08 (D386). The assay was performed using horse erythrocytes for all subjects. The following endpoints were derived:
- Individual titers ratios V07/V06, and V08/V06
- Titer ≥32* (1/dil) on A/Vietnam and titer ≥40 (1/dil) on A/Indonesia on V06, V07 and V08
- Significant increase of titer (titer ≥8 [1/dil] on A/Vietnam and titer ≥10 [1/dil] on A/Indonesia at V06 and ≥4-fold increase of the titer after injection) or a seroconversion rate (titer <8 [1/dil] on A/Vietnam and titer <10 [1/dil] on A/Indonesia at V06 and post-injection titer ≥32 [1/dil] on A/Vietnam and titer ≥40 [1/dil] on A/Indonesia) at V07, and V08
- Detectable Abs (titer ≥8 [1/dil] on A/Vietnam and titer ≥10 [1/dil] on A/Indonesia) on V06, V07, and V08

  *due to the starting dilution of 8 titer ≥32 (1/dil) was considered
- SN Ab titers obtained on V06 (D365), V07 (D372) and V08 (D386). The following endpoints were derived:
  - Individual titers ratios V07/V06, and V08/V06
  - Titer ≥40 (1/dil) on V06, V07 and V08
  - 4-fold increase of titers from V06 to V07, and to V08
  - Detectable Abs (titer ≥10 [1/dil]) on V06, V07, and V08

Ab persistence against A/Indonesia:

Anti-HA Ab titers (HIH method) and seroneutralizing Ab titers (SN method) obtained after the booster vaccination on V09 (M18), V10 (M24) and V11 (M30):

- Detectable Abs (titers ≥10 [1/dil] for HIH and SN methods) on V09 (M18), V10 (M24) and V11 (M30)
- Titer ≥40 (1/dil) (HI and SN methods) on V06 (M12), V09 (M18), V10 (M24) and V11 (M30)

Cross reactivity:

A cross-reactivity evaluation was performed between A/Vietnam and A/Indonesia antigens (see endpoints described above).

Observational Cellular-Mediated Immune Response (Primary Series and Booster)

Secretion of a panel of Th1 (IFN-gamma, TNF-alpha, IL-2), and Th2 (IL-5, IL-4, IL-13) cytokines by peripheral blood mononuclear cells (PBMCs), lymphoproliferation and intracellular cytokine staining (ICS) upon in vitro re-stimulation with vaccine antigens were quantified in two different subsets of subjects before the first injection (D0), 7 days after the second injection (D28), before and 7 days after the booster injection (D365 and D372).

Sample size (Number of Subjects):

	Step 1	Step 2 (D0 to D386)					
		1.9µg + AF03	3.75µg + AF03	7.5µg + AF03	15µg + AF03	7.5µg (control)	Total
Planned sample size	15	50	50	50	50	50	250
Subjects enrolled	15	51	50	50	50	50	251
Sample size for primary analysis	**15**	**51**	**50**	**50**	**50**	**50**	**251**
Subjects discontinued at D42	0	1	1	2	0	0	4
Subjects completed primary series (D42)	15	50	49	48	50	50	247
Subjects discontinued before booster (D365)	NA	3	1	0	1	1	6
Sample size for booster analysis	**NA**	**47**	**48**	**48**	**49**	**49**	**241**
Subjects completed D386	NA	47	48	48	49	47	239
Subjects completed D545	NA	45	43	46	46	46	226
Subjects completed D910	NA	42	43	45	45	42	217
Subjects discontinued from Booster (D365) to D910	NA	5	5	3	4	7	24

Schedules of Vaccination and Specimen Collection:

Step 1:

Subjects received two injections of the investigational vaccine separated by 21 days.

Eight blood samples for biological safety (10 mL each) and/or serology (30 mL each) were taken on D-21, D0, D2, D8, D21, D23, D29 and D42.

Step 2:

Subjects received two injections of the investigational or the control vaccine separated by 21 days. All subjects received a booster injection 12 months after the first injection.

For all subjects: eleven blood samples were taken for serology (30 mL each). Blood samples were taken on D0, D21, D42, D90 (M3), D180 (M6), D365 (M12, prior to booster injection), D372 (M12 + 7 days), D386 (M12 + 21 days), D545 (M18), D730 (M24) and D910 (M30).

For subjects included in Center 3: an additional 20 mL blood sample were taken in each group for CMI analysis on D0, D28 (7 days post-dose 2), D365 (booster vaccination) and D372 (7 days post-booster).

Product Under Investigation:

H5N1 inactivated split-virion pandemic flu vaccine made in embryonated eggs, adjuvanted with AF03

Form/Dose/Route:

Primary Series

Liquid/ Intramuscular (IM) injection into the deltoid muscle

	Vaccine formulations/group			
	Extemporaneous mixing in AF03 vial			
Formulations used for preparation	*1.9µg* + AF03 (Group 1)*	*3.75µg* + AF03 (Group 2)*	*7.5µg*+ AF03 (Group 3)*	*15µg* + AF03 (Group 4)*
Pandemic influenza vaccine				
Formulation 1: 15 µg/mL HA	0.4 mL	0.7 mL		
Formulation 2: 30 µg/mL HA			0.7 mL	
Formulation 3: 60 µg/mL HA				0.7 mL
Adjuvant				
AF03	0.7 mL	0.7 mL	0.7 mL	0.7 mL
Volume after mixing	1.1 mL	1.4 mL	1.4 mL	1.4 mL
Volume injected per vaccination	0.4 mL	0.6 mL	0.6 mL	0.6 mL

* As the concentration of each of the three pandemic influenza vaccine batches prepared for this phase I study are under the values of 15, 30 or 60 µg of HA/mL (13, 27 or 48.4 µg/mL respectively), and in order to have the same quantity of adjuvant whatever the group, volumes have been adapted in order to reach as closely as possible the targeted values of 1.9, 3.75, 7.5 or 15 µg of HA per vaccine dose (1.9, 3.9, 8.1 or 14.5 µg of HA respectively).

Booster Vaccination

Liquid/0.5 mL/ Intramuscular (IM) injection into the deltoid muscle

Batch number:

Primary Series

Pandemic influenza vaccine, formulation 1: S4144

Pandemic influenza vaccine, formulation 2: S4146

Pandemic influenza vaccine, formulation 3: S4147

AF03 adjuvant: S4124

Booster Vaccination

Pandemic influenza vaccine, booster formulation: S4189

AF03 adjuvant: S4124

Control Product:

H5N1 inactivated split-virion pandemic flu vaccine made in embryonated eggs

Form/Dose/Route:

Primary Series

Liquid/0.3 mL/ IM injection into the deltoid muscle

Booster Vaccination

Liquid/0.5 mL/ IM injection into the deltoid muscle

Batch number:

Primary Series: S4146 **Booster Vaccination:** S4189

Other Product(s): Not applicable

Statistical methods

All analyses were descriptive. 95% confidence intervals (CIs) of point estimates were calculated using normal approximation for quantitative data and exact binomial distribution (Clopper-Pearson method) for proportions.

Additionally, after each injection, safety data were presented according to the safety criteria quoted in the EMEA Note for Guidance on harmonization requirements for influenza vaccines for adults (CPMP/BWP/214/96).

Sample Size:

The sample size was arbitrarily set to 15 subjects for clinical and biological safety analysis (step 1) and 50 subjects per group for step 2 so that there was a 95% probability of observing at least one event that had a true incidence of 6% using the binomial distribution.

Results summary:

Preliminary safety data from Step 1, i.e. solicited and unsolicited reactions as well as biological parameters, evaluated 2 and 8 days after each vaccination, were reviewed and validated for the initiation of Step 2.

Demography

Step 1:

A total of 15 subjects from 18.6 to 36.9 years were recruited. No subjects discontinued the study. The mean age was 24.8 ± 4.7 years and the male/female ratio was 1.5.

All the 15 subjects were included in the Full Analysis Set (FAS). No subjects presented with any protocol deviations.

Step 2 Primary Series:

A total of 251 subjects from 18.1 to 40.3 years were recruited and the number of subjects was fairly equally balanced between the five groups. Four subjects discontinued the study: two for AE, one for SAE unrelated to the vaccine and one for non-compliance to the protocol. Baseline characteristics were similar between the five groups. Among the five groups, the mean age ranged from 22.9 ± 4.8 to 24.1 ± 6.1 years and the male/female ratio ranged from 0.6-0.7.

All the 251 randomized subjects were included in the Full Analysis Set (FAS). Eight subjects presented with at least one protocol deviation and were excluded from the per-protocol analysis set (PPAS).

Step 2 Booster Vaccination:

Among the 247 subjects who completed the Step 2 primary vaccination series, a total of 241 attended V06 (D365) and received the booster vaccination. Three subjects had missing blood samples.

Step 2 Booster 6-Month Follow-up:

Among the 241 subjects who received the booster vaccination, 226 attended V09 (M18) and 10 subjects who did not attend V09 had the 6-month safety follow-up completed by phone. One subject had missing blood samples.

Step 2 Booster 12- and 18-Month Follow-up:

Among the 241 who received the booster vaccination, 224 attended V10 (M24) and 217 subjects attended V11 (M30). A total of 24 subjects out of 241 did not complete the study.

SAFETY– PRIMARY SERIES:

No immediate unsolicited events or reactions were reported post-vaccination (primary and booster) in any step.

Step 1 Primary Series:

Within 7 days after any vaccination, all subjects reported at least one solicited injection site reaction and 80% reported at least one solicited systemic reaction.

Two subjects reported at least one reaction listed in the EMEA NfG for the registration of seasonal influenza vaccine within 3 days after the first vaccination and one subject after the second vaccination. All those reactions were mild and spontaneously resolved within 3 days.

No subjects reported any SAE or any AE leading to study discontinuation within the D0-D42 study period. No clinically significant changes were reported in biological and physical parameters.

Step 2 Primary Series:

-Solicited Reactions:

Within 7 days after the first vaccination, 98.0 to 100.0% of subjects in the four adjuvanted groups reported at least one solicited reaction versus 78.0% in the control group:

- 96.1 to 100.0% in the four adjuvanted groups reported at least one solicited injection site reaction versus 58.0% in the control group.
- 45.1 to 64.0% in the four adjuvanted groups reported at least one solicited systemic reaction versus 60.0% in the control group.

Within 7 days after the second vaccination, 82.0 to 91.8% of subjects in the four adjuvanted groups reported at least one solicited reaction versus 62.0% in the control group:

- 76.0 to 87.8% in the four adjuvanted groups reported at least one solicited injection site reaction versus 38.0% in the control group.
- 30.0 to 56.0% in the four adjuvanted groups reported at least one solicited systemic reaction versus 48.0% in the control group.

For all groups, the most frequently reported solicited injection site reactions after any vaccination were:

- Injection site pain with 96.1 to 100% of subjects in the adjuvanted groups versus 56.0% in the control group
- Induration with 26.0 to 46.0% of subjects in the adjuvanted groups versus 20.0% in the control group. Most of those reactions appeared within 3 days, were mild to moderate and spontaneously resolved within 3 days.

For all groups, the most frequently reported solicited systemic reactions after any vaccinations were:

- Headache with 47.1 to 62.0% of subjects in the adjuvanted groups versus 54.0% in the control group,
- Malaise with 27.5 to 42.0% of subjects in the adjuvanted groups versus 38.0% in the control group,
- Myalgia with 11.8 to 40.0% of subjects in the adjuvanted groups versus 26.0% in the control group.

Most of those reactions appeared within 3 days, were mild to moderate and spontaneously resolved within 3 days.

-EMEA reactions:

After the first vaccination, 19.6 to 36.0% of subjects in the four adjuvanted groups reported at least one reaction listed in the EMEA NfG versus 32.0% in the control group. Malaise was the most frequently reported reaction. Injection site ecchymosis and shivering were also reported.

After the second vaccination, 14.6 to 22.0% of subjects in the four adjuvanted groups reported at least one reaction listed in the EMEA NfG versus 28.0% in the control group. Malaise remains the most frequently reported reaction.

-Unsolicited Events:

Within 21 days after the first vaccination, 28.0 to 48.0% of subjects in the four adjuvanted groups reported at least one unsolicited event versus 32.0% in the control group:

- Only zero to 2.0% in the four adjuvanted groups reported at least one unsolicited injection site reaction versus 4.0% in the control group.
- 4.0 to 14.0% in the four adjuvanted groups reported at least one unsolicited systemic reaction versus 10.0% in the control group.

Within 21 days after the second vaccination, 20.4 to 46.0% of subjects in the four adjuvanted groups reported at least one unsolicited event versus 22.0% in the control group:

- Only zero to 2.1% in the four adjuvanted groups reported at least one unsolicited injection site reaction versus 2.0% in the control group.
- 2.1 to 10.0% in the four adjuvanted groups reported at least one unsolicited systemic reaction versus zero in the control group.

SAFETY – BOOSTER (STEP 2 ONLY):

-Solicited Reactions:

Within 7 days after the booster vaccination, 89.8 to 97.9% of subjects in the four adjuvanted groups reported at least one solicited reaction versus 63.3% in the control group:

- 89.6 to 97.9% in the four adjuvanted groups reported at least one solicited injection site reaction versus 51.0% in the control group.
- 36.2 to 60.4% in the four adjuvanted groups reported at least one solicited systemic reaction versus 44.9% in the control group.

For all groups, the most frequently reported solicited injection site reactions after booster vaccination were:

- Injection site pain with 83.3 to 91.5% of subjects in the adjuvanted groups versus 12.9% in the control group,
- Induration with 27.7 to 42.9% of subjects in the adjuvanted groups versus 16.3% in the control group.
- Erythema with 20.8 to 24.5% of subjects in the adjuvanted groups versus 22.4% in the control group.

Most of those reactions appeared within 3 days, were mild to moderate and spontaneously resolved within 3 days.

For all groups, the most frequently reported solicited systemic reactions after booster vaccinations were:

- Headache with 25.5 to 50.0% of subjects in the adjuvanted groups versus 40.8% in the control group,
- Malaise with 18.4 to 27.1% of subjects in the adjuvanted groups versus 18.4% in the control group,
- Myalgia with 17.0 to 27.1% of subjects in the adjuvanted groups versus 18.4% in the control group.

Most of those reactions appeared within 3 days, were mild to moderate and spontaneously resolved within 3 days.

-EMEA reactions:

24.5 to 31.3% of subjects in the four adjuvanted groups reported at least one reaction listed in the EMEA NfG versus 24.5% in the control group. Malaise was the most frequently reported reaction. Injection site ecchymosis and shivering were also reported.

-Unsolicited Events:

Within 21 days after booster vaccination, 25.5 to 36.7% of subjects in the four adjuvanted groups reported at least one unsolicited event versus 28.6% in the control group:

- Zero to 4.2% in the four adjuvanted groups reported at least one unsolicited injection site reaction versus none in the control group.
- 2.1to 16.3% in the four adjuvanted groups reported at least one unsolicited systemic reaction versus 6.1% in the control group.

SAFETY - AE LEADING TO DISCONTINUATION AND SAE

Step 1 and Step 2 Primary Series:

Two subjects from adjuvanted groups discontinued the study following an AE: one subject experienced moderate nausea and headache not related to the study vaccine. Another subject reported mild injection site pain as well as mild solicited systemic reactions as malaise and myalgia that prevented his normal activity as a dedicated sportsman.

One SAE was reported during the D0-D42 study period: one subject from the 7.5µgHA+AF03 group experienced a vasovagal syncope during the first vaccination, considered as related to the study procedures but not related to the vaccine. This SAE spontaneously resolved the same day and the subject was discontinued from the study. In addition, 8 subjects reported an SAE, all unrelated to the vaccine and occurring more than 21 days after vaccination.

No deaths were reported during the D0-D365 study period.

Step 2 booster and 6-month follow-up:

No SAEs were reported within 21 days after booster vaccination.

One subject reported an unrelated SAE (acute appendicitis) approximately 6 months after the booster vaccination.

The subject continued to participate in the study.

No deaths were reported during the D365-D545 period.

Step 2,18-month post-booster safety follow-up (from D545 to D910):

No SAEs were reported within the period from 6 months to18 months post-booster as only related SAEs were to be collected over this period.

IMMUNOGENICITY - PRIMARY SERIES:

Results were similar between the two methods of assessment used: HIH horse and SN methods.

Serological status at baseline was similar between the groups, i.e. with both methods, all subjects from both steps had no detectable Ab titer at D0, except one subject from the 7.5 µg+AF03 group in Step 2 who had a low detectable Ab titer (8 [1/dil]).

Humoral Immune Response - Primary Series:

HIH Test Against A/Vietnam:

Step 1 Primary Series:

In the FAS, an immune response was observed after the first vaccination with a GMTR of 2.83 and detectable Ab titers in 60.0% of the subjects. This immune response was then amplified after the second vaccination, so that each of the three EMEA criteria was met, i.e. percentage of seroconversion or significant increase in anti-HA antibodies >40%, percentage of subjects with HI titer ≥32 (1/dil) >70%, and GMTR >2.5.

Step 2 Primary Series:

In the FAS, an immune response was observed after the first vaccination in all the groups with GMTRs ranging from 2.47 to 4.39 and detectable antibodies in 42.0 to 66.7% of the subjects.

For all the adjuvanted groups, this immune response was then amplified after the second vaccination, i.e. each of the three EMEA criteria was met. For the control group, this immune response was less amplified after the second vaccination, i.e. only one of the three EMEA criteria was met. These results demonstrated the effect of the adjuvant to substantially increase the immune response to vaccine, even with low doses of antigen.

Overall, the immune response of the four adjuvanted groups followed a dose-dependent trend from 1.9 to 7.5 µg with no further increased immunogenicity when increasing the dose to 15 µg.

Although the 3.75µgHA+AF03, the 7.5µgHA+AF03 and the 15µgHA+AF03 formulations induced a higher immune response than the 1.9µgHA+AF03, each of the three EMEA criteria was met for the four adjuvanted groups after the second vaccination.

Similar results were observed in the PPAS.

SN Method Against A/Vietnam:

Step 1 Primary Series:

In the FAS, an immune response against A/Vietnam/1203 (homologous strain) was observed 21 days after the first vaccination with a GMTR of 3.02 and detectable Ab titers (titers ≥10 [1/dil]) in 53.3% of the subjects. This immune response is confirmed by the increase observed in the percentage of subjects presenting a two-fold and a four-fold increase in SN Ab titers on D21 and on D42:

- On D21, 53.3% and 46.7% of the subjects reported, respectively, a two-fold and a four-fold increase in SN Ab titers.
- On D42, all subjects and 93.3% of the subjects reported, respectively, a two-fold and four-fold increase in SN Ab titers.

Step 2 Primary Series:

In the FAS, an immune response against A/Vietnam/1203/2004 (homologous strain) was observed in the five groups 21 days after the first vaccination with GMTRs ranging from 2.05 to 3.62, detectable antibodies in 35.3 to 55.1% of the subjects and SN Ab titers ≥20 (1/dil) in 21.6 to 38.8% of the subjects.

In all the adjuvanted groups, this immune response was confirmed by the increase observed in the percentage of subjects presenting a two-fold and a four-fold increase in SN Ab titers on D21 and on D42:

- On D21, 35.3 to 55.1% and 21.6 to 38.8% of the subjects reported, respectively, a two-fold and a four-fold increase in SN Ab titers.
- On D42, 93.9 to 98.0% of the subjects and 87.8 to 96.0% of the subjects reported, respectively, a two-fold and four-fold increase in SN Ab titers.

These proportions were much lower in the control group, with 38.0% and 24.0% of subjects presenting respectively a two-fold and a four-fold increase in SN Ab titers on D21, increasing respectively to 54.0% and 42.0% on D42.

Similar results were observed in the PPAS.

Antibody Persistence Against A/Vietnam (Step 2 only) – Primary Series:

Three, 6 and 12 months after vaccination, a decrease in GMTs was observed in all groups. However, all adjuvanted groups maintained a higher proportion of subjects with detectable Ab titers.

Within the adjuvanted groups, Group 2 (3.75µgHA+AF03), Group 3 (7.5µgHA+AF03) and Group 4 (15µgHA+AF03) were comparable, whereas the antibody persistence in Group 1 (1.9µgHA+AF03) was lower.

The same trend was observed with the SN method.

Observational Immunogenicity - Primary Series:

Immunogenicity Serostatus Against A/NewCaledonia/7/2009 (H1N1):

Comparison of pre and post vaccination titers against A/New Caledonia (H1N1) did not demonstrate any circulation of the A/H1N1 virus which might have had an impact on the immune response against the A/H5N1 strain. However, it is interesting to note the increase in GMTs between D0 and D21 in all groups, and no further increase between D21 and D42.

Cross-Reactive Immunogenicity Against A/*Indonesia/02/2005_RG2(H5N1) Strain:*

A cross-reactivity evaluation was performed on D42 with the A/Indonesia/05/2005 (H5N1) RG2 strain using both HIH and SN methods. When considering the percentage of subjects positive by HIH (titer≥8 [1/dil]), the four adjuvanted groups showed a strong cross-reactivity response: 35.4-58.3% of the subjects from the adjuvanted groups versus 7.4% of the subjects from the control group.

IMMUNOGENICITY – 12-MONTH A/INDONESIA BOOSTER AFTER THE FIRST VACCINATION (STEP 2 ONLY)

Humoral Immune Response - Booster:

HIH test Against A/Indonesia:

As soon as 7 days (D372) after booster vaccination with A/Indonesia, a higher immune response was observed in the four adjuvanted groups with a GMTR [D372/D365] of 8.92 compared to 1.94 for the control group. Twenty-one days after booster vaccination, this immune response was amplified for the four adjuvanted groups. This response remained comparable across the adjuvanted groups with 95.8 to 100.0% of subjects with detectable HI Ab titers compared to 48.9% of subjects in the control group. Group 1 (1.9µgHA+AF03/3.75µgHA+AF03) had a lower proportion of subjects with HI Ab titers ≥40 (1/dil) and a lower proportion of seroconverted subjects compared to the three other adjuvanted groups.

Regarding EMEA criteria, all three criteria were met 21 days after booster vaccination for all the adjuvanted groups, as soon as 7 days after booster vaccination for Group 2 (3.75µgHA+AF03/ 3.75µgHA+AF03) and Group 3 (7.5µgHA+AF03/3.75µgHA+AF03). None of these criteria were met for the control group.

SN Method Against A/Indonesia:

As soon as 7 days after booster vaccination (D372) with A/Indonesia, an immune response was observed in the four adjuvanted groups with a GMTR [D372/D365] of 32.7 compared to 5.19 for the control group. The 2-fold and 4-fold increase was respectively 99.0% and 95.3% for all the adjuvanted groups compared to 70.2% and 53.2% for the control group. Twenty-one days after booster vaccination (D386), this immune response was amplified for the four adjuvanted groups. This response remained comparable across the adjuvanted groups with 97.9 to 100.0% of subjects with detectable SN Ab titers compared to 72.3% of subjects in the control group. Once again, the 2-fold and 4-fold increase was higher in all the adjuvanted groups.

Cross reactivity - Booster:

HIH Test Against A/Vietnam:

As soon as 7 days after booster vaccination (D372) with A/Indonesia, a higher immune response against A/Vietnam was observed in the four adjuvanted groups with GMTR [D372/D365] of 6.69 compared to 2.14 for the control group. Twenty-one days after booster vaccination (D386) with A/Indonesia, the proportion of subjects with detectable HI Ab titers against A/Vietnam increased to 99.0% for all adjuvanted groups, with 81.3% of subjects showing significant increase. For the control group, the proportion of subjects with detectable HI Ab titers against A/Vietnam remained comparable to D372.

Regarding EMEA criteria, all three criteria were met as soon as 7 days after booster vaccination for all the adjuvanted groups, whereas none of these criteria were met for the control group, not even 21 days after booster vaccination.

SN Method Against A/Vietnam:

The trend observed with SN antibody titers was the same as the one observed with HIH titers.

Antibody Persistence Post-booster (Step 2 only) Against A/Indonesia – (up to 18 Months after Booster Vaccination):

HIH test Against A/Indonesia:

HIH Ab titers were still detectable (≥ 10 [1/dil]) up to 18 months (M30) after booster vaccination in a majority of subjects who received the adjuvanted vaccine as booster, regardless of the adjuvanted formulation received for priming (all adjuvanted groups), i.e. around 57% of the subjects, and in around 10% of subjects who received the unadjuvanted vaccine, i.e. control group. The percentage of subjects with seroprotective titers (HIH Ab titers ≥ 40 [1/dil]) was also higher in all adjuvanted vaccine groups than in the control group. In all adjuvanted groups, GMTs were 5.24 (1/dil) before booster (M12) and decreased from 18.7 (1/dil) at M18 to 11.38 (1/dil) at M30. Approximately one third of the subjects still had seroprotective titers 6 months after booster vaccination and this proportion was divided by two 12 months later (M18 post-booster). Seroprotection rates were similar across adjuvanted groups. In the control group, GMTs were 5.04 (1/dil) before booster (M12) and remained extremely low up to M30 (5.39 [1/dil]). No subjects presented with seroprotective titers (HIH Ab titers ≥ 40 [1/dil]) 12 months after the booster (M24) vaccination.

SN Method Against A/Indonesia:

As observed with HIH Ab titers, SN Ab titers were still detectable up to 18 months after booster vaccination (M30) in a majority of subjects in all adjuvanted groups, i.e. around 95%, and around 40% in the control group. Results were similar across adjuvanted groups and were all above those measured before booster vaccination (M12). The percentage of subjects with SN Ab titers ≥40 [1/dil] was higher in all adjuvanted groups (approximately 70%) than in the control group (approximately 17%) and these proportions appeared to be stable over time from 6 to 18 months after the booster vaccination.

CMI RESPONSE – PRIMARY VACCINATION SERIES AND BOOSTER (STEP 2 ONLY):

The primary vaccination series and the booster vaccination increased the influenza specific lymphoproliferative response, the IFN-γ and IL-13 cytokine secretion and the multi-cytokine producing CD4 T-cell frequencies in all groups. No clear antigen dose-effect or significant difference between the four adjuvanted groups was observed after the primary or booster vaccinations. The booster vaccination did not improve the cellular response compared to the primary vaccination series. The Th1/Th2 balance was not significantly impacted by vaccination.

Conclusions:

Regarding safety, the A/H5N1 vaccine was in general well tolerated regardless of the dose and strain tested:

- After primary vaccination series, the proportion of subjects with at least one solicited injection site reaction was higher in the adjuvanted groups than in the control group. The same trend was observed after booster vaccination.
- Regarding EMEA criteria, the safety profile was found acceptable whatever the dose and strain: no injection site induration equal or greater than 5 cm for more than 3 consecutive days was reported, and only 4 subjects reported fever above 38°C (3 after primary vaccination series and 1 after booster).
- After primary vaccination series, the occurrence of adverse events was lower after the second vaccination in all groups.
- Only one SAE, not related to the vaccine, was reported within 21 days of any vaccination.
- In addition, vaccination did not show a significant clinical impact on biological parameters and vital signs of any subjects (Step 1 only).

Regarding immunogenicity after the primary vaccination series with A/Vietnam, both HIH and SN methods provided convergent results in terms of required dose and formulation to induce the highest immune response:

- Two doses, 21 days apart, are sufficient to induce a significant immune response.
- All four adjuvanted formulations induced a higher immune response against A/Vietnam than the formulation without adjuvant.
- After the second vaccination with A/Vietnam, each of the three EMEA criteria was met for the four adjuvanted groups whereas only one criterion was met for the control group.
- The immune response against A/Vietnam of the four adjuvanted groups followed a dose-dependent trend from 1.9 to 7.5 µg with no further increased immunogenicity when increasing the dose to 15 µg. Therefore, the advantage in increasing the level of HA beyond 7.5 µg appears to be limited when using AF03 adjuvant.
- The four adjuvanted groups showed a strong cross-reactivity response against the A/Indonesia/02/2005_RG2 strain.
- The 3.75µgHA+AF03, the 7.5µgHA+AF03 and the 15µgHA+AF03 formulations induced a higher immune response against A/Vietnam than the 1.9µgHA+AF03.
- The antibody persistence against A/Vietnam 3, 6 and 12 months after vaccination remained higher in the adjuvanted groups (3.75 µg, 7.5 µg and 15 µg) than in the control group.

Regarding immunogenicity after booster vaccination with A/Indonesia, both HIH and SN methods provided convergent results:

- An influenza A/Indonesia booster vaccination given one year after primary vaccination with A/Vietnam induced rapid anamnestic immune responses against both the priming and booster strains.
- All three EMEA criteria were met against both A/Vietnam and A/Indonesia 21 days after booster vaccination with A/Indonesia for all the adjuvanted groups, as soon as 7 days after booster vaccination for Group 2 (3.75µgHA+AF03/ 3.75µgHA+AF03) and Group 3 (7.5µgHA+AF03/3.75µgHA+AF03).
- The antibody persistence against A/Indonesia strain 6, 12 and 18 months after booster vaccination remained higher in the adjuvanted groups (3.75 µgHA+AF03) than in the control group (7.5µgHA).

In conclusion, two doses of all four adjuvanted formulations followed by a 12-month booster dose with the Flu H5N1 3.75µgHA+AF03 formulation induced a strong immune response and were characterized by satisfactory safety profile, thereby meeting the criteria of the EMEA guideline for registration of a prototype pandemic vaccine.

The new oil-in-water emulsion adjuvant AF03 has significant dose-sparing effect when added to a candidate pandemic influenza vaccine. Even at doses as low as 3.75 µg, the adjuvanted vaccine was able to elicit high levels of antibodies, including cross-reactive antibodies, in an H5N1-naïve adult population. In addition, a booster with A/Indonesia vaccine at a dose of 3.75 µgHA+AF03 induced rapid anamnestic immune responses against both the priming and booster strains, i.e. A/Vietnam and A/Indonesia, respectively. In the event of an influenza pandemic, this could have a major impact on the number of people who can be protected.

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