Sponsor:	Novartis Vaccines and Diagnostics S.r.l Via Fiorentina, 1 53100 Siena, Italy
Investigational Product:	MF59-adjuvanted trivalent influenza subunit vaccine
Indication:	Prophylaxis against Influenza
Protocol Number:	V70P4S
Protocol Title:	A Phase II, Open Label, Uncontrolled, Multi Center Study to Evaluate Safety and Immunogenicity of FLUAD® Surface Antigen, Inactivated, (Adjuvanted with MF59C.1) Influenza Vaccine, Formulation 2006-2007, When Administered to Elderly Subjects
Phase of Development:	Phase II
Study Period:	
Date of first enrolment:	03 JUL 06
Date of last visit:	28 JUL 06

Methodology:

Approximately 60 subjects aged 65 years and over were enrolled in the open label study. Subjects were observed for 30 minutes after vaccination on Day 0 for any immediate reactions. Each subject was instructed to complete a diary card for 3 more days post-immunization to describe local (pain, erythema, ecchymosis, swelling and induration) and systemic reactions [fever (i.e., axillary temperature 38°C), chills/shivering, malaise, headache, myalgia, arthralgia, sweating, fatigue] and was contacted by phone on Day 4 after immunization to obtain local and systemic reaction data and to determine subject's clinical status. All adverse events were collected during Day 0 to Day 3. All adverse events necessitating a physician's visit or consultation and/or leading to premature study discontinuation and all serious adverse events were collected throughout the trial. Subjects were informed that in the event of severe inter-current infection, they must telephone the Investigator who would take a nasal and/or pharyngeal swab for the diagnosis of influenza or any other respiratory infection of viral origin.
Blood samples for immunogenicity assays were collected before vaccination (Day 0) and after 21 (Day 21, window: 20-24) days.

Number of Subjects (planned and analyzed):

At least 50 evaluable subjects 65 years were required for the study. Considering a drop- out rate of 20% overall, approximately 60 subjects were planned to be enrolled.

Subjects who received the immunization were included in safety analyses. Subjects who provided evaluable blood samples at Day 0 and Day 21 were included in immunogenicity analyses.

Study Centers:

Two study centers in Italy.

Publication (reference) and/or ClinicalTrials.gov National Clinical Trial (NCT) Number:

NCT00329927

Objectives:

To evaluate the safety of the administration of a single intramuscular (IM) injection of FLUAD vaccine (formulation 2006/2007) in elderly subjects (≥ 65 years).

To evaluate the antibody response to each influenza vaccine antigen, as measured by Single Radial Hemolysis (SRH) at 21 days post-immunization in elderly subjects in compliance with the requirements of the current EU recommendations for the evaluation of the immunogenicity for a new formulation of a licensed flu vaccine (CPMP/BWP/214/96).

Test Product, Dose, Mode of Administration, Lot Number:

A single 0.5 mL dose of the MF59-adjuvanted trivalent influenza subunit vaccine contained 45µg of viral hemagglutinin protein composed of 15µg from each of influenza A/H1N1, A/H3N2 and B strain was administered intramuscularly in the deltoid muscle of the non dominant arm.

Lot no. W52P09H1

Duration of Study:

Approximately 3 weeks of participation for each research participant.

Reference Therapy, Dose, Mode of Administration, Lot Number:

None

Statistical Methods:

Only subjects who contributed evaluable serum samples both before immunization and at 21 days after immunization (acceptable time interval 20-24 days) were to be included in the immunogenicity analyses.

For each vaccine antigen, geometric mean areas were calculated by exponentiating (base 10) the mean of the log-transformed (base 10) titers. Day 21 to Day 0 geometric mean ratios (GMRs) of areas were computed as the geometric mean of the ratios of the Day 21

area to the Day 0 area of each subject. Percentages of subjects (with seroconversion, significant increase or protected) were also computed separately for each vaccine antigen.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion Criteria

Individuals eligible for enrollment into this study were male and female adult volunteers who were:

1. 65 years of age or older, mentally competent, willing and able to give written informed consent prior to study entry;
2. able to comply with all the study requirements;
3. in good health as determined by:
   - medical history;
   - physical examination;
   - clinical judgment of the investigator;

Informed consent was obtained for all the subjects before enrollment in the study.

Exclusion Criteria

Individuals were not to be enrolled into the study if:

1. they had any serious disease such as:
   - cancer (leukemia, lymphomas, neoplasm) except for benign or localized skin cancer and non metastatic prostate cancer not presently treated with chemotherapy;
   - autoimmune disease (including rheumatoid arthritis);
   - advanced arteriosclerotic disease or insulin dependent diabetes mellitus;
   - chronic obstructive pulmonary disease (COPD) that requires oxygen therapy;
   - acute or progressive hepatic disease;
   - acute or progressive renal disease;
   - congestive heart failure;
2. they were hypersensitive to ovalbumin, chicken protein, chicken feathers, influenza viral protein, neomycin or polymyxin or any other component of the vaccine;
3. they had a history of neurological symptoms or signs, or anaphylactic shock following administration of any vaccine;
4. they had a known or suspected (or have a high risk of developing) impairment/ alteration of immune function (excluding that normally associated with advanced age) resulting, for example, from:
   - receipt of immunosuppressive therapy (any parenteral or oral cortical steroid or cancer chemotherapy/radiotherapy) within the past 60 days and for the full length of the study;
   - receipt of immunostimulants;
   - receipt of parenteral immunoglobulin preparation, blood products and/or plasma derivates within the past 3 months and for the full length of the study;

suspected or known Human Immuno-deficiency Virus (HIV) infection or HIV-related disease;

5. they had a known or suspected history of drug or alcohol abuse;
6. they had a bleeding diathesis or conditions associated with prolonged bleeding time that in the investigator's opinion would interfere with the safety of the subject;
7. within the past 12 months, they had:
   received more than one injection of influenza vaccine; within the past 6 months,
8. they had:
   had laboratory confirmed influenza disease;
   received influenza vaccine,
9. within the past 4 weeks they had received:
   another vaccine;
   any investigational agent;
10. within the past 7 days, they had experienced:
    any acute disease;
    infections requiring systemic antibiotic or antiviral therapy (chronic antibiotic therapy for urinary tract prophylaxis is acceptable);
11. they had experienced an acute exacerbation of a COPD (chronic obstructive pulmonary disease) within the past 14 days;
12. within the past 3 days, they had experienced:fever (ie, body temperature $\geq$ 38°C);
13. they were taking part in another clinical study;
14. they had any condition which, in the opinion of the investigator, might interfere with the evaluation of the study objective.

Criteria for Evaluation:

For each of the three virus strains, at least one of the following criteria had to be met in subjects aged 65 years and over, approximately 3 weeks after vaccination:

- number of seroconversions or significant increases in antibody titer > 30%
- mean geometric increase >2.0
- percentage of subjects achieving an SRH area $\geq$25 mm^2 > 60%

Results:

Table 1 Overview of Data Sets - As Enrolled Set

	Number (%) of Research Participants
	≥ 65 Years N = 58
All Enrolled Set	58 (100%)
Immunogenicity - PPS	56 (97%)
Safety	57 (98%)

Abbreviations: PPS, per protocol set.

Table 2 Summary of Study Terminations - All Enrolled Set

	Number (%) of Research Participants
	≥ 65 Years N = 58
Total Number of Subjects Enrolled	58 (100%)
Completed	57 (98%)
Completed Protocol	57 (98%)
Premature Withdrawal	1 (2%)
Withdrawal of Consent	1 (2%)

Table 3 Demographic and Other Baseline Characteristics - All Enrolled Set

	≥ 65 Years N = 58
Age (Years):	74.2 ± 6.9
Sex:	
Male	25 (43%)
Female	33 (57%)
Ethnic Origin:	
Caucasian	58 (100%)
Weight (kg):	71.9 ± 13.2
Height (cm):	161.6 ± 7.3
Previous Influenza Vaccination:	
Yes	58 (100%)
Study Criteria Fulfilled:	
Yes	58 (100%)

Categorical parameters: N (%), non-categorical parameters: mean ± standard deviation.

Table 4 Vaccine Immunogenicity at Day 21 for subjects aged 65 years and over

		≥ 65 Years N = 58					
Strains		A/ (H1N1)		A/ (H3N2)		B	
PREVACCINATION							
		[1]n/N	%	n/N	%	n/N	%
[2]Seroprotection rate		44/56	79	20/56	36	48/56	86
[3]95% CI		66-88		23-50		74-94	
POSTVACCINATION							
	requirements	n/N	%	n/N	%	n/N	%
[4]Seroconversion rate or significant increase	>30%	19/56	34	31/56	55	18/56	32
95% CI		22-48		41-69		20-46	
Mean GMT Increase (GMR)	>2	1.59/56		3.15/56		1.58/56	
95% CI		1.27 - 1.99		2.26 - 4.39		1.25 - 2	
Seroprotection rate	>60%	52/56	93	44/56	79	56/56	100
95% CI		83-98		66-88		94-100	

Abbreviations: CI, confidence interval; SRH, single radial hemolysis.

[1] n/N: responders (n) as part of number of subjects of the (sub) population (N), i.e. seroconversion or significant increase

[2] Seroprotection rate: proportion of subjects with a protective titer pre- or postvaccination = SRH-test $25mm^2$

[3] 95% CI: 95% confidence interval

[4] Seroconversion rate or significant increase: proportion of subjects with either seroconversion or significant increase in antibody titer

Table 5 Overview of Subjects with at Least One Reactogenicity Sign in subjects aged 65 years and over

	≥ 65 Years N = 57	
	n	%
Any	24	42
Local	14	25
Systemic	15	26

Table 6 Overview of Local and systemic reactions after the administration of FLUAD in subjects aged 65 years and over

	≥ 65 years N = 57	
	n	%
Local reactions	14	25%
Pain	12	21%
Erythema	2	4%
Ecchymosis	0	0%
Swelling	0	0%
Induration	2	4%
Systemic Reactions	15	26%
Fever	1	2%
Chills/Shivering	1	2%
Malaise	3	5%
Headache	4	7%
Myalgia	9	16%
Arthralgia	4	7%
Sweating	2	4%
Fatigue	4	7%

Table 7 Overview of Research Participants with Unsolicited Adverse Events from Day 1 Through Day 22 – Unsolicited Safety Set

	Number (%) of Research Participants with Unsolicited Adverse Events
	≥ 65 years N = 57
Any AEs	0
At least possibly or probably related AEs	0
Serious AEs	0
At least possibly or probably related SAEs	0
Premature withdrawals due to AEs	0
Death	0

Abbreviations: AEs, adverse events; SAEs: serious adverse events.

Table 8 Number (Percentages) of Subjects with Serious Adverse Events by Preferred Term, sorted by System Organ Class

None reported

Table 9 Number (Percentages) of Subjects with Unsolicited Adverse Events Reported in > 5 % of Subjects by Preferred Term sorted by System Organ Class

None reported

Conclusion:

The immunogenicity of Fluad was measured by SRH against the three strains of influenza contained in the vaccine and evaluated based on the CPMP/BWP/214/96 guidance. Against the A/H1N1 and B antigens, two criteria were met (i.e., the proportion of subjects with seroconversion was >30% and the proportion of subjects with a SRH area ≥ 25 mm2 was > 60%). Against the A/H3N2 antigen all three criteria were met (i.e., the proportion of subjects with seroconversion was >30%, the mean GMT increase was >2 and the proportion of subjects with a SRH area ≥ 25 mm2 was > 60%).

In regards to safety, the incidence of pain at the injection site (21%) were consistent with that seen in earlier studies (32%, Clinical Expert Report for Mutual Recognition Procedure) and the incidence of other local reactions (0-4%) was lower than that seen in earlier studies.Systemic reactions were infrequent and their incidence (range 2% to 16%) was similar to that previously observed and most of reactions were mild and all resolved shortly after immunization.

The 2006/2007 Fluad adjuvanted influenza vaccine was safe and immunogenic and complied with CHMP criteria for approval of influenza vaccines.

Date of Clinical Trial Report: **31 Jul 06**