Sponsor:	Chiron S.r.l
Investigational Product:	Adjuvanted trivalent influenza virus vaccine (surface antigen, inactivated, adjuvanted with MF59C.1, egg-derived)
Indication:	Prophylaxis: Influenza
Protocol Number:	V70P3S
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 2005-2006, When Administered to Elderly Subjects
Phase of Development:	Phase II
Study Period:	
Date of first enrollment:	21 JUN 05
Date of last visit:	14 JUL 05

Methodology:

At least 50 subjects aged 65 years and over were to be enrolled in this 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 postimmunization 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 Days 0 to 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. 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):

A total of 61 subjects were enrolled but after screening based on the protocol inclusion/exclusion criteria only 60 subjects met the entry criteria. The average age was 74 years (standard deviation, 5.6 years); 29 males (48%) and 32 females (52%) participated. All 61 subjects were Caucasian (100%).

Study Centers:

Two study centers in Italy.

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

NCT00316628

Objectives:

Immunogenicity Objectives

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).

Safety Objectives

To evaluate the safety of the administration of a single intramuscular (IM) injection of FLUAD® in elderly subjects.

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

A single 0.5 mL dose of the adjuvanted trivalent influenza virus vaccine for the 2005/2006 season was administered intramuscularly in the deltoid muscle of the non dominant arm. (Lot number V52P98H1).

Duration of Study:

Two weeks enrollment, three weeks participation per subject.

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 of areas were computed as the ratio of the day 21 geometric mean to the day 0 geometric mean. 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 (except for benign or localized skin cancer and nonmetastatic prostate cancer not presently treated with chemotherapy)
- Autoimmune disease (including rheumatoid arthritis)
- advanced arteriosclerotic disease or complicated diabetes mellitus
- Chronic obstructive pulmonary disease (COPD) that required oxygen therapy

- Acute or progressive hepatic disease
- Acute or progressive renal disease
- Congestive heart failure

2. They were hypersensitive to eggs, 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 had 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)
- Receipt of immunostimulants
- Receipt of parenteral immunoglobulin preparation, blood products and/or plasma derivates within the past 3 months
- Suspected or known HIV infection or HIV-related disease

5. They had a bleeding diathesis or conditions associated with prolonged bleeding time.

6. Within the past 12 months, they had:

- Received more than one injection of influenza vaccine

7. Within the past 6 months, they had:

- Had laboratory confirmed influenza disease
- Received influenza vaccine

8. Within the past 4 weeks they had received:

- Another vaccine
- Any investigational agent

9. 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 was acceptable)

10. Within the past 3 days, they had experienced:

- Fever (ie, axillary temperature ≥38°C)

11. They were taking part in another clinical study.

12. They had any condition which, in the opinion of the investigator, might interfere with the evaluation of the study objective.

Criteria for Evaluation:

Immunogenicity objectives:

Immunogenicity was assessed by analyzing if at least one of the following assessments met the indicated requirements for each strain:

- Elderly subjects ≥ 65 years
  - Number of seroconversions[1] or significant increase in antibody titer[2] > 30%.
  - Mean geometric increase > 2.0.
  - The proportion of subjects achieving an SRH area ≥ 25 mm^2 should be > 60%.

Safety Objectives:

Safety was assessed in accordance with available safety data on influenza vaccines.

[1] Seroconversion is defined as negative prevaccination serum/postvaccination serum area ≥ 25 mm^2.
[2] Significant increase in antibody titer is defined as at least a 50% increase in area.

Results:

Table 1 Overview of Subject Populations

	Number (%) of Subjects Fluad (N=61)
Population	
Enrolled	61 (100%)
Immunogenicity (per-protocol set)	57 (93%)
Safety	59 (97%)

Table 2 Summary of Study Terminations

Primary Withdrawal Reason	Number (%) of Subjects Fluad
Total number of subjects enrolled	61
Completed	58 (95%)
Completed protocol	58 (95%)
Premature withdrawal	3 (5%)
Death	0
Adverse event	1 (2%)
Lost to follow-up	1 (2%)
Inappropriate enrollment	1 (2%)

Table 3 **Demographic and Other Baseline Characteristics - All Enrolled Set**

	Fluad (N=61)
Age (years)	74.0±5.6
Sex	
Male	29 (48%)
Female	32 (52%)
Ethnic origin	
Caucasian	61 (100%)
Weight (kg)	71.8±12.6
Height (cm)	162.1±8.8
Previous influenza vaccinations	
Yes	60 (98%)
No	1 (2%)
Study criteria fulfilled	
Yes	60 (98%)
No	1 (2%)

Table 4 Vaccine Immunogenicity at Day 21 for Subjects Aged 65 Years and Over

		Elderly (≥65 years) N=57					
Strains		**A/H1N1**		**A/H3N2**		**B**	
PRE-VACCINATION							
		[1]n/N	%	[1]n/N	%	[1]n/N	%
[2]**GMA**		23		8.17		38	
[3]**95% CI**		17-31		6.26-11		28-50	
[4]**Seroprotection rate**		38/57	67	11/57	19	46/57	81
95% CI		53-79		10-32		68-90	
POST-VACCINATION							
	Requirements	[1]n/N	%	[1]n/N	%	[1]n/N	%
[5]**Seroconversion rate**		13/15	87	26/36	72	8/9	89
[6]**Significant increase in antibody titer**		19/42	45	9/21	43	17/48	35
[7]**Seroconversion rate or significant increase**	**> 30%**	32/57	56	35/57	61	25/57	44
95% CI		42-69		48-74		31-58	
GMA		51		38		67	
95% CI		43-60		28-52		59-77	
Mean GMA Increase	**> 2**	2.2		4.68		1.79	
95% CI		1.67-2.88		3.22-6.79		1.41-2.28	
Seroprotection rate	**> 60%**	53/57	93	43/57	75	55/57	96
95% CI		83-98		62-86		88-100	

[1] n/N: responders (n) as part of number of subjects of the (sub) population (N), i.e. seroconversion or significant increase. [2] GMA: geometric meanarea. [3] 95% CI: 95% confidence interval. [4] Seroprotection rate: proportion of subjects with a protective titer pre- or postvaccination = SRH-test ³ 25mm². [5] Seroconversion rate: proportion of subjects with antibody increase from prevaccination (seronegative) to postvaccination area ³ 25mm². [6] Significant increase: proportion of subjects with a significant increase in antibody titer, i.e. at least a 50% increase in area. [7] Seroconversion rate or significant increase: proportion of subjects with either seroconversion or significant increase in antibody titer.

Table 5 Overview of Solicited Adverse Events - Safety Set

	Number (%) of Subjects Fluad (N=59)
Any	18 (31)
Injection site	14 (24)
Systemic	10 (17)

Table 6 Local and Systemic Reactions After the Administration of Fluad in Subjects Aged 65 years and over

	Number (%) of Subjects Fluad (N=59)
Local reactions	14 (24)
Pain	9 (15)
Erythema	5 (8)
Ecchymosis	4 (7)
Swelling	3 (5)
Induration	7 (12)
Systemic reactions	10 (17)
Fever	0 (0)
Chills/Shivering	3 (5)
Malaise	4 (7)
Headache	5 (8)
Myalgia	3 (5)
Arthralgia	4 (7)
Sweating	4 (7)
Fatigue	5 (8)

Table 7 Overview of Unsolicited Adverse Events - Safety Set

	Number (%) of Subjects
	Fluad (N=59)
Any adverse events (AEs)	1 (2)
Serious AEs	1 (2)
AEs leading to premature withdrawal	1 (2)
Death	0

Table 8 Serious Adverse Events by Preferred Term, Sorted by System Organ Class

	Number (%) of Subjects
MedDRA System Organ Class	**Fluad**
MedDRA Preferred Term	**(N=59)**
Any SAEs	1 (2)
Eye disorders	
Uveitis	1 (2)

Abbreviations: MedDRA = Medical Dictionary for Regulatory Affairs; SAEs = serious adverse events.

Table 9 Other Adverse Events Reported in > 5 % of Subjects by Preferred Term Sorted by System Organ Class

None reported.

Conclusion:

The results of the present study enable us to draw the following conclusions:

For the A/H1N1 and A/H3N2 antigens all three criteria were met (ie, the proportion of subjects with seroconversion was >30%, the mean GMA increase was >2 and the proportion of subjects with an SRH area $\geq$ 25 mm^2 was > 60%). For the B antigen two criteria were met (ie, the proportion of subjects with seroconversion was >30% and the proportion of subjects with an SRH area $\geq$ 25 mm^2 was > 60%).

With regard to side effects, we can conclude the following:

1. The incidence of pain at the injection site (15%) was lower than that seen in earlier studies (32%)[3] and the incidence of other local reactions (5-12%) was similar than that seen in earlier studies.
2. Systemic reactions were infrequent and their incidence (range 0% to 8%) was similar to that previously observed.
3. Most of reactions were mild and all resolved shortly after immunization.

We can therefore conclude that the 2005/2006 Fluad adjuvanted influenza vaccine has a very good immunogenicity and safety profile and complies with Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines.

Date of Clinical Trial Report: 18 JUL 05

[3] Podda, A. The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. Vaccine (2001) 19: 2673–2680