Sponsor:	Novartis Vaccine and Diagnostics
Investigational Product:	Adjuvanted Trivalent Influenza Virus Vaccine (Surface Antigen, Inactivated, Adjuvanted with MF59C.1, egg-derived) (FLUAD)
Indication:	Prophylaxis: Influenza
Protocol Number:	V70_25S
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 2010-2011, when Administered to Elderly Subjects
Phase of Development:	Phase II

Study Period:

Date of first enrolment:	14 JUN 10
Date of last visit:	12 JUL 10

Methodology:

At least 50 subjects aged 65 years and older were to be evaluable in this open label study. Blood samples, approx. 10 mL, for the determination of antibody titers were drawn on Day 1 prior to vaccination and on Day 22 (-1/+5 days) (end of individual study participation).

After immunization on Day 1, subjects were observed for approximately 30 minutes for any immediate reactions. Each subject was instructed to complete a diary card for three days following immunization to collect local (ecchymosis, erythema, induration, swelling and pain at the injection site) and systemic reactions (chills/shivering, malaise, myalgia, arthralgia, headache, sweating, fatigue and fever [i.e., axillary temperature ≥ 38°C]). Subjects were contacted by phone on Day 5 (window: 0/+2) after immunization to ensure that local and systemic reaction data were collected on the Subject's Diary Card and also to determine the subject's clinical status. All adverse events (solicited and unsolicited) were collected during Day 1 to 4. All adverse events necessitating a physician's visit or consul-

tation 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 (i.e., any severe flu like symptoms) during the study period until Day 22 (window: -1/+5), he/she had to contact the Investigator who would take a nasal and/or pharyngeal swab for the diagnosis of influenza or any other respiratory infection of viral origin. For confirmatory purpose specimens were planned to be analyzed via Quick test and RT-PCR or culture.

Number of Subjects (planned and analyzed):

Approximately 63 subjects (aged 65 years and older) were planned to be enrolled. This sample size allowed for up to 13 non evaluable subjects (non-evaluable subjects were enclosed in the per protocol set exclusions due to protocol deviation as predefined in the analysis plan).

In total 64 subjects were actually enrolled. All enrolled subjects were included in the safety analysis. 62 subjects were included in the immunogenicity analysis [per protocol set (PPS)].

Study Centers: 6 sites in Italy.

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

NCT01152814.

Objectives:

Immunogenicity:

Primary

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 clinical trials related to yearly licensing of influenza vaccines.

Antibodies may be additionally quantified using the hemagglutination inhibition (HI) test for confirmation purposes (Note for Guidance on Harmonization of Requirements for Influenza Vaccines, CPMP/BWP/214/96: 12 March 1997).

Safety:

To evaluate the safety of a single intramuscular (IM) injection of FLUAD in elderly subjects in compliance with the requirements of the current EU recommendations for clinical trials related to yearly licensing of influenza vaccines (CPMP/BWP/214/96).

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

A single 0.5mL dose of FLUAD, influenza subunit vaccine, adjuvanted with MF59C.1, for the Northern Hemisphere (NH) influenza season 2010/2011 was IM administered. Lot No.: 104003, Date of expiry April 2011.

Duration of Study:

Each subject participated approximately for 3 weeks after enrolment into the study.

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

None.

Statistical Methods:

There was no statistical null hypothesis associated with the immunogenicity objective. Statistical analyses were carried out descriptively.

This study is in compliance with the sample size requirements of the current CHMP guideline on harmonization of requirements for influenza vaccines (CPMP/BWP/214/96).

Diagnosis and Main Criteria for Inclusion and Exclusion:

The study population consisted of healthy male and female adults who are ≥ 65 years of age, mentally competent, willing and able to give informed consent prior to study entry. They were eligible if they did not receive any seasonal or pandemic influenza vaccine or did not have a laboratory confirmed seasonal or pandemic influenza disease within the past 6 months.

Criteria for Evaluation:

Immunogenicity analyses were performed by SRH assay and assessed according to CPMP/BWP/214/96. In elderly subjects aged 65 years and older at least one of the following criteria was to meet the indicated requirements (CPMP/BWP/214/96) for each strain: i.e., seroprotection rate > 60%; seroconversion or significant increase rate > 30%; post/pre-vaccination GMR > 2.0.

Safety

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

Table 1: Time and Events

Study Periods	Vaccination	Post-vaccination	
Clinic Visit (Yes/No)[a]	Yes	No	Yes
Study Day	1	5	22
Study Visit Window	n/a	0/+2	-1/+5
ICF	x		
Exclusion/Inclusion	x[b]		
Medical History	x[b]		
Physical Examination[c]	x		x
Investigational vaccine administered	x		
Serology Blood draw (10mL)	x[b]		x
Diary Card Dispensed [d]	x		
Diary Card Collected and/or Reviewed [d]		x	x
Assess Local/Systemic Reactions[e]	x	x	x
Assess AEs and SAEs [f]	x	x	x
Concomitant Medications	x	x	x
Study Termination			x

[a] Clinic visit "no" refers to telephone contact only with subject.

[b] Performed prior to vaccination.

[c] Physical examination were performed by a qualified health professional designated within the Site Responsibility Delegation Log. Brief physical exam was performed at Study Day 1 and 22. Physical examination of injection site and complaint-focused physical examination were performed at visits on Day1 and 22.

[d] Diary card review was performed over the phone for Day 5 and at Day 22 clinic visit. Diaries were returned at Day 22 clinic visit.

[e] Data on local and systemic reactions were observed by the study personnel for all subjects for approximately 30 minutes after vaccination. Subjects recorded local and systemic reactions on the diary card daily for 3 days after study vaccination (Study Days 1-4).

[f] All adverse events were collected for three days post immunization (Study Days 1-4). Thereafter, only 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.

Results:

Table 2: Overview of Subject Populations

	FLUAD N=64
Population:	
Enrolled	64 (100%)
Immunogenicity (FAS)	62 (97%)
Immunogenicity (PPS)	62 (97%)
Exposed	64 (100%)
Safety	64 (100%)
Safety After Study Day 4	64 (100%)

Table 3: Summary of Study Terminations - All Enrolled Subjects

	Number (%) of Subjects
	FLUAD
	N=64
Enrolled	64 (100%)
Completed study	62 (97%)
Premature withdrawals	2 (3%)
Lost to follow up	2 (3%)

Table 4: Demographic and Other Baseline Characteristics - All Enrolled Subjects

	FLUAD N=64
Age (Yrs):	73.3 ± 5.1
Gender	
Male	30 (47%)
Female	34 (53%)
Ethnic Origin:	
Caucasian	64 (100%)
Weight (kg):	72.66 ± 12.93
Height (cm):	163.6 ± 6.7
Body Mass Index:	27.08 ± 4.09
Prev. Seasonal Influenza Vaccination:	
No	2 (3%)
Yes	62 (97%)
If Yes Date:	
Jun-2003	1 (2%)
Jun-2009	29 (45%)
Nov-2009	20 (31%)
Oct-2009	11 (17%)
Sep-2009	1 (2%)
Not Available	2
Prev. Pandemic Influenza Vaccination:	
No	35 (55%)
Yes	29 (45%)
Met Entry Criteria:	
Yes	64 (100%)

Categorical parameters: N(%), non-categorical parameters: Mean ± Std

Table 5: Vaccine Immunogenicity Assessed by SRH Assay (Per-Protocol Set)

		Elderly (≥ 65 YOA) N=62					
Strains		**A/H1N1**		**A/H3N2**		**B**	
PRE-VACCINATION		n/N[1]	%	n/N	%	n/N	%
GMA[2]		24		15		39	
95% CI[3]		19-29		13-18		35-44	
Seroprotection rate[4]		37/62	60%	14/62	23%	52/62	84%
95% CI[3]		46-72		13-35		72-92	
POST-VACCINATION							
	CHMP Requirements						
Seroconversion rate[5]		5/6	83%	2/4	50%	0/0	-
Significant increase[6]		23/56	41%	36/58	62%	17/62	27%
Seroconversion or significant increase [7]	**> 30%**	28/62	**45%**	38/62	**61%**	17/62	27%
95% CI[3]		32%-58%		48%-73%		17%-40%	
GMA[2]		46		28		52	
95% CI[3]		42-51		24-32		48-56	
Geometric mean increase	> 2	1.96		1.88		1.32	
95% CI[3]		1.59-2.42		1.64-2.16		1.22-1.43	
Seroconversion rate[4]	**> 60%**	60/62	**97%**	44/62	**71%**	62/62	**100%**
95% CI[3]		89%-100%		58%-82%		94%-100%	

YOA = years of age

[1] n/N: responders (n) as part of number of subjects of the (sub-) population (N). [2] GMA: geometric mean area. [3] 95% CI: 95% confidence interval.

[4] Seroprotection rate: proportion of subjects with a pre- or post-vaccination area ≥ 25 mm^2. [5] Seroconversion rate: proportion of subjects with negative pre-vaccination serum and a post-vaccination serum area ≥ 25 mm^2. [6] Significant increase: proportion of subjects with at least a 50% increase in area from positive pre-vaccination serum. [7] Seroconversion or significant increase: proportion of subjects with either seroconversion or significant increase.

Table 6: Immunogenicity Results Assessed by SRH Assay on Day 22 – Subjects Seronegative at Baseline

		≥ 65 YOA
A/California/7/2009 (H1N1)-like strain	**Number of subjects**	N=6
	GMA [1]	46
	Geometric mean increase	**11**
	Seroprotection rates	**83%**
	Seroconversion or significant increase [3]	**83%**
A/Perth/16/2009 (H3N2)-like strain	**Number of subjects**	N=4
	GMA [1]	20
	Geometric mean increase	**4.92**
	Seroprotection rates [2]	50%
	Seroconversion or significant increase [3]	**50%**
B/Brisbane/60/2008-like strain	**Number of subjects**	N=0

Bold = CHMP criteria met; YOA = years of age

[1] GMA=Geometric mean area;

[2] Seroprotection rate: proportion of subjects with a pre- or post-vaccination area ≥ 25 mm^2.

[3] Seroconversion or significant increase: proportion of subjects with either seroconversion or significant increase (for subjects seronegative at baseline significant increase is not applicable).

Seroconversion: proportion of subjects with negative pre-vaccination serum and a post-vaccination serum area ≥ 25 mm^2.

Significant increase: proportion of subjects with at least a 50% increase in area from positive pre-vaccination serum.

Table 7: CHMP Criteria Met by SRH Assay

Viral Strain		A/H1N1	A/H3N2	B
≥ 65 Years	Seroprotection	+	+	+
	Geometric mean increase	-	-	-
	Seroconversion or significant increase	+	+	-

"+" CHMP criteria met: "-" CHMP criteria not met

Table 8: Vaccine Immunogenicity Assessed by HI Assay (Per-Protocol Set)

		Elderly (≥ 65 YOA) N=62					
Strains		**A/H1N1**		**A/H3N2**		**B**	
PRE-VACCINATION		n/N[1]	%	n/N	%	n/N	%
GMT[2]		14		71		36	
95% CI[3]		10-19		52-96		27-49	
Seroprotection rate[4]		11/62	18%	47/62	76%	32/62	52%
95% CI[3]		9-30%		63-86%		39-65%	
POST-VACCINATION							
	CHMP Requirements						
Seroconversion rate[5]		20/29	69%	1/1	100%	3/5	60%
Significant increase[6]		20/33	61%	41/61	67%	13/57	23%
Seroconversion or significant increase [7]	**> 30%**	40/62	**65%**	42/62	**68%**	16/62	26%
95% CI[3]		51-76%		55-79%		16-38%	
GMT[2]		90		398		77	
95% CI[3]		62-130		302-524		62-96	
Geometric mean increase	**> 2**	**6.51**		**5.63**		**2.13**	
95% CI[3]		4.44-9.55		4.18-7.57		1.74-2.6	
Seroconversion rate[4]	**> 60%**	52/62	**84%**	62/62	**100%**	55/62	**89%**
95% CI[3]		72-92%		94-100%		78-95%	

YOA = years of age

[1] n/N: responders (n) as part of number of subjects of the (sub-) population (N); [2] GMT: geometric mean titer; [3] 95% CI: 95% confidence interval. [4] Seroprotection rate: proportion of subjects with a protective titer pre- or post-vaccination (titer ≥ 40); [5] Seroconversion rate: proportion of subjects with antibody increase from < 10 pre- vaccination to > 40 post-vaccination; [6] Significant increase: proportion of subjects with an antibody titer of ≥10 pre-vaccination and 4-fold antibody increase post-vaccination; [7] Seroconversion or significant increase: proportion of subjects with either seroconversion or significant increase

Table 9: CHMP Criteria Met by HI Assay – PPS

Viral Strain		A/H1N1	A/H3N2	B
≥ 65 Years	Seroprotection	+	+	+
	Geometric mean increase	+	+	+
	Seroconversion or significant increase	+	+	-

"+" CHMP criteria met: "-" CHMP criteria not met

Table 10: Overview of Solicited Reactions

	Number (%) of Subjects With Solicited Reactions
	FLUAD N=62
Any[1]	22 (35)
Local	19 (31)
Systemic	14 (23)

[1]Number and percent of subjects with one or more local and systemic reactions. Hence, number and percent of local and systemic reactions may not sum to number and percent of subjects with any reactions.

Table 11: Overview of Solicited Local Reactions (1-4 Days Post-Vaccination)

		Number (%) of Subjects With Injection Site Reactions
		FLUAD N=62
Ecchymosis (mm)	Any	2 (3)
	>50 mm	0
Erythema (mm)	Any	4 (6)
	>50 mm	0
Induration (mm)	Any	2 (3)
	>50 mm	0
Swelling (mm)	Any	0
	>50 mm	0
Pain	Any	15 (24)
	Severe	0

Note: The numbers (N) in the header is the total number of subjects with documented reactions. Two subjects were lost to follow up and have not provided any reactogenicity data.

Categorization of erythema, swelling, ecchymosis and induration: none (diameter <10mm), mild (diameter 10-25mm), moderate (diameter 26-50mm) and severe (diameter >50mm).

Table 12: Overview of Solicited Systemic Reactions (1-4 Days Post-Vaccination)

		Number (%) of Subjects With Systemic Reactions
		FLUAD N=62
Chills/Shivering	Any	2 (3)
	Severe	0
Malaise	Any	2 (3)
	Severe	0
Myalgia	Any	7 (11)
	Severe	0
Arthralgia	Any	4 (6)
	Severe	0
Headache	Any	5 (8)
	Severe	0
Sweating	Any	7 (11)
	Severe	0
Fatigue	Any	5 (8)
	Severe	0
Fever (≥ 38°C)	Any	0 / N=59
	Severe	

Note: The numbers (N) in the header is the total number of subjects with documented reactions. Two subjects were lost to follow up and have not provided any reactogenicity data. Further 3 subjects have not provided data for body temperature.

Table 13: Overview of Unsolicited AEs

	Number (%) of Subjects with Adverse Events
	FLUAD N=64
Any AEs	4 (6)
At least possibly related AEs	3 (5)
Serious AEs	0
At least possibly related SAEs	0
AEs leading to discontinuation	0
Death	0

Table 14: Serious Adverse Events by Preferred Term Sorted By System Organ Class

None reported.

Table 15: Unsolicited AEs Reported by ≥ 5% of Subjects by Preferred Term Sorted by System Organ Class

None reported.

Conclusion:

FLUAD 2010/2011 NH formulation is immunogenic and has a good tolerability and safety profile and complies with the CHMP criteria for approval of influenza vaccines.

Date of Clinical Trial Report: 20 JUL 2010