Sponsor: Novartis Vaccines and Diagnostics Srl.

Investigational Product: Fluad-H5N1 influenza vaccine containing 7.5 μg of the A/Vietnam/1194/2004-like (H5N1) influenza antigen

Indication: Prophylaxis: A/H5N1 avian influenza

Protocol Number: V87P3

Protocol Title: A Phase I, Single-centre, Exploratory Study to Evaluate Safety and Immunogenicity of Two Doses of Fluad®-H5N1 Influenza Vaccine in Adults Unprimed and Primed with MF59-adjuvanted or Non-adjuvanted H5N3 Influenza Vaccines.

Phase of Development: Phase I

Study Period:

Date of first enrolment: 14 May 07

Date of last visit: 19 Jan 08

Methodology:

The study investigated 2x7.5 vaccinations of Fluad-H5N1, 3 weeks apart on subjects aged 18 to 65 years which were either unprimed or primed with MF59-adjuvanted or Non-adjuvanted H5N3 Influenza Vaccines. Subjects were observed for 30 minutes after each vaccination for any immediate reactions. All subjects were instructed to complete a diary card to record solicited local (i.e., ecchymosis, erythema, induration, swelling and pain at injection site) and solicited systemic reactions (i.e., chills, malaise, myalgia, arthralgia, nausea, headache, sweating, fatigue) and axillary temperature starting on the day of vaccination (after 6 hours) and for each of the 6 days following each vaccination. All adverse events (AEs) were collected during visit 1 to visit 5. All AEs necessitating a physician's visit or consultation and/or leading to premature study discontinuation and all serious AEs (SAEs) were collected throughout the entire trial and data were reconciled at study termination visit (visit 6). Blood samples for immunogenicity assays were obtained on Day 1 (pre-vaccination), 8, 15, 22 (pre-vaccination), 43 and 202 (20 mL each). Blood samples for Cell-mediated Immunity (CMI) assays were obtained on Day 1 (pre-vaccination), 22 (prevaccination), 43 and 202 (80 mL each).
Antibody response was evaluated in serum by hemagglutination inhibition (HI), micro-neutralization (MN), and single-radial hemolysis (SRH) in all subjects.
CMI was assessed by evaluating frequency and functionality of circulating H5N1-specific T and ASC-precursor (i.e., memory B cells) by means of:

Intracellular staining/Fluorescence Activated Cell Sorting (ICS/FACS) analysis of circulating CD4+ T cells producing cytokines in response to a short in vitro-pulse with H5-peptide pools or the H5N1 protein.
Limiting Dilution Assay - Enzyme-Linked Immuno-Sorbent Assay (LDA-ELISA) assessment of numbers of circulating B lymphocytes producing H5N1- IgG specific antibodies after in vitro activation with polyclonal stimuli.

Number of Subjects (planned and analyzed):

Planned enrollment for this study was at least 60 subjects overall including 30 primed subjects (i.e., who previously received vaccine with an H5 antigen) and 30 non-primed subjects (i.e., with no prior vaccination with an H5 antigen) as control subjects. Control subjects were to be matched for age within an interval of ± 5 years.

The Full Analysis Set (FAS) and safety populations consisted of 24 primed (12 primed with MF-59 adjuvanted H5N3 vaccine and 12 primed with non-adjuvanted H5N3 vaccine), 30 unprimed, and 4 subjects with unclear priming status.

Study Centers:

One center in United Kingdom.

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

NCT00478816

Objectives:

Immunogenicity:

To evaluate the magnitude of antibody responses to two 0.5 mL intramuscular (IM) doses of an MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5 µg of H5N1 antigen, in subjects primed by previous vaccination with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve).

To determine the kinetics of the antibody response to MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine in subjects primed by previous vaccination with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine, and in subjects not primed with an H5 antigen (immunologically naïve).

To evaluate the breadth of immune responses induced by MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine with respect to a representative range of antigenically distinct H5N1 viruses (wild-type and attenuated) in subjects primed by previous vaccination with either MF59-adjuvanted or non- adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine.

To evaluate cell-mediated immunity (CMI) (frequency and functionality of Ag specific T and B lymphocytes) following two doses of MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5µg of H5N1 antigen, in subjects primed by previous vaccination with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve).

Safety:

To evaluate the safety of the administration of two 0.5 mL IM doses of an MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5 µg of H5N1 antigen, in subjects primed with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve).

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

Two 0.5 mL doses of MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) hemagglutinin (HA) subvirion influenza vaccine (Lot number: W52PO7H1B, Expiry date: August 2007), containing 7.5 µg of H5N1 antigen, administered 3 weeks apart, IM in the deltoid muscle, preferably of the non-dominant arm.

Duration of Study:

The actual subject enrollment interval was approximately 2 weeks. Duration of individual subject's participation was approximately 7 months. The total duration of the study was 25 months including a laboratory phase of 18 months after the last blood sample.

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

None

Statistical Methods:

There was no statistical null hypothesis associated with any of the immunogenicity objectives, which were analyzed descriptively. The statistical evaluations of the results were performed by Biostatistics and Clinical Data Management (BCDM) as predefined in the Analysis Plan.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Subjects eligible for enrollment into this study were male and female adult volunteers who were: 18 to 65 years of age, mentally competent, willing and able to give written informed consent prior to study entry; able to comply with all the study requirements in general good health as determined by medical history, physical examination, and clinical judgement of the investigator. Subjects in the primed group had received at least two previous doses of an H5N3 vaccine. Informed consent was obtained for all the subjects before enrollment in the study.

Criteria for Evaluation:

Immunogenicity:

To evaluate magnitude, kinetics and breadth of the antibody response after one and two doses of MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5 mg of H5N1 antigen, in subjects primed by previous vaccination with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve), with respect to the vaccine strain and representative range of antigenically distinct H5N1 viruses (wild type and attenuated) as assessed by hemagglutination inhibition (HI), micro-neutralization (MN), and possibly single-radial hemolysis (SRH). In the interpretation of HI and SRH immunogenicity results, CHMP criteria (CPMP/BWP/214/96) were considered, except for seroconversion or significant increase for HI measured at Health Protection Agency, UK, which were re-defined as follows: seroconversion was defined as negative pre-vaccination serum (< 8) and positive post-vaccination titer (≥ 32) (Note: initial cut-off for negative titers was <10 and for positive titers was ≥ 40); and significant increase in anti-body titer was defined as at least a 4-fold increase from non-negative pre-vaccination serum (≥ 8).

To evaluate cell-mediated immunity (CMI) (frequency and functionality of Ag specific T and B lymphocytes) following two doses of MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5mg of H5N1 antigen, in subjects primed by previous vaccination with either MF59- adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve) assessed by ICS/FACS analysis of circulating CD4+ T cells and LDA-ELISA assessment of circulating H5N1-specific T and ASC-precursor (i.e., memory B cells) cells.

Safety:

To evaluate the safety of the administration of two 0.5 mL IM doses of an MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) influenza vaccine, each containing 7.5 µg of H5N1 antigen, in subjects primed with either MF59-adjuvanted or non-adjuvanted A/Duck/Singapore/97 (H5N3 Clade 0) influenza vaccine and in subjects not primed with an H5 antigen (immunologically naïve).

Results:

Table 1: Overview of Subject Populations

	Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed	Unclear Priming
	N = 12	N = 12	N = 30	N = 4
Enrolled Population	12 (100%)	12 (100%)	30 (100%)	4 (100%)
Exposed Population	12 (100%)	12 (100%)	30 (100%)	4 (100%)
Safety Population	12 (100%)	12 (100%)	30 (100%)	4 (100%)
Full Analysis Population	12 (100%)	12 (100%)	30 (100%)	4 (100%)
Per Protocol Population	11 (92%)	12 (100%)	30 (100%)	3 (75%)

Table 2: Summary of Study Terminations - Enrolled Set

	Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed	Unclear Priming
	N = 12	N = 12	N = 30	N = 4
Completed	12 (100%)	11 (92%)	26 (87%)	0
Completed protocol	12 (100%)	11 (92%)	26 (87%)	0
Premature withdrawal	0	1 (8%)	4 (13%)	4 (100%)
Lost to follow-up	0	1 (8%)	4 (13%)	4 (100%)

Table 3: Demography and Baseline Characteristics by Priming Status – Enrolled Population

	Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed	Unclear Priming
	N = 12	**N = 12**	**N = 30**	**N = 4**
Age (Years):	35.1±6.3	36.8±6.8	38.2±10.5	48.3±15.6
Sex				
Male	5 (42%)	3 (25%)	13 (43%)	3 (75%)
Female	7 (58%)	9 (75%)	17 (57%)	1 (25%)
Race				
Asian	1 (8%)	0	10 (33%)	0
Caucasian	11 (92%)	12 (100%)	20 (67%)	4 (100%)
Weight (kg):	76.58±17.70	78.08±23.64	74.16±15.59	78.00±4.83
Height (cm):	167.8±9.7	168.2±12.2	168.6±10.5	178.0±3.6
Previous Influenza Vaccine				
Yes	5 (42%)	3 (25%)	12 (40%)	4 (100%)
No	7 (58%)	8 (67%)	18 (60%)	0
Unknown	0	1 (8%)	0	0
Meeting Entry Criteria				
Yes	12(100%)	12 (100%)	29 (97%)	4 (100%)
No	0	0	1(3%)	0

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

Table 4: Geometric Mean HI Titers and GMRs - GMR Calculated from Day 1 - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

		Adjuvanted H5N3	Non adjuvanted H5N3	Unprimed
Time Point	**Variable**	**N = 12**	**N = 12**	**N = 30**
Day 1	GMT (95% CI)	4.00 (4.00-4.00)	4.00 (4.00-4.00)	4.00 (4.00-4.00)
Day 8	GMT (95% CI)	128 (51-323) N=10	51 (19-135) N=9	5.15 (2.90-9.15) N=26
Day 8/Day 1	GMR (95% CI)	32 (13-81) N=10	13 (4.79-34) N=9	1.29 (0.73-2.29) N=26
Day 15	GMT (95% CI)	378 (134-1067) N=8	79 (31-200) N=10	7.29 (3.90-14) N=22
Day 15/Day 1	GMR (95% CI)	95 (33-267) N=8	20 (7.80-50) N=10	1.82 (0.97-3.40) N=22
Day 22	GMT (95% CI)	287 (129-641)	52 (23-117)	8.03 (4.84-13)
Day 22/Day 1	GMR (95% CI)	72 (32-160)	13 (5.86-29)	2.01 (1.21-3.34)
Day 43	GMT (95% CI)	181 (75-436)	44 (18-106)	26 (15-47) N=29
Day 43/Day 1	GMR (95% CI)	45 (19-109)	11 (4.57-26)	6.62 (3.76-12) N=29
Day 202	GMT (95% CI)	171 (88-332)	27 (13-53) N=11	7.28 (4.63-11) N=26
Day 202/Day 1	GMR (95% CI)	43 (22-83)	6.63 (3.31-13) N=11	1.82 (1.16-2.86) N=26

GMR - the geometric mean of the respective study day to day 1 titer ratio.

Table 5: Percentages of Subjects with Seroconversion or significant increase in HI Titers - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

	n/N[a] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	**N = 12**	**N = 30**
Day 8/Day 1	9 (90%) (55-100) N=10	5 (56%) (21-86) N=9	2 (8%) (1-25) N=26
Day 15/Day 1	8 (100%) (63-100) N=8	6 (60%) (26-88) N=10	3 (14%) (3-35) N=22
Day 22/Day 1	12 (100%) (74-100)	7 (58%) (28-85)	7 (23%) (10-42)
Day 43/Day 1	11 (92%) (62-100)	7 (58%) (28-85)	15 (52%) (33-71) N=29
Day 202/Day 1	11 (92%) (62-100)	7 (64%) (31-89) N=11	2 (8%) (1-25) N=26

[a] n/N - responders (n) [i.e., subjects who met the HI definition of seroconversion or significant increase] as part of the total number of subjects in the population (N); Seroconversion: negative pre-vaccination serum (i.e., HI titer < 8) and post-vaccination HI titer ≥ 32, Significant increase - at least a 4-fold increase in HI titers in subjects who were positive pre-vaccination (i.e., HI titer ≥ 8)

Table 6: Seroprotection (CHMP) by HI Assay - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

	n/N[a] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	**N = 12**	**N = 30**
Day 1	0 (0%) (0-26)	0 (0) (0-26)	0 (0%) (0-12)
Day 8	9 (90%) (55-100) N=10	5 (56%) (21-86) N=9	2 (8%) (1-25) N=26
Day 15	8 (100%) (63-100) N=8	6 (60%) (26-88) N=10	3 (14%) (3-35) N=22
Day 22	12 (100%) (74-100)	7 (58%) (28-85)	7 (23%) (10-42)
Day 43	11 (92%) (62-100)	7 (58%) (28-85)	15 (52%) (33-71) N=29
Day 202	11 (92%) (62-100)	7 (64%) (31-89) N=11	2 (8%) (1-25) N=26

Seroprotection - HI titers ≥ 32;

Table 7: Geometric Mean HI Titers and GMRs - GMR Calculated from Day 43 - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

		Adjuvanted H5N3	**Non Adjuvanted H5N3**	**Unprimed**
		N = 12	**N = 12**	**N = 29**
Day 43	GMT (95% CI)	181 (75-436)	44 (18-106)	26 (15-47)
Day 202	GMT (95% CI)	171 (88-332)	27 (13-53) N=11	7.28 (4.63-11) N=26
Day 202/Day 43	GMR (95% CI)	0.94 (0.52-1.71)	0.48 (0.26-0.90) N=11	0.30 (0.20-0.44) N=26

GMR - the geometric mean of the respective day 202 to day 43 titer ratio

Table 7: Geometric Mean HI Titers and GMRs - GMR Calculated from Day 1 - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

		Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed
Time Point	**Variable**	**N = 12**	**N = 12**	**N = 30**
Day 1	GMT (95% CI)	5.34 (4.08-6.99)	4.00 (3.06-5.24)	4.00 (3.37-4.74)
Day 8	GMT (95% CI)	119 (52-272) N=10	47 (20-112) N=9	4.77 (2.86-7.95) N=26
Day 8/Day 1	GMR (95% CI)	21 (8.85-50) N=10	12 (4.70-29) N=9	1.19 (0.70-2.04) N=26
Day 15	GMT (95% CI)	347 (152-793) N=8	111 (53-234) N=10	5.66 (3.43-9.32) N=22
Day 15/Day 1	GMR (95% CI)	56 (21-147) N=8	28 (12-66) N=10	1.41 (0.79-2.53) N=22
Day 22	GMT (95% CI)	279 (152-513)	99 (54-181)	6.43 (4.37-9.44)
Day 22/Day 1	GMR (95% CI)	52 (26-104)	25 (12-49)	1.61 (1.04-2.49
Day 43	GMT (95% CI)	242 (123-475)	66 (34-129)	7.93 (5.13-12) N=29
Day 43/Day 1	GMR (95% CI)	45 (21-96)	16 (7.79-35)	1.98 (1.22-3.21) N=29

GMR - the geometric mean of the respective study day to day 1 titer ratio.

Table 8: Percentages of Subjects with Seroconversion or significant increase in HI Titers - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

	n/N[a] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	**N = 12**	**N = 30**
Day 8/Day 1	8 (80%) (44-97) N=10	5 (56%) (21-86) N=9	2 (8%) (1-25) N=26
Day 15/Day 1	7 (88%) (47-100) N=8	8 (80%) (44-97) N=10	3 (14%) (3-35) N=22
Day 22/Day 1	11 (92%) (62-100)	10 (83%) (52-98)	4 (13%) (4-31)
Day 43/Day 1	11 (92%) (62-100)	9 (75%) (43-95)	6 (21%) (8-40) N=29

[a] n/N - responders (n) [i.e., subjects who met the HI definition of seroconversion or significant increase] as part of the total number of subjects in the population (N); Seroconversion - negative pre-vaccination serum (i.e., HI titer <8) and post-vaccination HI titer ≥ 32, Significant increase - at least a 4-fold increase in HI titers in subjects who were positive pre-vaccination (i.e., HI titer ≥ 8).

Table 9: Seroprotection (CHMP) by HI Assay - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

	n/N[a] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	N = 12	N = 30
Day 1	1 (8%) (0-38)	0 (0%) (0-26)	0 (0%) (0-12)
Day 8	9 (90%) (55-100) N=10	5 (56%) (21-86) N=9	2 (8%) (1-25) N=26
Day 15	8 (100%) (63-100) N=8	8 (80%) (44-97) N=10	3 (14%) (3-35) N=22
Day 22	12 (100%) (74-100)	10 (83%) (52-98)	4 (13%) (4-31)
Day 43	12 (100) (74-100)	9 (75%) (43-95)	6 (21%) (8-40) N=29

Seroprotection - HI titers ≥ 32;

Table 10: Geometric Mean SRH Areas and GMAs - GMA Calculated from Day 1 - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (Montomoli Laboratory)

		Adjuvanted H5N3 primed	Non Adjuvanted	Unprimed
Time Point	**Variable**	**N = 12**	**N = 12**	**N = 30**
Day 1	GMA (95% CI)	4.00 (3.03-5.28)	4.77 (3.61-6.29)	4.69 (3.93-5.59)
Day 8	GMA (95% CI)	45 (25-82) N=10	44 (23-83) N=9	10 (7.10-15) N=26
Day 8/Day 1	GMR (95% CI)	11 (5.91-21) N=10	8.73 (4.44-17) N=9	2.15 (1.44-3.20) N=26
Day 15	GMA (95% CI)	73 (42-128) N=8	56 (34-92) N=10	19 (14-27) N=22
Day 15/Day 1	GMR (95% CI)	18 (9.28-36) N=8	11 (6.18-21) N=10	3.86 (2.56-5.81) N=22
Day 22	GMA (95% CI)	70 (44-111)	55 (34-87)	18 (14-24)
Day 22/Day 1	GMR (95% CI)	18 (10-30)	11 (6.66-20)	3.86 (2.74-5.43)
Day 43	GMA (95% CI)	72 (55-95)	59 (45-78)	44 (36-52) N=29
Day 43/Day 1	GMR (95% CI)	18 (12-27)	12 (8.42-18)	9.69 (7.54-12) N=29
Day 202	GMA (95% CI)	66 (44-100)	40 (26-62) N=11	17 (13-23) N=26
Day 202/Day 1	GMR (95% CI)	17 (11-26)	8.29 (5.21-13) N=11	3.75 (2.78-5.08) N=26

GMR – the geometric mean of the respective study day to day 1 SRH area ratio.

Table 11: Percentages of Subjects with Seroconversion or significant increase in SRH Area - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (Montomoli Laboratory)

	n/N[b] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	N = 12	N = 30
Day 8/Day 1	9 (90%) (55-100) N=10	6 (67%) (30-93) N=9	6 (23%) (9-44) N=26
Day 15/Day 1	8 (100%) (63-100) N=8	8 (80%) (44-97) N=10	12 (55%) (32-76) N=22
Day 22/Day 1	12 (100%) (74-100)	10 (83%) (52-98)	15 (50%) (31-69)
Day 43/Day 1	12 (100%) (74-100)	11 (92%) (62-100)	26 (90%) (73-98) N=29
Day 202/Day 1	12 (100%) (74-100)	9 (82%) (48-98) N=11	13 (50%) (30-70) N=26

[b] n/N - responders (n) [i.e., subjects who met the SRH definition of seroconversion or significant increase] as part of the total number of subjects in the population (N); Seroconversion – negative pre-vaccination serum (i.e., SRH area $\leq 4mm^2$) and post-vaccination SRH area $\geq 25\ mm^2$; Significant increase – at least a 50% increase in SRH area in subjects who were positive pre-vaccination (i.e., SRH area $> 4mm^2$).

Table 12: Seroprotection (CHMP) by SRH Assay - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (Montomoli Laboratory)

	n/N[a] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3**	**Unprimed**
	N = 12	N = 12	N = 30
Day 1	0 (0%) (0-26)	1 (8%) (0-38)	1 (3%) (0.084-17)
Day 8	9 (90%) (55-100) N=10	7 (78%) (40-97) N=9	7 (27%) (12-48) N=26
Day 15	8 (100%) (63-100) N=8	9 (90%) (55-100) N=10	13 (59%) (36-79) N=22
Day 22	12 (100%) (74-100)	11 (92%) (62-100)	16 (53%) (34-72)
Day 43	12 (100%) (74-100)	12 (100%) (74-100)	27 (93%) (77-99) N=29
Day 202	12 (100%) (74-100)	10 (91%) (59-100) N=11	14 (54%) (33-73) N=26

Seroprotection – SRH area $\geq 25mm^2$

Table 13: Geometric Mean SRH Areas and GMAs - GMA Calculated from Day 1 - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (Montomoli Laboratory)

		Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed
Time Point	**Variable**	N = 12	N = 12	N = 30
Day 1	GMT (95% CI)	4.00 (3.17-5.04)	4.70 (3.73-5.93)	4.55 (3.93-5.27)
Day 8	GMT (95% CI)	44 (26-76) N=10	41 (24-73) N=9	8.94 (6.42-12) N=26
Day 8/Day 1	GMR (95% CI)	11 (6.20-20) N=10	8.34 (4.52-15) N=9	1.93 (1.34-2.76) N=26
Day 15	GMT (95% CI)	72 (44-117) N=8	54 (35-84) N=10	16 (12-21) N=22
Day 15/Day 1	GMR (95% CI)	18 (9.75-33) N=8	11 (6.44-19) N=10	3.26 (2.26-4.72) N=22
Day 22	GMT (95% CI)	68 (45-101)	52 (35-78)	16 (12-20)
Day 22/Day 1	GMR (95% CI)	17 (10-27)	11 (6.85-18)	3.48 (2.57-4.70)
Day 43	GMT (95% CI)	69 (53-90)	55 (42-72)	34 (28-40) N=29
Day 43/Day 1	GMR (95% CI)	17 (12-25)	12 (8.17-17)	7.67 (6.09-9.67) N=29
Day 202	GMT (95% CI)	61 (40-92)	36 (23-55) N=11	14 (11-18) N=26
Day 202/Day 1	GMR (95% CI)	15 (9.94-24)	7.51 (4.79-12) N=11	3.14 (2.34-4.20) N=26

GMR – the geometric mean of the respective study day to day 1 SRH area ratio.

Table 14: Percentages of Subjects with Seroconversion or significant increase in SRH Areas - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (Montomoli Laboratory)

	n/N[b] (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	N = 12	N = 30
Day 8/Day 1	9 (90%) (55-100) N=10	6 (67%) (30-93) N=9	4 (15%) (4-35) N=26
Day 15/Day 1	8 (100%) (63-100) N=8	8 (80%) (44-97) N=10	7 (32%) (14-55) N=22
Day 22/Day 1	12 (100%) (74-100)	10 (83%) (52-98)	9 (30%) (15-49)
Day 43/Day 1	12 (100%) (74-100)	11 (92%) (62-100)	25 (86%) (68-96) N=29
Day 202/Day 1	12 (100%) (74-100)	8 (73%) (39-94) N=11	10 (38%) (20-59) N=26

[b] n/N - responders (n) [i.e., subjects who met the HI definition of seroconversion or significant increase] as part of the total number of subjects in the population (N); Seroconversion – negative pre-vaccination serum (i.e., SRH titer $\leq 4mm^2$) and post-vaccination SRH area $\geq 25\ mm^2$; Significant increase – at least a 50% increase in SRH area in subjects who were positive pre-vaccination (i.e., SRH area $> 4mm^2$).

Table 15: Seroprotection (CHMP) by SRH Assay - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (Montomoli Laboratory)

	n/N (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	N = 12	N = 30
Day 1	0 (0%) (0-26)	1 (8%) (0-38)	0 (0%) (0-12)
Day 8	9 (90%) (55-100) N=10	7 (78%) (40-97) N=9	4 (15%) (4-35) N=26
Day 15	8 (100%) (63-100) N=8	9 (90%) (55-100) N=10	7 (32%) (14-55) N=22
Day 22	12 (100%) (74-100)	11 (92%) (62-100)	9 (30%) (15-49)
Day 43	12 (100%) (74-100)	12 (100%) (74-100)	26 (90%) (73-98) N=29
Day 202	12 (100%) (74-100)	9 (82%) (48-98) N=11	11 (42%) (23-63) N=26

Seroprotection – SRH area $\geq 25mm^2$.

Table 16: Geometric Mean MN Titers and GMRs - GMR Calculated From Day 1 - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

		Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed
Time Point	Variable	N = 12	N = 12	N = 30
Day 1	GMT (95% CI)	10 (10-10)	10 (10-10)	10 (10-10)
Day 8	GMT (95% CI)	407 (168-988) N=10	151 (59-385) N=9	11 (6.33-19) N=26
Day 8/Day 1	GMR (95% CI)	41 (17-99) N=10	15 (5.93-38) N=9	1.10 (0.63-1.90) N=26
Day 15	GMT (95% CI)	1754 (802-3835) N=8	473 (235-952) N=10	12 (7.75-20) N=22
Day 15/Day 1	GMR (95% CI)	175 (80-384) N=8	47 (23-95) N=10	1.24 (0.78-1.99) N=22
Day 22	GMT (95% CI)	1067 (574-1986)	324 (174-603)	12 (8.01-18)
Day 22/Day 1	GMR (95% CI)	107 (57-199)	32 (17-60)	1.19 (0.80-1.76)
Day 43	GMT (95% CI)	938 (477-1847)	241 (123-475)	33 (22-51) N=29
Day 43/Day 1	GMR (95% CI)	94 (48-185)	24 (12-47)	3.32 (2.15-5.14) N=29
Day 202	GMT (95% CI)	215 (123-375)	34 (19-60) N=11	21 (14-30) N=26
Day 202/Day 1	GMR (95% C	22 (12-38)	3.36 (1.88-6.02) N=11	2.08 (1.43-3.04) N=26

GMR – the geometric mean of the respective study day to day 1 titer ratio.

Table 17: Subjects With MN Titers ≥ 1:40 - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

	n/N (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	N = 12	N = 30
Day 1	0 (0%) (0-26)	0 (0%) (0-26)	0 (0%) (0-12)
Day 8	9 (90%) (55-100) N=10	5 (56%) (21-86) N=9	1 (4%) (0.097-20) N=26
Day 15	8 (100%) (63-100) N=8	10 (100%) (69-100) N=10	2 (9%) (1-29) N=22
Day 22	12 (100%) (74-100)	11 (92%) (62-100)	3 (10%) (2-27)
Day 43	12 (100%) (74-100)	10 (83%) (52-98)	16 (55%) (36-74) N=29
Day 202	11 (92%) (62-100)	5 (45%) (17-77) N=11	3 (12%) (2-30) N=26

Table 18: Subjects with Four-fold Increase in MN Titer above Baseline - FAS - A/Vietnam/1194/2004 NIBRG-14 (clade 1) strain (HPA Laboratory)

	n/N (%) and 95%		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3**	**Unprimed**
	N = 12	N = 12	N = 30
Day 8/Day 1	9 (90%) (55-100) N=10	5 (56%) (21-86) N=9	1 (4%) (0.097-20) N=26
Day 15/Day 1	8 (100%) (63-100) N=8	10 (100%) (69-100) N=10	2 (9%) (1-29) N=22
Day 22/Day 1	12 (100%) (74-100)	11 (92%) (62-100)	3 (10%) (2-27)
Day 43/Day 1	12 (100%) (74-100)	10 (83%) (52-98)	16 (55%) (36-74) N=29
Day 202/Day 1	11 (92%) (62-100)	5 (45%) (17-77) N=11	3 (12%) (2-30) N=26

Table 19: Geometric Mean MN Titers and GMRs - GMR Calculated from Day 1 - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

		Adjuvanted H5N3 primed	Non Adjuvanted H5N3 primed	Unprimed
Time Point	**Variable**	**N=12**	**N=12**	**N=30**
Day 1	GMT (95% CI)	10 (10-11)	10 (9.74-10)	10 (9.84-10)
Day 8	GMT (95% CI)	495 (240-1022) N=10	214 (99-458) N=9	10 (6.38-16) N=26
Day 8/Day 1	GMR (95% CI)	48 (23-99) N=10	21 (9.94-46) N=9	1.00 (0.64-1.57) N=26
Day 15	GMT (95% CI)	2128 (1052-4307)	538 (286-1010) N=10	12 (8.13-19) N=22
Day 15/Day 1	GMR (95% CI)	204 (102-409) N=8	54 (29-100) N=10	1.24 (0.82-1.89) N=22
Day 22	GMT (95% CI)	1372 (818-2300)	409 (244-686)	12 (8.29-16)
Day 22/Day 1	GMR (95% CI)	133 (80-222)	41 (25-68)	1.15 (0.83-1.59)
Day 43	GMT (95% CI)	1046 (602-1817)	289 (167-502)	16 (11-23) N=29
Day 43/Day 1	GMR (95% CI)	102 (59-176)	29 (17-50)	1.59 (1.12-2.26) N=29

GMR – the geometric mean of the respective study day to day 1 titer ratio.

Table 20: Subjects With MN Titers ≥ 1:40 - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

	n/N (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	**N = 12**	**N = 3**
Day 1	0 (0%) (0-26)	0 (0%) (0-26)	0 (0%) (0-12)
Day 8	9 (90%) (55-100) N=10	7 (78%) (40-97) N=9	0 (0%) (0-13) N=26
Day 15	8 (100%) (63-100) N=8	10 (100%) (69-100) N=10	2 (9%) (1-29) N=22
Day 22	12 (100%) (74-100)	11 (92%) (62-100)	2 (7%) (1-22)
Day 43	12 (100%) (74-100)	12 (100%) (74-100)	3 (10%) (2-27) N=29

Table 21: Subjects with Four-fold Increase in MN Titer above Baseline - FAS – A/turkey/Turkey/1/05 NIBRG-23 (clade 2.2) strain (HPA Laboratory)

	n/N (%) and 95% CI		
	Adjuvanted H5N3 primed	**Non Adjuvanted H5N3 primed**	**Unprimed**
	N = 12	**N = 12**	**N**
Day 8/Day 1	9 (90%) (55-100) N=10	7 (78%) (40-97) N=9	0 (0%) (0-13) N=26
Day 15/Day 1	8 (100%) (63-100) N=8	10 (100%) (69-100) N=10	2 (9%) (1-29) N=22
Day 22/Day 1	12 (100%) (74-100)	11 (92%) (62-100)	2 (7%) (1-22)
Day 43/Day 1	12 (100%) (74-100)	12 (100%) (74-100)	3 (10%) (2-27) N=29

Table 22: Overview of Subjects with Solicited Reactions, by Injection - Safety Set

		Number (%) of Subjects With Solicited Reactions				
		Adjuvanted H5N3	**Non Adjuvanted**	**Unprimed**	**Unclear Priming**	**Total**
		N = 12	N = 12	N = 30	N = 4	N = 58
Injection 1	Any	12 (100)	11 (92)	21 (70)	3 (75)	47 (81)
	Local	11 (92)	9 (75)	15 (50)	3 (75)	38 (66)
	Systemic	9 (75)	8 (67)	15 (50)	1 (25)	33 (57)
	Other	6 (50)	3 (25)	6 (20)	0	15 (26)
Injection 2	Any	10 (83)	9 (75)	16 (53)	1 (25)	36 (62)
	Local	7 (58)	8 (67)	14 (47)	1 (25)	30 (52)
	Systemic	6(50)	2 (17)	7 (23)	1 (25)	16 (28)
	Other	3 (25)	3 (25)	4 (13)	1 (25)	11 (19)

Table 23 Solicited Local Reactions by Severity, by Injection

			Number (%) of Subjects with Local Reactions				
			Fluad-H5N1				
			Adjuvanted H5N3 primed N=12	Non Adjuvanted H5N3 primed N=12	Unprimed N=30	Unclear Priming N=4	Total N=58
Injection 1	Ecchymosis (mm)	Any	2 (17)	0	2 (7)	1 (25)	5 (9)
		> 50 mm	0	0	0	0	0
	Erythema (mm)	Any	2 (17)	1 (8)	3 (10)	0	6 (10)
		> 50 mm	0	0	0	0	0
	Induration (mm)	Any	0	0	3 (10)	0	3 (5)
		> 50 mm	0	0	0	0	0
	Swelling (mm)	Any	1 (8)	0	2 (7)	0	3 (5)
		> 50 mm	0	0	0	0	0
	Pain	Any	10 (83)	9 (75)	14 (47)	3 (75)	36 (62)
		Severe	0	0	0	0	0
Injection 2	Ecchymosis (mm)	Any	0	0	0	0	0
		> 50 mm	0	0	0	0	0
	Erythema (mm)	Any	1 (8)	0	0	0	1 (2)
		> 50 mm	0	0	0	0	0
	Induration (mm)	Any	0	2 (17)	3 (10)	1 (25)	6 (10)
		> 50 mm	0	0	0	0	0
	Swelling (mm)	Any	1 (8)	0	1 (3)	0	2 (3)
		> 50 mm	0	0	0	0	0
	Pain	Any	7 (58)	7 (58)	14 (47)	1 (25)	29 (50)
		Severe	0	0	0	0	0

Table 24 Solicited Systemic Reactions by Severity, by Injection

		Number (%) of Subjects With Systemic Reactions				
				Fluad-		
		Adjuvanted H5N3 primed N = 12	Non Adjuvanted H5N3 primed N = 12	Unprimed N = 30	Unclear Priming N = 4	Total N = 58
Injection 1						
Chills	Any	0	1 (8)	0	0	1 (2)
	Severe	0	0	0	0	0
Malaise	Any	2 (17)	1 (8)	1 (3)	1 (25)	5 (9)
	Severe	0	0	0	0	0
Myalgia	Any	3 (25)	7 (58)	6 (20)	1 (25)	17 (29)
	Severe	0	0	0	0	0
Arthralgia	Any	2 (17)	2 (17)	0	1 (25)	5 (9)
	Severe	0	0	0	0	0
Nausea	Any	0	0	4 (13)	0	4 (7)
	Severe	0	0	0	0	0
Headache	Any	8 (67)	3 (25)	9 (30)	0	20 (34)
	Severe	0	0	0	0	0
Sweating	Any	1 (8)	1 (8)	0	1 (25)	3 (5)
	Severe	0	0	0	0	0
Fatigue	Any	3 (25)	0	2 (7)	0	5 (9)
	Severe	0	0	0	0	0
Other Indicators of Reactogenicity						
Temp. (C)	<38 C	12 (100)	12 (100)	30 (100)	4 (100)	58 (100)
	≥ 40 C	0	0	0	0	0
Stay Home:	Yes	0	0	0	0	0
Analge. Antipyr. Med.Used:	Yes	6 (50)	3 (25)	6 (20)	0	15 (26)
Injection 2						
Chills	Any	1 (8)	1 (8)	1 (3)	0	3 (5)
	Severe	1 (8)	0	0	0	1 (2)
Malaise	Any	1 (8)	1 (8)	3 (10)	1 (25)	6 (10)
	Severe	1 (8)	0	0	0	1 (2)
Myalgia	Any	2 (17)	2 (17)	5 (17)	1 (25)	10 (17)
	Severe	1 (8)	0	0	0	1 (2)
Arthralgia	Any	2 (17)	1 (8)	0	1 (25)	4 (7)

		Number (%) of Subjects With Systemic Reactions				
	Severe	1 (8)	0	0	0	1 (2)
Nausea	Any	1 (8)	0	1 (3)	0	2 (3)
	Severe	1 (8)	0	0	0	1 (2)
Headache	Any	5 (42)	2 (17)	3 (10)	0	10 (17)
	Severe	1 (8)	0	0	0	1 (2)
Sweating	Any	1 (8)	2 (17)	1 (3)	1 (25)	5 (9)
	Severe	1 (8)	0	0	0	1 (2)
Fatigue	Any	3 (25)	0	2 (7)	0	5 (9)
	Severe	1 (8)	0	0	0	1 (2)
Other Indicators of Reactogenicity						
Temp. (C)	<38 C	11 (92)	12 (100)	29 (97)	4 (100)	56 (97)
	≥ 40 C	0	0	0	0	0
Stay Home:	Yes	1 (8)	0	1/29 (3)	0	2/57 (4)
Analge. Antipyr. Med.Used:	Yes	3 (25)	3 (25)	3 (10)	1 (25)	10 (17)

Table 25: Overview of Other AEs by Period

		Number (%) of Subjects with Adverse Events				
		Adjuvanted H5N3 primed	**Non Adjuvantec H5N3 primed**	**Unprimed**	**Unclear Priming**	**Total**
Day 1 to Day 43		N=12	N=12	N=30	N=4	N=58
	Any AEs	4 (33)	2 (17)	7 (23)	1 (25)	14 (24)
	At least possibly related	1 (8)	2 (17)	3 (10)	1 (25)	7 (12)
Day 43 to Day 202		N=12	N=11	N=26	N=0	N=49
	Any AEs	3 (25)	3 (27)	4 (15)		10 (20)
	At least possibly related	0	0	0		0

Table 26: Overview of Other Specific AEs by Period

		Number (%) of Subjects with Adverse Events				
		Adjuvanted H5N3 primed	**Non Adjuvanted H5N3**	**Unprimed**	**Unclear Priming**	**Total**
Day 1 to Day 202		**N = 12**	**N = 12**	**N = 30**	**N = 4**	**N= 58**
	Any AEs	2 (17)	2 (17)	2 (7)	0	6 (10)
	At least possibly related AEs	0	0	0	0	0
Day 1 to Day 43		**N=12**	**N=12**	**N=30**	**N=4**	**N=58**
	Any AEs	1 (8)	0	0	0	1 (2)
	At least possibly related AEs	0	0	0	0	0
Day 43 to Day 202		**N=12**	**N=11**	**N=26**	**N=0**	**N=49**
	Any AEs	1 (8)	2 (18)	2 (8)		5 (10)
	At least possibly related AEs	0	0	0		0

Table 27: Number (Percentages) of Subjects with Serious Adverse Events by Preferred Term, sorted by System Organ Class

	Number (%) of Subjects with Adverse Events			
MedDRA System Organ Class MedDRA Preferred Term	**Adjuvanted H5N3 primed**	**Non Adjuvantec H5N3 primed**	**Unprimed**	**Unclear Priming**
Any Serious Adverse Event	0	1 (8%)	1 (3%)	0
Hepato-Biliary Disorders				
Cholelithiasis	0	1 (8%)	0	0
Injury & Poisoning				
Foot Fracture	0	0	1 (3%)	0

Table 28: Number (Percentages) of Subjects with Unsolicited Adverse Events Reported in > 5 % of Subjects by Preferred Term sorted by System Organ Class

	Number (%) of Subjects With Solicited Reactions				
MedDRA System Organ Class MedDRA Preferred Term	**Adjuvanted H5N3 primed**	**Non Adjuvanted H5N3 primed**	**Unprimed**	**Unclear Priming**	**Total**
	N = 12	N = 12	N = 30	N = 4	N = 58
Gastrointestinal Disorders					
Abdominal Pain	0	1 (8%)	0	0	1 (2%)
Nausea	0	1 (8%)	0	0	1 (2%)
Hepato-Biliary Disorders					
Cholelithiasis	0	1 (8%)	0	0	1 (2%)
Resp., Thoracic & Mediastinal					
Cough	1 (8%)	0	0	0	1 (2%)
Infections & Infestations					
Eczema Infected	1 (8%)	0	0	0	1 (2%)
Nasopharyngitis	1 (8%)	0	0	0	1 (2%)
Injury & Poisoning					
Foot Fracture	0	0	1 (3%)	0	1 (2%)
Endocrine Disorders					
Hyperthyroidism	0	0	1 (3%)	0	1 (2%)

Conclusion:

All CHMP criteria were met by Fluad-H5N1 vaccine after the first and second vaccination in the adjuvanted H5N3 primed group for all strains including the wild type H5N3 duck/Singapore/97 strain by both HI and SRH assays. In the non-adjuvanted primed group, at least two CHMP criteria were met by Fluad-H5N1 vaccine after the first and second vaccinations for all strains including the wild type H5N3 duck/Singapore/97 strain by both HI and SRH assays. In the unprimed group, either no CHMP criterion was met or a maximum of two CHMP criteria were met by Fluad-H5N1 vaccine after the first and second vaccinations for all strains including the wild type H5N3 duck/Singapore/97 strain, except in reassortant Vietnam and turkey strains when all the three CHMP criteria were met after the second vaccination when evaluated by SRH assay.

In the adjuvanted H5N3 primed and non-adjuvanted primed groups, very high antibody titers were seen at day 8 which reached a plateau at day 15 followed by slight decrease at the following visits. Whereas in the unprimed group, titers increased gradually and constantly up to the last visit but the titers remained low in comparison to that seen in the primed groups. Thus, priming enhanced the ability of cross-reactivity as demonstrated by high antibody titers across all the strains in the primed groups.

H5-specific and H5N1-specific CD4 T-cells and the frequency of H5N1-IgG memory B-cells were only analyzed descriptively since the number of subjects was too low to detect significant differences between vaccination groups or doses.

H5-specific and H5N1-specific CD4 T-cells increased substantially over baseline in all the groups after any vaccination.

The frequency of H5N1-IgG memory B-cells increased above baseline in all the groups after any vaccination and more pronounced increase was seen in the adjuvanted H5N3 primed group than the non adjuvanted H5N3 primed and unprimed groups.

Two injections of Fluad-H5N1 vaccine were safe and well tolerated across all the groups. Solicited local and systemic reactions after vaccination were mostly mild or moderate in severity. No possibly/probably related deaths or SAEs were reported.

Date of Clinical Trial Report: **20 FEB 09**