Sponsor: Novartis Vaccines and Diagnostics Srl.

Investigational Product: MF59-eH5N1

Indication: Prophylaxis of A/H5N1 avian influenza

Protocol Number: V87P2

Protocol Title: A Phase II, Randomized, Controlled, Observer-blind, Single-Center Study to Evaluate Safety and Immunogenicity of Two Doses, Administered Three Weeks Apart, and a Six Month Booster Dose of Two FLUAD-like (Surface Antigen Adjuvanted with MF59C.1) Influenza Vaccines Containing 7.5 µg or 15 µg of A/H5N1 Influenza Antigen and of a Nonadjuvanted Influenza Vaccine Containing 15 µg of A/H5N1 Influenza Antigen, in Adults

Phase of Development: Phase II

Study Period:

Date of first enrolment: 27 NOV 06

Date of last visit: 10 JAN 08

Methodology:

Subjects were enrolled and randomly assigned in a 1:1:1 ratio to receive two 0.5 mL vaccinations, 3 weeks apart followed by a booster vaccination of the same vaccine 6 months after the second vaccination:

- MF59-eH5N1 7.5 (i.e., 7.5 µg/vaccination MF59-adjuvanted H5N1 HA)
- MF59-eH5N1 15 (i.e., 15 µg/vaccination MF59-adjuvanted H5N1 HA)
- Non-adjuvanted 15 (i.e., 15 µg/vaccination non-adjuvanted H5N1 HA).

Number of Subjects (planned and analyzed):

At least 45 subjects were planned for this study but the actual number of subjects enrolled was 40. Of these, 14 were assigned to receive the MF59-eH5N1 influenza vaccine containing 7.5 µg of A/H5N1 influenza antigen, 13 to receive the MF59-eH5N1 influenza vaccine containing 15 µg of A/H5N1 influenza antigen, and 13 to receive the non-adjuvanted influenza vaccine containing 15 µg of A/H5N1 influenza antigen.

Study Centers:

One study center in Italy.

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

PMID: 19237568; NCT00382187.

Objectives:

Immunogenicity Objectives
To evaluate cell-mediated immunity (frequency and functionality of Ag-specific T and B lymphocytes) and immunogenicity (as measured by hemagglutination inhibition [HI], microneutralization [MN] and single radial hemolysis [SRH] assay) after 1, 2 and 3, 0.5 ml intramuscular (IM) injections of 2 MF59-eH5N1 influenza vaccines containing either 7.5 μg or 15 μg of A/H5N1 influenza antigen and of a non-adjuvanted influenza vaccine containing 15 μg of A/H5N1 influenza antigen.
Safety Objectives
To evaluate the safety of the administration of 3, 0.5 mL IM injections of 2 MF59-eH5N1 influenza vaccines containing either 7.5 μg or 15 μg of A/H5N1 influenza antigen and of a non-adjuvanted influenza vaccine containing 15 μg of A/H5N1 influenza antigen.

Test Product, Dose, Mode of Administration, Lot Number:
MF59-eH5N1 7.5 (Lot number: W52P07H1A, expiry date August 2007) contained 7.5 μg of MF59-eH5N1 influenza vaccine and MF59-eH5N1 15 (Lot number: W52P06H1A, expiry date August 2007) contained 15 μg of MF59-eH5N1 influenza vaccine.
The 2 formulations contained either 7.5 μg or 15 μg HA per vaccination of an A/Vietnam/1194/2004-like (H5N1) MF59-adjuvanted vaccine.
Two 0.5 ml IM injections of either vaccine MF59-eH5N1 7.5 or MF59-eH5N1 15 were administered 3 weeks apart in the deltoid muscle of the (preferably) non-dominant arm. A booster injection of the same vaccine was administered 6 months after the second injection.

Duration of Study:
Approximately 4 weeks for enrollment and 381 Days of follow-up after first injection per subject.

Reference Therapy, Dose, Mode of Administration, Lot Number:
Two 0.5 mL IM injections of non-adjuvanted 15 μg (H5N1) vaccine were administered at the same dose. A booster injection of the same vaccine was administered 6 months after the second injection.
Non-adjuvanted 15 (Lot number: W51P85H1, expiry date August 2007) contained 15 μg HA of A/Vietnam/1194/2004-like (H5N1) non-adjuvanted influenza vaccine.

Statistical Methods:

All analyses were done descriptively and this study was not powered to detect significant differences between vaccines or injections.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion Criteria
Individuals eligible for enrollment were male and female adult volunteers who were:

1. 18 to 60 years of age, 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 general 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 non metastatic 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 kanamicin 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 corticosteroid or cancer chemotherapy/radiotherapy) within 60 Days prior to enrollment and for the full length of the study
   - receipt of immunostimulants

- receipt of parenteral immunoglobulin preparation, blood products and/or plasma derivates within 3 months prior to enrollment
- suspected or known human immunodeficiency virus (HIV) infection or HIV-related disease;

5. Women who were pregnant, or women able to bear children but not willing to practice acceptable contraception for the duration of the trial (382 Days);
6. Within 4 weeks prior to enrollment they had received:
   - another vaccine
   - any investigational agent;
7. Within 7 Days prior to enrollment, they had experienced:
   - any acute diseaseinfections requiring systemic antibiotic or antiviral therapy (chronic antibiotic therapy for urinary tract prophylaxis was acceptable);
8. Within 3 Days prior to enrollment, they had experienced:
   - fever (i.e., axillary temperature ≥ 38°C);
9. They were taking part in another clinical study;
10. They had surgery planned during the study period;
11. They had any anemia, hypotension or any other medical condition which at the investigator's discretion could constitute a contraindication to the blood sampling foreseen by the study protocol;
12. They had any condition, which, in the opinion of the investigator, could have interfered with the evaluation of the study objective.

Criteria for Evaluation:

Immunogenicity: To evaluate immune response and persistence (both serology and cell-mediated immunity) after primary and booster vaccinations of MF59-eH5N1 influenza vaccines containing either 7.5 μg or 15 μg of H5N1 influenza antigen and a non-adjuvanted influenza vaccine containing 15 μg of H5N1 influenza antigen.

In the interpretation of HI and SRH immunogenicity results, Committee for Medicinal Products for Human Use (CHMP) Nfg criteria (CPMP/BWP/214/96) were taken into consideration.

Cell-mediated Immunity (CMI) analysis: Priming of H5-specific CD4 T lymphocytes was analyzed by polychromatic flow cytometry [Darrah, P.A., *et al.* Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. *Nat Med* **13**, 843- 850 (2007)] after in vitro stimulation with a library of peptides (18-mers overlapping by 10) spanning the whole H5 A/Vietnam/1194/2004 protein, with H5N1, the subunit antigen preparation contained in the vaccine or with further peptide pools or proteins.

Priming of H5N1-specific memory B lymphocytes was analyzed by an enzyme-linked immunosorbent assay (ELISA)-coupled limiting dilution assay described elsewhere [Bernasconi, N.L., Traggiai, E. & Lanzavecchia, A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* **298**, 2199-2202 (2002)].

Safety: The administration of three 0.5 mL IM injections of 2 MF59-eH5N1 influenza vaccines containing either 7.5 μg or 15 μg of A/H5N1 influenza antigen and of a non-adjuvanted influenza vaccine containing 15 μg of A/H5N1 influenza antigen was assessed in accordance with available safety data on influenza vaccines.

Results:

Table 1. Time and Events

Study Day	Day 1	Day 22	Day 43	Day 130	Day 202	Day 223	Day 382
Estimated Study Day and Window	**1**	**22 (-1/+3)**	**43 (-1/+3)**	**130 (-3/+4)**	**202 (-10/+28)**	**223 (-1/+3)**	**382 (-4/+4)**
Extended Window							
Visit No. →	1	2	3	3.1	4	5	6
Informed Consent	X			X			
Inclusion/Exclusion	X	X			X		
Medical History	X						
Physical exam/assessment	X	X	X		X	X	X
Blood draw (80 mL each)	X	X	X		X	X	X
Blood draw (30mL)				X			
Injection							
Diary Card	X	X	X		X	X	
Assess Local/Systemic Reactions	X	X	X		X	X	
Assess AEs	X	X	X		X	X	X
Concomitant medications	X	X	X		X	X	X

AEs: Adverse Events

Table 2. Summary of Study Terminations - Enrolled Population

	Number of Subjects (% of Total)			
Primary Withdrawal Reason	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**	**Total**
Total Number Of Subjects Enrolled	14	13	13	40
Completed	12 (86%)	10 (77%)	11 (85%)	33 (83%)
Premature Withdrawal	2 (14%)	3 (23%)	2 (15%)	7 (18%)
Withdrawal Of Consent	1 (7%)	2 (15%)	2 (15%)	5 (13%)
Lost To Follow-Up	1 (7%)	1 (8%)	0	2 (5%)

Table 3. Population Analyzed

Population Total:	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	Total N=40
Enrolled	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Full Analysis Set Visit 1	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Full Analysis Set Visit 2	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Full Analysis Set Visit 3	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Full Analysis Set Visit 3.1	14 (100%)	12 (92%)	13 (100%)	39 (98%)
Full Analysis Set Visit 4	13 (93%)	12 (92%)	12 (92%)	37 (93%)
Full Analysis Set Visit 5	13 (93%)	12 (92%)	11 (85%)	36 (90%)
Full Analysis Set Visit 6	12 (86%)	10 (77%)	11 (85%)	33 (83%)
Per Protocol Set Booster	11 (79%)	8 (62%)	8 (62%)	27 (68%)
Safety Reactogenicity Imm. 1	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Safety Reactogenicity Imm. 2	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Safety Reactogenicity Imm. 3	13 (93%)	12 (92%)	11 (85%)	36 (90%)
Safety Adverse Events Imm. 1	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Safety Adverse Events Imm. 2	14 (100%)	13 (100%)	13 (100%)	40 (100%)
Safety Adverse Events Imm. 3	13 (93%)	12 (92%)	11 (85%)	36 (90%)

Table 4. Demography and Baseline Characteristics – Full analysis set (FAS)

	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	Total N=40
Age (Years)	34.7±7.5	33.8±7.2	35.8±8.6	34.8±7.6
Sex :				
Male	8 (57%)	8 (62%)	9 (69%)	25 (63%)
Female	6 (43%)	5 (38%)	4 (31%)	15 (38%)
Race :				
Asian	0	0	1 (8%)	1 (3%)
Caucasian	14 (100%)	13 (100%)	12 (92%)	39 (98%)
Weight (kg) :	67.9±13.4	64.1±9.7	69.5±10.6	67.2±11.4
Height (cm) :	173.1±8.6	171.2±13.0	173.5±6.8	172.6±9.6
Previous Influenza Vaccine?				
Yes	5 (36%)	1 (8%)	5 (38%)	11 (28%)
No	9 (64%)	12 (92%)	8 (62%)	29 (73%)
Meeting Entry Criteria?				
Yes	9 (64%)	12 (92%)	9 (69%)	30 (75%)
No	5 (36%)	1 (8%)	4 (31%)	10 (25%)

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

Table 5. IL-2$^+$/IFN-γ- H5-Specific CD4 T Cells[a] (Peptide Pool: H5 Pool of 70 - Visit 1-6 Fresh Samples) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	71	53	61
	Median	48	46	49
	SD	74	44	63
	Min, max	0,242	0,149	0,188
Day 22	N			
	Mean	368	342	116
	Median	284	260	71
	SD	366	321	127
	Min, max	21,1338	32,1189	0,429
Day 43	N			
	Mean	420	259	124
	Median	309	145	84
	SD	387	248	139
	Min, max	71,1222	0,790	0,519
Day 130	N		12	
	Mean	197	182	106
	Median	156	169	80
	SD	130	97	93
	Min, max	81,476	10,312	0,316
Day 202	N	13	12	12
	Mean	208	225	123
	Median	169	177	112
	SD	174	188	98
	Min, max	52,683	13,590	2,362
Day 223	N	13	12	11
	Mean	567	647	165
	Median	405	553	86
	SD	562	380	138
	Min, max	120,2160	240,1474	24,378
Day 382	N	12	10	11
	Mean	303	223	99
	Median	194	170	68
	SD	338	181	85
	Min, max	39,1150	39,589	16,290

[a] Numbers of IL-2$^+$/IFN-γ-H5-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 6. IL-2$^+$/IFN-γ$^+$ H5-Specific CD4 T Cells[a] (Peptide Pool: H5 Pool of 70 - Visit 1-6 Fresh Samples) – FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	51	18	28
	Median	27	12	22
	SD	60	19	24
	Min, max	0,183	0,59	0,81
Day 22	N			
	Mean	99	99	62
	Median	57	75	45
	SD	95	94	53
	Min, max	0,299	8.5,339	0,188
Day 43	N			
	Mean	107	96	70
	Median	74	72	38
	SD	97	92	82
	Min, max	27,399	0,293	0,297
Day 130	N		12	
	Mean	122	90	72
	Median	111	77	32
	SD	95	75	68
	Min, max	0,248	12,308	0,182
Day 202	N	13	12	12
	Mean	127	96	70
	Median	87	84	51
	SD	106	67	59
	Min, max	0,381	6.8,231	18,206
Day 223	N	13	12	11
	Mean	205	250	114
	Median	153	198	127
	SD	208	181	80
	Min, max	27,833	67,741	23,284
Day 382	N	12	10	11
	Mean	161	92	61
	Median	97	81	54
	SD	168	63	45
	Min, max	18,595	8.3,224	21,178

[a] Numbers of IL-2$^+$/IFN-γ^+ H5-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 7. IL-2-/IFN-γ^+ H5-Specific CD4 T Cells[a] (Peptide Pool: H5 Pool of 70 - Visit 1-6 Fresh Samples) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	38	27	44
	Median	29	21	32
	SD	32	23	35
	Min, max	0,90	6,95	0,127
Day 22	N			
	Mean	57	33	67
	Median	47	32	21
	SD	58	26	159
	Min, max	0,244	2.4,87	0,593
Day 43	N			
	Mean	51	24	34
	Median	31	21	25
	SD	50	20	27
	Min, max	0,160	0,63	0,92
Day 130	N		12	
	Mean	44	15	29
	Median	37	10	28
	SD	38	20	22
	Min, max	0,152	0,69	0,69
Day 202	N	13	12	12
	Mean	36	15	27
	Median	16	16	14
	SD	48	14	23
	Min, max	0,163	0,41	0,57
Day 223	N	13	12	11
	Mean	60	50	29
	Median	42	47	19
	SD	52	33	25
	Min, max	0,142	9.61,116	8.93,78
Day 382	N	12	10	11
	Mean	42	10	7.91

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
	Median	36	9.5	3.4
	SD	33	8.31	12
	Min, max	8.4,105	0,24	0,32

[a] Numbers of IL-2-/IFN- γ^+ H5-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 8. CK^+ H5-Specific CD4 T Cells[a] [excluding IL2-/IL13-/INF-γ^-/TNF-α^+] (Peptide Pool: H5 Pool of 70 - Visit 1-6 Fresh Samples) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	188	122	179
	Median	162	123	175
	SD	146	75	98
	Min, max	10,544	33,277	23,399
Day 22	N			
	Mean	536	477	250
	Median	381	330	191
	SD	450	406	215
	Min, max	50,1710	43,1451	56,677
Day 43	N			
	Mean	592	384	232
	Median	489	359	170
	SD	476	328	233
	Min, max	123,1788	4,948	12,899
Day 130	N		12	
	Mean	363	287	208
	Median	278	264	193
	SD	216	146	156
	Min, max	113,748	116,615	14,550
Day 202	N	13	12	12
	Mean	372	355	224
	Median	293	282	167
	SD	249	283	158
	Min, max	98,950	40,1079	73,614
Day 223	N	13	12	11

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
	Mean	845	962	316
	Median	608	774	303
	SD	781	514	195
	Min, max	226,3176	408,1987	79,622
Day 382	N	12	10	11
	Mean	507	328	169
	Median	353	245	109
	SD	503	236	121
	Min, max	114,1786	68,755	52,403

[a] Numbers of CK^+ H5-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 9. IL-13^+ H5-Specific CD4 T Cells[a] [excluding IL2-/IL13-/INF-γ^-/TNF-α^+] (Peptide Pool: H5 Pool of 70 - Visit 1-6 Fresh Samples) – FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	55	47	63
	Median	56	30	71
	SD	52	48	45
	Min, max	0,185	0,139	10,120
Day 22	N			
	Mean	25	28	30
	Median	19	21	14
	SD	23	33	41
	Min, max	0.6,83	0,108	0,119
Day 43	N			
	Mean	33	23	14
	Median	17	12	8.9
	SD	37	27	17
	Min, max	0,105	0,92	0,58
Day 202	N	13	12	12
	Mean	22	50	17
	Median	8.7	6.1	4.6
	SD	28	155	24
	Min, max	0,97	0,542	0,68

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
Day 223	N	13	12	11
	Mean	30	40	12
	Median	18	26	0
	SD	42	42	26
	Min, max	0,160	0,136	0,87
Day 382	N	12	10	11
	Mean	17	19	3.14
	Median	0.05	6.25	0
	SD	38	33	5.53
	Min, max	0,118	0,106	0,18

[a] Numbers of IL-13^{+}- H5-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 10. IL-2^{+}/IFN-γ^{-} H5N1-Specific CD4 T Cells[a] (Subunit H5N1 (Visit 1-3 Frozen samples, Visit 3.1-6 Fresh samples)) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59-eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	151	45	42
	Median	23	27	25
	SD	374	44	56
	Min, max	0,1420	0,136	0,176
Day 22	N	14	13	13
	Mean	326	179	81
	Median	202	98	30
	SD	369	205	106
	Min, max	10,1149	0,651	0,335
Day 43	N	14	12	13
	Mean	420	175	141
	Median	251	138	81
	SD	553	180	177
	Min, max	7.1,1958	0,455	0,584
Day 130	N	14	12	13
	Mean	364	377	253
	Median	331	297	191
	SD	263	308	133
	Min, max	54,958	130,1204	95,482
Day 202	N	13	12	12
	Mean	490	541	286
	Median	401	443	289
	SD	345	325	159
	Min, max	108,1417	196,1124	42,622
Day 223	N	13	12	11
	Mean	1150	1175	416
	Median	745	1073	334
	SD	918	551	251
	Min, max	141,2962	242,2079	228,1083
Day 382	N	12	10	11
	Mean	402	295	110
	Median	232	151	103
	SD	467	296	69
	Min, max	70,1603	70,1038	31,257

[a] Numbers of IL-2^{+}/IFN-γ^{-} H5N1-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells
The number of subjects for (FAS) are only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 11. IL-2^{+}/IFN-γ^{+} H5N1-Specific CD4 T Cells[a] (Subunit H5N1 (Visit 1-3 Frozen samples, Visit 3.1-6 Fresh samples)) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	67	53	75
	Median	38	28	57
	SD	78	63	74
	Min, max	0,225	0,188	0,278
Day 22	N	14	13	13
	Mean	140	97	73
	Median	76	39	48
	SD	182	119	87
	Min, max	0,692	0,369	0,283
Day 43	N	14	12	13
	Mean	165	88	110
	Median	102	79	89
	SD	205	94	111
	Min, max	0,798	0,269	0,369
Day 130	N	14	12	13
	Mean	265	323	274
	Median	209	273	260
	SD	220	188	144
	Min, max	66,935	163,834	108,633
Day 202	N	13	12	12
	Mean	368	460	361
	Median	295	476	418
	SD	307	184	169
	Min, max	0,1269	90,789	103,555
Day 223	N	13	12	11
	Mean	710	991	456
	Median	569	952	552
	SD	562	419	234
	Min, max	72,1966	408,1755	146,818
Day 382	N	12	10	11
	Mean	329	305	119
	Median	228	218	107

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
	SD	387	340	60
	Min, max	13,1448	92,1251	24,234

[a] Numbers of IL-2^+/IFN-γ^+ H5N1-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 12. IL-2-/IFN-γ^+ H5N1-Specific CD4 T Cells[a] (Subunit H5N1 (Visit 1-3 Frozen samples, Visit 3.1-6 Fresh samples)) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	57	52	62
	Median	34	36	43
	SD	63	44	56
	Min, max	0,240	0,145	0,178
Day 22	N			
	Mean	146	211	75
	Median	87	53	53
	SD	129	328	328
	Min, max	17,480	7.7,1009	7.7,1009
Day 43	N	13	12	
	Mean	198	232	86
	Median	145	101	57
	SD	166	304	101
	Min, max	37,677	0,851	0,378
Day 130	N		12	13
	Mean	67	50	39
	Median	48	46	32
	SD	59	31	30
	Min, max	20,243	0,113	4.9,101
Day 202	N	13	12	12
	Mean	67	60	38
	Median	35	46	31
	SD	88	41	22
	Min, max	0,308	7.1,130	13,84
Day 223	N	13	12	11
	Mean	188	253	63
	Median	184	167	62

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
	SD	138	175	33
	Min, max	0,380	96,625	19,134
Day 382	N	12	10	11
	Mean	54	28	16
	Median	43	14	15
	SD	44	31	12
	Min, max	3.9,160	7.4,101	1.3,40

[a] Numbers of IL-2-/IFN-γ^+ H5N1-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 13. CK^+ H5N1-Specific CD4 T Cells[a] [excluding IL2-/IL13-/INF-γ^-/TNF-α^+] (Subunit H5N1 [Visit 1-3 Frozen samples, Visit 3.1-6 Fresh samples]) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	291	170	191
	Median	134	148	158
	SD	388	101	130
	Min, max	34,1506	26,360	33,387
Day 22	N			
	Mean	634	622	249
	Median	435	274	154
	SD	570	657	224
	Min, max	118,2157	7.7,2248	67,719
Day 43	N		12	
	Mean	886	582	359
	Median	710	452	279
	SD	780	520	310
	Min, max	210,2757	0,1770	33,972
Day 130	N		12	
	Mean	695	750	566
	Median	666	654	478
	SD	439	470	237
	Min, max	211,2005	350,2117	238,1099
Day 202	N	13	12	12
	Mean	931	1064	689
	Median	769	982	701
	SD	530	487	292
	Min, max	110,2055	322,1826	173,1196
Day 223	N	13	12	11
	Mean	2053	2433	938
	Median	1255	2392	936
	SD	1499	892	427
	Min, max	235,5335	966,4208	419,1696
Day 382	N	12	10	11
	Mean	790	628	247
	Median	448	403	261
	SD	876	647	89
	Min, max	161,3177	242,2391	75,382

[a] Numbers of CK^+ H5N1-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 14. IL-13^+ H5N1-Specific CD4 T Cells[a] (Subunit H5N1 [Visit 1-3 Frozen samples, Visit 3.1-6 Fresh samples]) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	39	39	31
	Median	16	18	0
	SD	60	56	61
	Min, max	0,203	0,207	0,215
Day 22	N			
	Mean	37	216	42
	Median	22	9.6	7.2
	SD	42	579	105
	Min, max	0,154	0,2070	0,384
Day 43	N		12	
	Mean	162	179	50
	Median	35	16	14
	SD	389	420	126
	Min, max	0,1474	0,1481	0,467
Day 202	N	13	12	12
	Mean	29	42	26
	Median	21	29	21
	SD	33	33	24
	Min, max	0,106	15,133	0,90
Day 223	N	13	12	11
	Mean	48	59	7.71
	Median	18	63	0
	SD	95	34	13
	Min, max	0,359	11,106	0,32
Day 382	N	12	10	11
	Mean	35	22	3.71
	Median	8.45	8.45	3.5
	SD	64	64	4.52
	Min, max	0,204	0,204	0,15

[a] Numbers of IL-13^+ H5N1-specific CD4 T cells are expressed as cells/1.000.000 total CD4 T cells. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given for Day 1.

Table 15. B Cells Summary Statistics, Change Over Day 1 (H5N1-IgG MBC - % of Total IgG MBC) - FAS

		Vaccine Group		
Timepoint	**Variable**	**MF59-eH5N1 7.5**	**MF59- eH5N1 15**	**NON-ADJ 15**
Day 1	N	14	13	13
	Mean	1.05	0.41	0.36
	Median	0.33	0	0.28
	SD	2.04	0.83	0.31
	Min, max	(0, 6.79)	(0, 2.48)	(0, 0.93)
Day 22-Day 1	N	11	12	11
	Mean	1.94	1.56	0.46
	Median	1.16	1.56	0.23
	SD	2.72	1.85	0.76
	Min, max	(-2.965, 6.71)	(-1.408, 5.22)	(-0.515, 1.98)
Day 43-Day 1	N	11	12	11
	Mean	4.52	2.65	0.72
	Median	2.26	2.59	0.51
	SD	5.38	1.79	0.82
	Min, max	(0.19, 16)	(0.45, 6.76)	(-0.515, 2.05)
Day 202-Day 1	N	8	9	8
	Mean	4.18	4.16	1.21
	Median	2.37	1.55	0.55
	SD	9.15	7.33	1.5
	Min, max	(-6.097, 22)	(0.23, 23)	(-0.052, 3.81)
Day 223-Day 1	N	12	12	11
	Mean	10	11	3.03
	Median	9.37	8.8	0.9
	SD	9.04	9.72	8.58
	Min, max	(-4.872, 27)	(2.06, 32)	(-0.925, 29)
Day 382-Day 1	N	12	10	11
	Mean	9.85	9.05	0.89
	Median	1.59	5.98	0.61
	SD	16	11	1
	Min, max	(-3.682, 54)	(0.56, 38)	(-0.515, 3.18)

Table 16. Percentages of Subjects with HI Titer ≥ 40 - FAS

Timepoint	n/N (%) and 95% CI		
	MF59-eH5N1 7.5 **N=14**	**MF59-eH5N1 15** **N=13**	**NON-ADJ 15** **N=13**
Day 1	1 (7%) (0-34)	0 (0%) (0-25)	2 (15%) (2-45)
Day 22	5 (36%) (13-65)	2 (15%) (2-45)	3 (23%) (5-54)
Day 43	12 (86%) (57-98)	6 (46%) (19-75)	4 (31%) (9-61)
Day 130	10 (71%) (42-92) N=14	2 (17%) (2-48) N=12	3 (23%) (5-54) N=13
Day 202	4 (31%) (9-61) N=13	1 (8%) (0-38) N=12	3 (25%) (5-57) N=12
Day 233	11 (85%) (55-98) N=13	8 (67%) (35-90) N=12	5 (45%) (17-77) N=11
Day 382	7 (58%) (28-85) N=12	6 (60%) (26-88) N=10	4 (36%) (11-69) N=11

The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading

Table 17. Percentages of Subjects with Seroconversion or Significant Increase in HI Titers –FAS

Timepoint	n/N[a] (%) and 95% CI		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Day 22[b]	4 (29%) (8-58)	2 (15%) (2-45)	1 (8%) (0-36)
Day 43[b]	11 (79%) (49-95)	6 (46%) (19-75)	2 (15%) (2-45)
Day 130[b]	9 (64%) (35-87) N=14	2 (17%) (2-48) N=12	1 (8%) (0-36) N=13
Day 202[b]	3 (23%) (5-54) N=13	1 (8%) (0-38) N=12	1 (8%) (0-38) N=12
Day 233[c]	10 (77%) (46-95) N=13	7 (58%) (28-85) N=12	2 (18%) (2-52) N=11
Day 382[c]	5 (42%) (15-72) N=12	5 (50%) (19-81) N=10	1 (9%) (0-41) N=11

[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 <10) and post-vaccination HI titer ≥40; Significant increase - at least a 4-fold increase in HI titers in subjects who were positive pre-vaccination (i.e., HI titer ≥10). [b] referring to pre-vaccination Day 1, [c] referring to pre-booster vaccination Day 202.

The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading

Table 18. HI GMTs and GMRs-FAS

Timepoint	Variable	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Pre-vaccination (Day 1)	n	14	13	13
	GMT (95% CI)	6.4 (4.08-10)	5 (3.13-7.99)	8.07 (5.05-13)
Post-1st injection (Day 22)	n	14	13	13
	GMT (95% CI)	16 (7.03-36)	8.08 (3.44-19)	12 (5.26-29)
	GMR[a] (95% CI)	2.5 (1.25-4.98)	1.62 (0.79-3.31)	1.53 (0.75-3.13)
Post-2nd injection (Day 43)	n	14	13	13
	GMT (95% CI)	135 (52-350)	30 (11-81)	19 (7.01-51)
	GMR[a] (95% CI)	21 (8.41-52)	5.97 (2.31-15)	2.35 (0.91-6.07)
Persistence (Day 130)	n	14	12	13
	GMT (95% CI)	44 (20-96)	8.41 (3.63-19)	11 (4.95-25)
	GMR[a] (95% CI)	6.89 (3.58-13)	1.68 (0.83-3.42)	1.38 (0.7-2.72)
Pre-booster injection (Day 202)	n	13	12	12
	GMT (95% CI)	16 (7.26-34)	6.67 (2.99-15)	12 (5.32-27)
	GMR[a] (95% CI)	2.41 (1.38-4.22)	1.33 (0.75-2.39)	1.46 (0.81-2.61)
Post-booster injection (Day 223)	n	13	12	11
	GMT (95% CI)	160 (56-456)	90 (30-267)	23 (7.26-71)
	GMR[a] (95% CI)	10 (3.74-28)	13 (4.75-38)	1.76 (0.59-5.23)
6-months post-booster injection (Day 382)	n	12	10	11
	GMT (95% CI)	44 (15-126)	41 (13-133)	14 (4.66-43)
	GMR[a] (95% CI)	3 (1.34-6.69)	5.86 (2.43-14)	1.1 (0.48-2.54)

[a] GMR - the geometric Mean of the post-vaccination Day to Day 1 titer ratio; [b] GMR - the geometric Mean of the post-vaccination Day to Day 202 titer ratio.

Table 19. Percentages of Subjects with a MN Titer ≥40 - FAS

Timepoint	n/N (%) and 95% CI		
	MF59-eH5N1 7.5 N=14	**MF59-eH5N1 15 N=13**	**NON-ADJ 15 N=13**
Day 1	3 (21%) (5-51)	1 (8%) (0-36)	2 (15%) (2-45)
Day 22	4 (29%) (8-58)	2 (15%) (2-45)	3 (23%) (5-54)
Day 43	13 (93%) (66-100)	7 (54%) (25-81)	4 (31%) (9-61)
Day 130	10 (71%) (42-92) N=14	5 (42%) (15-72) N=12	3 (23%) (5-54) N=13
Day 202	4 (31%) (9-61) N=13	6 (50%) (21-79) N=12	3 (25%) (5-57) N=12
Day 223	12 (92%) (64-100) N=13	12 (100%) (74-100) N=12	4 (36%) (11-69) N=11
Day 382	9 (75%) (43-95) N=12	7 (70%) (35-93) N=10	2 (18%) (2-52) N=11

The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading.

Table 20. MN GMTs and GMRs-FAS

Timepoint	Variable	GMT and 95% CI		
		MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Pre-vaccination (Day 1)	n	14	13	13
	GMT (95% CI)	14 (9.9-20)	12 (8.05-17)	15 (11-22)
Post-1st injection (Day 22)	n	14	13	13
	GMT (95% CI)	25 (13-45)	16 (8.68-31)	23 (12-43)
	GMR[a] (95% CI)	1.76 (0.97-3.19)	1.41 (0.76-2.62)	1.52 (0.82-2.83)
Post-2nd injection (Day 43)	n	14	13	13
	GMT (95% CI)	114 (60-219)	67 (34-130)	24 (12-48)
	GMR[a] (95% CI)	8.14 (3.78-18)	5.75 (2.6-13)	1.62 (0.73-3.58)
Persistence (Day 130)	n	14	12	13
	GMT (95% CI)	59 (33-107)	38 (20-71)	17 (9.36-32)
	GMR[a] (95% CI)	4.22 (2.15-8.26)	3.21 (1.55-6.63)	1.14 (0.57-2.3)
Pre-booster injection (Day 202)	n	13	12	12
	GMT (95% CI)	30 (16-54)	31 (17-58)	17 (9.1-32)
	GMR[a] (95% CI)	2.05 (1.07-3.91)	3.12 (1.59-6.1)	1.08 (0.55-2.12)
Post-booster injection (Day 223)	n	13	12	11
	GMT (95% CI)	251 (136-465)	343 (181-651)	33 (17-65)
	GMR[b] (95% CI)	8.5 (4.86-15)	11 (6.15-20)	1.87 (1.02-3.45)
6-months post-booster injection (Day 382)	n	12	10	11
	GMT (95% CI)	83 (41-168)	70 (32-153)	15 (7.27-32)
	GMR[b] (95% CI)	2.55 (1.41-4.64)	2.56 (1.33-4.92)	0.85 (0.46-1.59)

MN titers below the limit of detection were set to half that limit (i.e., to 10); [a] GMR - the geometric Mean of the post-vaccination Day to Day 1 titer ratio; [b] GMR - the geometric Mean of the post-vaccination Day to Day 202 titer ratio.

Table 21. Percentages of Subjects with at Least a 4-fold Increase in MN Titer - FAS

Timepoint	n/N (%) and 95% CI		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Day 22[a]	4 (29%) (8-58)	2 (15%) (2-45)	3 (23%) (5-54)
Day 43[a]	12 (86%) (57-98)	7 (54%) (25-81)	3 (23%) (5-54)
Day 130[a]	8 (57%) (29-82)	5 (42%) (15-72)	2 (15%) (2-45)
Day 202[a]	3 (23%) (5-54) N=13	6 (50%) (21-79) N=12	2 (17%) (2-48) N=12
Day 223[b]	9 (69%) (39-91) N=13	9 (75%) (43-95) N=12	2 (18%) (2-52) N=11
Day 382[b]	3 (25%) (5-57) N=12	5 (50%) (19-81) N=10	0 (0%) (0-28) N=11

[a] referring to pre-vaccination Day 1, [b] referring to pre-booster vaccination Day 202. The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading.

Table 22. Percentages of Subjects with SRH Area ≥ 25 mm² - FAS

Timepoint	n/N (%) and 95% CI		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Visit 1 (Day 1)	0 (0%) (0-23)	0 (0%) (0-25)	2 (15%) (2-45)
Visit 2 (Day 22)	3 (21%) (5-51)	3 (23%) (5-54)	4 (31%) (9-61)
Visit 3 (Day 43)	12 (86%) (57-98)	5 (38%) (14-68)	6 (46%) (19-75)
Visit 3.1 (Day 130)	10 (71%) (42-92) N=14	4 (33%) (10-65) N=12	4 (31%) (9-61) N=13
Visit 4 (Day 202)	4 (31%) (9-61) N=13	3 (25%) (5-57) N=12	4 (33%) (10-65) N=12
Visit 5 (Day 223)	11 (85%) (55-98) N=13	12 (100%) (74-100) N=12	4 (36%) (11-69) N=11
Visit 6 (Day 382)	8 (67%) (35-90) N=12	7 (70%) (35-93) N=10	4 (36%) (11-69) N=11

The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading.

Table 23. Percentages of Subjects with Seroconversion or Significant Increase in SRH Areas- FAS

	n/N[a] (%) and 95% CI		
	MF59-eH5N1 7.5 N=14	**MF59-eH5N1 15 N=13**	**NON-ADJ 15 N=13**
Day 22[b]	3 (21%) (5-51)	3 (23%) (5-54)	2 (15%) (2-45)
Day 43[b]	12 (86%) (57-98)	5 (38%) (14-68)	4 (31%) (9-61)
Day 130[b]	10 (71%) (42-92) N=14	4 (33%) (10-65) N=12	2 (15%) (2-45) N=13
Day 202[b]	4 (31%) (9-61) N=13	4 (33%) (10-65) N=12	2 (17%) (2-48) N=12
Day 223[c]	9 (69%) (39-91) N=13	12 (100%) (74-100) N=12	1 (9%) (0-41) N=11
Day 382[c]	5 (42%) (15-72) N=12	7 (70%) (35-93) N=10	1 (9%) (0-41) N=11

[a] 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); (i.e. seroconversion is defined as negative pre-vaccination serum (<4 mm²) and post-vaccination SRH area ≥ 25 mm²; significant increase is defined as at least a 50% increase in SRH area in subjects who were positive pre-vaccination (i.e. SRH area ≥4 mm²). [b] referring to pre-vaccination Day 1, [c] referring to pre-booster vaccination Day 202.

The number of subjects for (FAS) is only mentioned in the respective cells if they deviate from the numbers given in the table heading.

Table 24. SRH GMAs and GMRs-FAS

Days post-vaccination	Variable	GMA/GMR and 95% CI		
		MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Pre-vaccination (Day 1)	n	14	13	13
	GMT (95% CI)	4.37 (3.21-5.96)	4.81 (3.49-6.63)	5.59 (4.06-7.7)
Post-1st injection (Day 22)	n	14	13	13
	GMT (95% CI)	7.07 (4.04-12)	7.6 (4.25-14)	8 (4.48-14)
	GMR[a] (95% CI)	1.62 (0.98-2.66)	1.58 (0.94-2.64)	1.43 (0.86-2.4)
Post-2nd injection (Day 43)	n	14	13	13
	GMT (95% CI)	36 (20-64)	11 (5.93-20)	11 (5.95-20)
	GMR[a] (95% CI)	8.12 (4.61-14)	2.27 (1.26-4.09)	1.96 (1.09-3.53)
Persistence (Day 130)	n	14	12	13
	GMT (95% CI)	22 (13-39)	8.55 (4.74-15)	7.97 (4.52-14)
	GMR[a] (95% CI)	5.11 (3.04-8.58)	1.94 (1.11-3.4)	1.43 (0.83-2.44)
Pre-booster injection (Day 202)	n	13	12	12
	GMT (95% CI)	15 (8.92-27)	8.45 (4.77-15)	8.33 (4.71-15)
	GMR[a] (95% CI)	3.51 (2.09-5.9)	1.73 (1.01-2.97)	1.45 (0.84-2.49)
Post-booster injection (Day 223)	n	13	12	11
	GMT (95% CI)	38 (24-62)	52 (32-86)	11 (6.68-19)
	GMR[b] (95% CI)	2.49 (1.56-3.98)	6.21 (3.81-10)	1.5 (0.9-2.49)
6-months post-booster injection (Day 382)	n	12	10	11
	GMT (95% CI)	28 (16-49)	22 (12-42)	8.97 (4.93-16)
	GMR[b] (95% CI)	1.84 (1.16-2.93)	2.98 (1.74-4.94)	1.2 (0.74-1.95)

[a] GMR - the geometric Mean of the post-vaccination Day to Day 1 SRH ratio; [b] GMR - the geometric Mean of the post-vaccination Day to Day 202 SRH ratio.

Table 25. Number (%) of Subjects with at Least One Reactogenicity Sign, by Vaccination - Safety Set

	Number (%) of Subjects with Solicited Reaction								
	1st injection			2nd injection			Booster injection		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Any	13 (93)	11 (85)	6 (46)	10 (71)	7 (54)	3 (23)	9 (69)	6 (50)	0
Local	12 (86)	7 (54)	3 (23)	8 (57)	5 (38)	3 (23)	8 (62)	4 (33)	0
Systemic	7 (50)	6 (46)	6 (46)	2 (14)	4 (31)	0	6 (46)	4 (33)	0
Other	1 (7)	0	1 (8)	0	0	0	1 (8)	0	0

Table 26. Comparison of Number (%) of Subjects with Each Solicited Local Reaction, by Vaccination - Safety Set

		Number (%) of Subjects with Injection Site Reaction								
		1st injection			2nd injection			Booster injection		
		MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Erythema (mm)	Any	1 (7)	1 (8)	1 (8)	1 (7)	1 (8)	1 (8)	2 (15)	1 (8)	0
	>50 mm	0	0	0	0	0	0	0	0	0
Induration (mm)	Any	3 (21)	1 (8)	0	3 (21)	1 (8)	0	4 (31)	1 (8)	0
	>50 mm	0	0	0	0	0	0	0	0	0
Swelling (mm)	Any	0	1 (8)	0	0	1 (8)	0	2 (15)	1 (8)	0
	>50 mm	0	0	0	0	0	0	0	0	0
Ecchymosis (mm)	Any	0	1 (8)	1 (8)	0	0	0	1 (8)	0	0
	>50 mm	0	0	0	0	0	0	0	0	0
Pain	Any	9 (64)	5 (38)	3 (23)	5 (36)	4 (31)	2 (15)	8 (62)	3 (25)	0
	Severe	0	0	0	0	0	0	1 (8)	0	0

Note: The numbers (N) in the header is the total number of subjects with documented reactions.

Table 27. Comparison of Number (%) of Subjects with Each Solicited Systemic Reaction, by Vaccination - Safety Set

		Number (%) of Subjects with Systemic Reaction								
		1st injection			2nd injection			Booster injection		
		MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Systemic										
Chills	Any	2 (14)	0	1 (8)	0	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	0	0
Malaise	Any	1 (7)	1 (8)	1 (8)	0	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	1 (8)	0	0
Myalgia	Any	5 (36)	5 (38)	2 (15)	1 (7)	3 (23)	0	6 (46)	3 (25)	0
	Severe	0	0	0	0	0	0	1 (8)	0	0
Arthralgia	Any	1 (7)	1 (8)	1 (8)	1 (7)	1 (8)	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	0	0
Headache	Any	3 (21)	0	4 (31)	0	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	1 (8)	0
Sweating	Any	1 (7)	0	1 (8)	0	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	0	0
Fatigue	Any	2 (14)	1 (8)	3 (23)	0	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	2 (17)	0
Nausea	Any	1 (7)	0	0	1 (7)	0	0	1 (8)	0	0
	Severe	0	0	0	0	0	0	0	0	0
Coughing	Any	1 (7)	0	2 (15)	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0
Wheezing	Any	1 (7)	0	1 (8)	0	0	0	1 (8)	0	0

		Number (%) of Subjects with Systemic Reaction								
		1st injection			2nd injection			Booster injection		
		MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Systemic										
Chest	Any	0	0	1 (8)	0	0	0	0	0	0
Tightness	Severe	0		0	0	0	0	0	0	0
Breath	Any	0	0	0	0	0	0	0	0	0
Difficulty	Severe	0	0	0	0	0	0	0	0	0
Sore Throat	Any	1 (7)	0	3 (23)	0	0	0	0	1 (8)	0
	Severe	0	0	0	0	0	0	0	0	0
Facial Edema	Any	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0
Red Eye	Any	0	0	0	0	0	0	0	0	0
	Severe	0	0	0	0	0	0	0	0	0
Fever (≥38°C)	Yes	0	0	0	0	0	0	0	0	0
Other										
Axillary Temperature (°C)	(<38)	14 (100)	13 (100)	13 (100)	14 (100)	13 (100)	13 (100)	13 (100)	12 (100)	11 (100)
	(≥40)	0	0	0	0	0	0	0	0	0
Stayed Home	Yes	0	0	1 (8)	0	0	0	0	0	0
Analgesic Antipyretic	Yes	1 (7)	0	1 (8)	0	0	0	1 (8)	0	0

Note: The numbers (N) in the header is the total number of subjects with documented reactions

Table 28. Overview of Other AEs - 3 weeks post-injection – Safety Set

Type of Reaction	Number (%) of Subjects with Adverse Event								
	1st injection			2nd injection			Booster injection		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Any AE	1 (7%)	0	3 (23%)	2 (14%)	0	0	1 (8%)	1 (8%)	0
At least possibly related AE	0	0	0	0	0	0	0	0	0
Serious AEs	0	0	0	0	0	0	0	0	0
AEs leading to discontinuation	0	0	0	0	0	0	0	0	0
At least possibly related SAE	0	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0	0

Table 29. Overview of Other AEs - 3 week to 6 month follow-up – Safety Set

Type of Reaction	Number (%) of Subjects with Adverse Event					
	2nd injection			Booster injection		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=12	NON-ADJ 15 N=13	MF59-eH5N1 7.5 N=13	MF59-eH5N1 15 N=12	NON-ADJ 15 N=11
Any AE	2 (14%)	0	0	1 (8%)	0	0
At least possibly related AE	0	0	0	0	0	0
Serious AEs	1 (7%)	0	0	0	0	0
AEs leading to discontinuation	0	0	0	0	0	0

Type of Reaction	Number (%) of Subjects with Adverse Event					
	2nd injection			Booster injection		
	MF59-eH5N1 7.5	MF59-eH5N1 15	NON-ADJ 15	MF59-eH5N1 7.5	MF59-eH5N1 15	NON-ADJ 15
	N=14	N=12	N=13	N=13	N=12	N=11
At least possibly related SAE	0	0	0	0	0	0
Death	0	0	0	0	0	0

Table 30. Serious Adverse Events by Preferred Term Sorted By System Organ Class-Safety Set

MedDRA System Organ Class MedDRA Preferred Term	Number (%) of Subjects[1]		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Any Serious Adverse Event	1 (7%)	0 (0%)	0
Surgical & Medical Procedure	1 (7%)	0	0
Appendicectomy	1 (7%)	0	0

[1]Number and percent of subjects with one or more events (as reported on Adverse Events form) that map to each MedDRA system organ class or MedDRA preferred term. Hence, MedDRA preferred term counts may not sum to MedDRA system organ class counts, and MedDRA system organ class counts may not sum to overall counts.

Table 31. Unsolicited AEs by Preferred Term Reported By >5% of Subjects Sorted By System Organ Class-Safety Set

MedDRA System Organ Class MedDRA Preferred Term	Number (%) of Subjects[1]		
	MF59-eH5N1 7.5 N=14	MF59-eH5N1 15 N=13	NON-ADJ 15 N=13
Nervous System Disorder			
Headache	1 (7%)	0 (0%)	1 (8%)
Infections & Infestations	0	0	1 (8%)
Abscess Oral			
Sinusitis	1 (7%)	0	0
Surgical And Medical Procedure			
Appendicectomy	1 (7%)	0	0
Resp., Thoracic & Mediastinal Dis.			
Cough	0	0	1 (8%)
Gen. Disorders & Admin. Site Cond			
Influenza Like Illness	1 (7%)	0	0
Injuries and Poisoning			
Meniscus Lesion	0	1 (8%)	0
Respiratory, thoracic and Mediasternal Dis.			
Pharyngolaryngeal Pain	1 (7%)	0	0
Gen. Disorders & Admin. Site Cond.			
Pyrexia	1 (7%)	0	0
Nervous System Disorder			
Somnolence	0	0	1 (8%)
Syncope	1 (7%)	0	0

[1]Number and percent of subjects with one or more events (as reported on Adverse Events form) that map to MedDRA preferred term. Hence, MedDRA preferred term counts may not sum to overall counts.

Conclusion:

- The immune responses, assessed by hemagglutination inhibition (HI), single radial hemolysis (SRH) and microneutralization (MN) assay, were generally higher for recipients of the adjuvanted than non-adjuvanted formulations.
- After the primary vaccination, immune responses were consistently higher in the MF59-eH5N17.5 than in the MF59-eH5N115 vaccine group. All Committee for Medicinal Products for Human Use (CHMP) criteria were met by subjects who received 7.5µg H5N1 adjuvanted vaccine when assessed by HI and SRH.
- After the booster vaccination at 6 months after the second injection the responses were similar for both MF59-eH5N1 groups and still low in the group of subjects, who received the non-adjuvanted vaccine. All CHMP criteria were met by subjects who received 7.5 µg H5N1-adjuvanted vaccine when assessed by HI and by subjects who received 15 µg H5N1-adjuvanted vaccine when assessed by SRH.
- Persistence determined 6 months after the booster vaccination was high for both MF59-eH5N1groups, without showing clinically relevant differences between the two groups in any parameter, and low for the non-adjuvanted 15 group.
- Both adjuvanted vaccines similarly induced higher frequencies of H5-specific CD4 memory T cells and of H5N1-specific memory B cells than the non-adjuvanted vaccine. Very few interleukin (IL)-13^+ H5-specific CD4 T cells were detected before and after vaccination regardless of the vaccine formulation thus suggesting that IL-13 production is not induced by non-adjuvanted or MF59-adjuvanted H5N1 vaccine. IL-13^+ is considered one of the hallmarks of a TH2 response which in turn can be associated with allergic responses. The results reported in this trial show that in humans MF59 (i.e., MF59-eH5N1) does not drive the immune response toward TH2, as it was reported in some experimental mouse models. Indeed, vaccination induced an increase in the frequency of H5-specific CD4 T cells with a memory TH0/TH1 phenotype, with high survival potential in vivo and the ability to expand and differentiate into effectors upon infection. Such increase in memory CD4 cells was higher in the groups receiving MF59-eH5N1 7.5 and MF59-eH5N1 15 compared with recipients of the non-adjuvanted formulation.
- Three injections of either adjuvanted or non-adjuvanted vaccine were safe and well tolerated.
- Overall reactogenicity was highest after the first vaccination, and decreased after the following vaccinations. Reactogenicity was higher for adjuvanted vaccine groups than for the non-adjuvanted 15 group. Injection site pain was the most frequently reported local reaction, while myalgia and headache were the most frequently reported systemic reactions.
- No possibly or probably related Adverse Events (AEs) were reported. One Serious Adverse Event (SAE) (appendicectomy, MF59-eH5N1 7.5) was reported, which was considered as not vaccine related.

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