The study listed may include approved and non-approved uses, formulations or treatment regimens. The results reported in any single study may not reflect the overall results obtained on studies of a product. Before prescribing any product mentioned in this Register, healthcare professionals should consult prescribing information for the product approved in their country.

Study No.: 104887 (FluAS25-004)

Title: A phase II, open, controlled study to evaluate the reactogenicity and the immunogenicity of GlaxoSmithKline Biologicals AS25 adjuvanted influenza vaccine (FluAS25) in elderly adults (≥ 66 years) previously vaccinated in FluAS25-003 clinical trial with the same candidate vaccine. *Fluarix*™ (known as α-Rix™ in Belgium) administered in young (19-41 years) and elderly (≥ 66 years) adults will be used as reference.
FluAS25: GlaxoSmithKline (GSK) Biologicals' AS25 adjuvanted influenza vaccine.
Fluarix™ (Flu): GSK Biologicals' licensed trivalent split influenza vaccine.

Rationale: The aim of this study was to evaluate the safety and immunogenicity of a re-vaccination dose of the FluAS25 vaccine (2006-2007 season) compared to that of the Flu vaccine (2006-2007 season) in adults aged 66 years or older who previously received the same vaccine in FluAS25-003 (104886) study.

Phase: II

Study Period: 23 October 2006 to 12 December 2006

Study Design: Single centre, open, controlled study with 3 parallel groups.

Centres: One study centre in Belgium.

Indication: Immunisation against influenza disease in an elderly population aged 66 years and older.

Treatment: The study groups were as follows:

- FluAS25: subjects (≥ 66 years of age) who previously received 1 dose of FluAS25 vaccine were administered 1 dose of FluAS25 vaccine in this study.
- Flu YNG: subjects (19-41 years of age) who previously received 1 dose of Flu vaccine were administered 1 dose of Flu vaccine in this study.
- Flu ELD: subjects (≥ 66 years of age) who previously received 1 dose of Flu vaccine were administered 1 dose of Flu vaccine in this study.

All vaccines were administered by intramuscular injection into the deltoid region of the non-dominant arm.

Objectives: To evaluate in elderly adults, the safety of repeated vaccination with FluAS25, during the 21 days following the intramuscular administration of the vaccine. Flu vaccine administered to young (19-41 years) and elderly (≥ 66 years) adults was used as reference.

Primary Outcome/Efficacy Variable:

- Percentage, intensity and relationship to vaccination of solicited local and general signs and symptoms during a 7-day follow-up period (i.e. day of vaccination and 6 subsequent days) after vaccination and overall.
- Percentage, intensity and relationship to vaccination of unsolicited local and general adverse events (AEs) during a 21-day follow-up period (i.e. day of vaccination and 20 subsequent days) after vaccination and overall.
- Occurrence of serious adverse events (SAEs) during the entire study period.

Secondary Outcome/Efficacy Variable(s):

Humoral immune response

- Geometric mean titres (GMTs) of haemagglutination-inhibition (HI) antibody titres at Days 0 and 21
- Seroconversion rates* at Day 21
- Seroconversion factors** at Day 21
- Seroprotection rates*** at Days 0 and 21

Anti 3-deacylated Monophosphoryl Lipid A (anti-MPL) response

- Geometric mean concentrations (GMCs) of serum anti-MPL antibodies at Days 0 and 21

* Seroconversion rate was defined as the percentage of vaccinees who had either a prevaccination titre < 1:10 and a post-vaccination titre ≥ 1:40 or a pre-vaccination titre ≥ 1:10 and at least a four-fold increase in post-vaccination titre.
**Seroconversion factor was defined as the fold increase in serum HI GMTs post-vaccination compared to Day 0.
***Seroprotection rate was defined as the percentage of vaccinees with a serum HI titre ≥ 1:40 that usually is accepted as indicating protection.

CMI response

- At Days 0 and 21:
  - Frequency of cytokine-positive CD4/CD8 cells per 10^6 in tests producing at least 2 different cytokines* [Cluster of differentiation-40L (CD40L), Interleukin-2 (IL-2), Tumour Necrosis Factor-alpha (TNF-α),

Interferon-gamma (IFN-γ)].
- Frequency of cytokine-positive CD4/CD8 cells per 10^6 in tests producing at least CD40L and another signal molecule (IL-2, IFN-γ, TNF-α).
- Frequency of cytokine -positive CD4/CD8 cells per 10^6 in tests producing at least IL-2 and another signal molecule (CD40L, IFN-γ, TNF-α).
- Frequency of cytokine-positive CD4/CD8 cells per 10^6 in tests producing at least IFN-γ and another signal molecule (IL2, CD40L, TNF-α).
- Frequency of cytokine-positive CD4/CD8 cells per 10^6 in tests producing at least TNF-α and another signal molecule (IL2, CD40L, IFN-γ).

- At Days 0 and 21: frequency of influenza-specific memory B-cells per 10^6 in tests, in each group.

* Final data for CD40L not available

Statistical Methods:

The analyses were performed on the Total Vaccinated Cohort, the According-To-Protocol (ATP) cohort for immunogenicity and the B-cells subset from ATP cohort for immunogenicity.

- The Total Vaccinated Cohort included all vaccinated subjects for whom data were available.
- The ATP cohort for immunogenicity included all evaluable subjects (i.e., those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) for whom data concerning immunogenicity measures were available. This included subjects for whom assay results were available for antibodies against at least one study vaccine antigen component after vaccination.
- For the B-cells subset from ATP cohort for immunogenicity, a sub-randomisation of 20 subjects per group and for each age sub-group in the FluAS25-003 study (65-72 years, ≥73 years and 18-40 years) was performed including subjects with available CMI blood samples in the FluAS25-003 study and in the 2 re-vaccination studies FluAS25-004 and FluAS25-008 EXT:003 Y2 (110263).

Immunogenicity

The analysis was performed on the ATP cohort for immunogenicity and the B-cells subset from ATP-cohort for immunogenicity.

Humoral immune response

At Day 0 and Day 21 after vaccination, seropositivity and seroprotection rates were calculated for all vaccine strains (together with their exact 95% CI). Also the GMT for HI-antibodies was calculated together with the 95% CI. At Day 21, the incidence of seroconverted subjects and the seroconversion factor for anti-HI antibodies were tabulated together with the 95% CI. For anti-MPL antibodies, the incidence of subjects with antibody concentrations ≥ the limit of quantitation (LOQ) level and GMCs were given together with their 95% CI.

CMI response

The frequency of influenza-specific cytokine-positive CD4/CD8 T-lymphocytes was summarised at Days 0 and 21, for each vaccine strain, using descriptive statistics. The frequency of influenza-specific memory B cells after in vitro stimulation with each vaccine strain was summarised (descriptive statistics) at Days 0 and 21.

Safety

The analysis was performed on the Total Vaccinated Cohort.

The percentages of subjects with at least one solicited local or general symptom in the 7-day (Days 0-6) follow-up period after vaccination were tabulated with their exact 95% Confidence Intervals (CI). The same calculations were performed for symptoms of Grade 3 intensity and for solicited general symptoms assessed by the investigator as related to vaccination. The incidence of unsolicited AEs during the 21-day follow-up period (Days 0-20) after vaccination, was tabulated according to Medical Dictionary of Regulatory Activities (MedDRA) preferred terms. The same tabulations were performed for Grade 3 AEs and for AEs with relationship to vaccination. The number of SAEs was also tabulated according to MedDRA preferred terms during the entire study period.

Study Population: A male or female aged 66 years or above at the time of the revaccination, who previously received FluAS25 (for the FluAS25 Group) or a male or female aged between, and including, 19 and 41 years of age (Flu YNG Group) or over 65 years (Flu ELD Group) at the time of revaccination, who previously received Flu vaccine. Subjects were to be healthy as established by medical history and clinical examination and had to have neither history of confirmed influenza infection since the date of previous vaccination, nor previous vaccination with the influenza vaccine season 2006-2007 before entering into the study. Written informed consent was obtained from the subject. If of childbearing potential, a female subject had to be abstinent or had to use adequate contraceptive precautions for 30 days prior to vaccination, to have had a negative pregnancy test and had agree to continue such precautions for 2 months after vaccination.

Number of subjects	Flu YNG Group	Flu ELD Group	FluAS25 Group
Planned, N	75	50	75

Randomised, N (Total Vaccinated Cohort)	55	45	62
Completed, n (%)	55 (100)	45 (100)	61 (98.4)
Total Number Subjects Withdrawn, n (%)	0 (0.0)	0 (0.0)	1 (1.6)
Withdrawn due to Adverse Events, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawn due to Lack of Efficacy, n (%)	Not applicable	Not applicable	Not applicable
Withdrawn for other reasons, n (%)	0 (0.0)	0 (0.0)	1 (1.6)
Demographics	**Flu YNG Group**	**Flu ELD Group**	**FluAS25 Group**
N (Total Vaccinated Cohort)	55	45	62
Females:Males	28:27	18:27	25:37
Mean Age, years (SD)	27.2 (6.02)	70.1 (3.37)	70.3 (3.08)
White/Caucasian, n (%)	53 (96.4)	45 (100)	62 (100)

Primary Efficacy Results:
Incidence of solicited local symptoms reported during the 7-day (Days 0-6) post-vaccination period (Total Vaccinated Cohort)

Symptom	Intensity	Flu YNG Group					Flu ELD Group					FluAS25 Group				
		N	n	%	95% CI		N	n	%	95% CI		N	n	%	95% CI	
					LL	UL				LL	UL				LL	UL
Ecchymosis	Any	55	3	5.5	1.1	15.1	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	> 50 mm	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
Pain	Any	55	40	72.7	59.0	83.9	45	8	17.8	8.0	32.1	62	47	75.8	63.3	85.8
	Grade 3	55	1	1.8	0.0	9.7	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
Redness	Any	55	8	14.5	6.5	26.7	45	1	2.2	0.1	11.8	62	12	19.4	10.4	31.4
	> 50 mm	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	7	11.3	4.7	21.9
Swelling	Any	55	7	12.7	5.3	24.5	45	3	6.7	1.4	18.3	62	11	17.7	9.2	29.5
	> 50 mm	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	5	8.1	2.7	17.8

N = number of subjects with the documented dose
n (%) = number (percentage) of subjects reporting at least once the symptom
95% CI = exact 95% confidence interval; LL = lower limit, UL = upper limit
Any = incidence of a particular symptom regardless of intensity
Grade 3 pain = pain that prevented normal activity

Primary Efficacy Results:
Incidence of solicited general symptoms reported during the 7-day (Days 0-6) post-vaccination period (Total Vaccinated Cohort)

Symptom	Intensity/ Relation-ship	Flu YNG Group					Flu ELD Group					FluAS25 Group				
		N	n	%	95% CI		N	n	%	95% CI		N	n	%	95% CI	
					LL	UL				LL	UL				LL	UL
Arthralgia	Any	55	1	1.8	0.0	9.7	45	6	13.3	5.1	26.8	62	8	12.9	5.7	23.9
	Grade 3	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	Related	55	1	1.8	0.0	9.7	45	2	4.4	0.5	15.1	62	7	11.3	4.7	21.9
Fatigue	Any	55	16	29.1	17.6	42.9	45	7	15.6	6.5	29.5	62	21	33.9	22.3	47.0
	Grade 3	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	Related	55	13	23.6	13.2	37.0	45	3	6.7	1.4	18.3	62	21	33.9	22.3	47.0
Fever (axillary)	≥ 37.5°C	55	0	0.0	0.0	6.5	45	1	2.2	0.1	11.8	62	9	14.5	6.9	25.8
	> 39.0°C	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	Related	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	8	12.9	5.7	23.9
Headache	Any	55	19	34.5	22.2	48.6	45	7	15.6	6.5	29.5	62	15	24.2	14.2	36.7
	Grade 3	55	1	1.8	0.0	9.7	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	Related	55	13	23.6	13.2	37.0	45	2	4.4	0.5	15.1	62	13	21.0	11.7	33.2
Myalgia	Any	55	13	23.6	13.2	37.0	45	6	13.3	5.1	26.8	62	15	24.2	14.2	36.7
	Grade 3	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	0	0.0	0.0	5.8
	Related	55	11	20.0	10.4	33.0	45	2	4.4	0.5	15.1	62	15	24.2	14.2	36.7
Shivering	Any	55	9	16.4	7.8	28.8	45	3	6.7	1.4	18.3	62	16	25.8	15.5	38.5
	Grade 3	55	0	0.0	0.0	6.5	45	0	0.0	0.0	7.9	62	1	1.6	0.0	8.7
	Related	55	7	12.7	5.3	24.5	45	1	2.2	0.1	11.8	62	16	25.8	15.5	38.5

N = number of subjects with the documented dose

n (%) = number (percentage) of subjects reporting at least once the symptom
95% CI = exact 95% confidence interval; LL = lower limit, UL = upper limit
Any = incidence of a particular symptom regardless of intensity or relationship
Grade 3 = symptom that prevented normal activity.
Related = symptom considered by the investigator to have a causal relationship to study vaccination

Secondary Outcome Variable (s):
Seropositivity rates, seroprotection rate and GMTs for HI antibody titre at Day 0 and 21 (ATP cohort for immunogenicity)

Vaccine strain	Group	Timing	N	≥ 1:10				≥ 1:40				GMT		
				n	%	95% CI		n	%	95% CI		Value	95% CI	
						LL	UL			LL	UL		LL	UL
A/New Caledonia	Flu YNG	PRE	55	55	100	93.5	100	54	98.2	90.3	100	268.3	194.6	369.8
		PI(D21)	55	55	100	93.5	100	55	100	93.5	100	479.1	357.1	642.7
	Flu ELD	PRE	45	44	97.8	88.2	99.9	33	73.3	58.1	85.4	63.4	46.6	86.4
		PI(D21)	45	45	100	92.1	100	44	97.8	88.2	99.9	111.5	88.9	139.8
	FluAS25	PRE	61	61	100	94.1	100	41	67.2	54.0	78.7	57.9	45.8	73.1
		PI(D21)	61	61	100	94.1	100	57	93.4	84.1	98.2	127.4	100.6	161.5
A/Wisconsin	Flu YNG	PRE	55	52	94.5	84.9	98.9	47	85.5	73.3	93.5	89.1	64.3	123.5
		PI(D21)	55	55	100	93.5	100	53	96.4	87.5	99.6	256.6	198.9	331.0
	Flu ELD	PRE	45	38	84.4	70.5	93.5	24	53.3	37.9	68.3	40.6	26.6	61.9
		PI(D21)	45	45	100	92.1	100	40	88.9	75.9	96.3	151.5	108.8	211.0
	FluAS25	PRE	61	57	93.4	84.1	98.2	43	70.5	57.4	81.5	62.2	45.2	85.7
		PI(D21)	61	61	100	94.1	100	60	98.4	91.2	100	439.9	337.5	573.3
B/Malaysia	Flu YNG	PRE	55	55	100	93.5	100	37	67.3	53.3	79.3	58.7	44.4	77.5
		PI(D21)	55	55	100	93.5	100	55	100	93.5	100	472.9	364.1	614.2
	Flu ELD	PRE	45	45	100	92.1	100	37	82.2	67.9	92.0	72.4	56.1	93.5
		PI(D21)	45	45	100	92.1	100	45	100	92.1	100	194.0	142.9	263.3
	FluAS25	PRE	61	61	100	94.1	100	47	77.0	64.5	86.8	74.7	58.3	95.6
		PI(D21)	61	61	100	94.1	100	61	100	94.1	100	277.6	226.4	340.4

N = number of subjects with available results
n (%) = number (percentage) of subjects with antibody titres above the specified cut-off values
95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit
PRE = pre-vaccination Dose 1 (Day 0)
PI(D21) = post-vaccination Dose 1 (Day 21)

Secondary Outcome Variable (s):
Seroconversion rates (SCR) for HI antibody titre at Day 21 (ATP cohort for immunogenicity)

Vaccine strain	Group	N	SCR			
			n	%	95% CI	
					LL	UL
A/New Caledonia	Flu YNG	55	8	14.5	6.5	26.7
	Flu ELD	45	9	20.0	9.6	34.6
	FluAS25	61	13	21.3	11.9	33.7
A/Wisconsin	Flu YNG	55	16	29.1	17.6	42.9
	Flu ELD	45	18	40.0	25.7	55.7
	FluAS25	61	42	68.9	55.7	80.1
B/Malaysia	Flu YNG	55	39	70.9	57.1	82.4
	Flu ELD	45	13	28.9	16.4	44.3
	FluAS25	61	30	49.2	36.1	62.3

N = number of subjects with pre- and post-vaccination results available
n (%) = number (percentage) of seroconverted subjects
95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit

Secondary Outcome Variable (s):
Seroconversion factor (SCF) for HI antibody titre at Day 21 (ATP cohort for immunogenicity)

Vaccine strain	Group	N	SCF		
			Value	95% CI	
				LL	UL
A/New Caledonia	Flu YNG	55	1.8	1.5	2.1

	Flu ELD	45	1.8	1.4	2.2
	FluAS25	61	2.2	1.9	2.6
A/Wisconsin	Flu YNG	55	2.9	2.3	3.7
	Flu ELD	45	3.7	2.6	5.3
	FluAS25	61	7.1	5.2	9.6
B/Malaysia	Flu YNG	55	8.1	5.8	11.1
	Flu ELD	45	2.7	2.1	3.4
	FluAS25	61	3.7	3.0	4.6

N = number of subjects with pre- and post-vaccination results available
SCF = Seroconversion Factor or geometric mean ratio (mean[log10(POST/PRE)])
95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit

Secondary Outcome Variable (s):
Percentage of subjects with concentrations higher or equal to the LOQ level and GMCs for anti-MPL antibodies at Day 0 and 21 (ATP cohort for immunogenicity)

Group	**Timing**	**N**	**≥ 59 EL.U/mL**				**GMC (EL.U/mL)**		
			n	**%**	**95% CI**		**Value**	**95% CI**	
					LL	**UL**		**LL**	**UL**
Flu YNG	PRE	55	34	61.8	47.7	74.6	84.4	65.8	108.3
	PI(D21)	55	37	67.3	53.3	79.3	90.7	71.5	115.1
Flu ELD	PRE	45	19	42.2	27.7	57.8	59.1	45.3	77.2
	PI(D21)	44	13	29.5	16.8	45.2	49.3	38.2	63.7
FluAS25	PRE	61	42	68.9	55.7	80.1	147.2	103.6	209.3
	PI(D21)	60	55	91.7	81.6	97.2	387.0	285.2	525.0

N = number of subjects with available results
n (%) = number (percentage) of subjects with antibody concentration ≥ 59 EL.U/mL
95% CI = 95% confidence interval, LL = Lower Limit, UL = Upper Limit
PRE = pre-vaccination Dose 1 (Day 0)
PI(D21) = post-vaccination Dose 1 (Day 21)

Secondary Outcome Variable (s):
Descriptive Statistics on the frequency cytokine-positive CD4 T-cells (per million CD4 T-cells) for each vaccine strain at Day 0 and Day 21 (ATP cohort for immunogenicity)

Test description	**Measurement method used**	**Group**	**Timing**	**N**	**Nmiss**	**GM**	**SD**	**Median**
CD4- ALL DOUBLES	A/New Caledonia	Flu YNG	PRE	54	1	832.21	526.59	833.50
			PI(D21)	53	2	1220.53	838.59	1155.00
		Flu ELD	PRE	43	2	460.76	317.30	516.00
			PI(D21)	44	1	664.25	512.99	794.50
		FluAS25	PRE	60	1	690.55	395.68	721.50
			PI(D21)	61	0	1184.25	621.10	1322.00
	A/Wisconsin	Flu YNG	PRE	53	2	421.52	422.04	499.00
			PI(D21)	52	3	518.45	568.43	620.50
		Flu ELD	PRE	44	1	174.28	251.79	318.50
			PI(D21)	44	1	377.11	429.82	432.50
		FluAS25	PRE	58	3	357.91	351.04	375.50
			PI(D21)	60	1	538.37	425.47	671.50
	B/Malaysia	Flu YNG	PRE	53	2	893.32	669.79	858.00
			PI(D21)	53	2	1524.88	996.00	1463.00
		Flu ELD	PRE	44	1	575.53	297.39	592.00
			PI(D21)	44	1	896.18	655.34	914.50
		FluAS25	PRE	60	1	733.38	490.47	808.50
			PI(D21)	61	0	1390.92	865.58	1326.00
CD4- IFNγ	A/New Caledonia	Flu YNG	PRE	54	1	475.92	374.89	426.50
			PI(D21)	53	2	680.61	589.86	667.00
		Flu ELD	PRE	43	2	213.06	241.88	204.00
			PI(D21)	44	1	313.95	348.75	396.50

		FluAS25	PRE	60	1	300.64	294.85	332.00
			PI(D21)	61	0	566.98	444.43	678.00
	A/Wisconsin	Flu YNG	PRE	53	2	248.49	317.17	263.00
			PI(D21)	52	3	294.92	414.42	388.00
		Flu ELD	PRE	44	1	103.75	142.25	157.50
			PI(D21)	44	1	180.52	235.78	248.00
		FluAS25	PRE	58	3	191.70	253.62	218.00
			PI(D21)	60	1	330.31	273.76	366.00
	B/Malaysia	Flu YNG	PRE	53	2	478.34	558.54	485.00
			PI(D21)	53	2	815.31	677.78	931.00
		Flu ELD	PRE	44	1	223.96	190.96	274.50
			PI(D21)	44	1	367.53	408.02	390.00
		FluAS25	PRE	60	1	297.06	382.92	404.00
			PI(D21)	61	0	680.13	608.17	711.00
CD4- IL2	A/New Caledonia	Flu YNG	PRE	54	1	743.64	440.01	730.50
			PI(D21)	53	2	1043.88	762.46	1019.00
		Flu ELD	PRE	43	2	403.53	292.59	430.00
			PI(D21)	44	1	572.53	477.48	671.00
		FluAS25	PRE	60	1	606.16	361.70	607.00
			PI(D21)	61	0	1042.30	558.90	1121.00
	A/Wisconsin	Flu YNG	PRE	53	2	372.53	327.25	460.00
			PI(D21)	52	3	463.63	463.21	551.50
		Flu ELD	PRE	44	1	152.06	239.55	278.00
			PI(D21)	44	1	315.96	389.30	365.00
		FluAS25	PRE	58	3	332.62	319.02	347.00
			PI(D21)	60	1	486.19	368.73	577.00
	B/Malaysia	Flu YNG	PRE	53	2	786.77	602.90	776.00
			PI(D21)	53	2	1353.10	928.41	1352.00
		Flu ELD	PRE	44	1	480.65	289.67	501.00
			PI(D21)	44	1	794.53	601.25	813.00
		FluAS25	PRE	60	1	660.54	451.70	713.50
			PI(D21)	61	0	1224.10	796.14	1168.00
CD4- TFNα	A/New Caledonia	Flu YNG	PRE	54	1	622.59	456.97	686.00
			PI(D21)	53	2	976.89	678.18	881.00
		Flu ELD	PRE	43	2	363.71	238.04	436.00
			PI(D21)	44	1	522.06	401.84	641.00
		FluAS25	PRE	60	1	556.98	362.60	556.00
			PI(D21)	61	0	890.93	538.72	960.00
	A/Wisconsin	Flu YNG	PRE	53	2	311.10	391.87	406.00
			PI(D21)	52	3	428.24	532.41	495.00
		Flu ELD	PRE	44	1	149.60	198.86	255.50
			PI(D21)	44	1	357.18	354.94	331.00
		FluAS25	PRE	58	3	272.59	309.63	324.50
			PI(D21)	60	1	447.84	372.86	542.00
	B/Malaysia	Flu YNG	PRE	53	2	735.18	632.64	719.00
			PI(D21)	53	2	1200.99	816.23	1208.00
		Flu ELD	PRE	44	1	459.47	256.10	470.50
			PI(D21)	44	1	676.29	510.05	656.00
		FluAS25	PRE	60	1	580.22	448.04	637.50
			PI(D21)	61	0	1049.68	749.46	1020.00

N = number of subjects with available results
Nmiss = number of subjects with missing results
GM= Geometric Mean
SD = Standard Deviation
All doubles: T-cells producing at least 2 cytokines (among IL-2, TNF-α, IFN-γ)
IFN-γ: T-cells producing at least IFN-γ and another cytokine (IL-2, TNF-α)

IL-2: T-cells producing at least IL-2 and another cytokine (TNF-α, IFN-γ)
TNF-α: T-cells producing at least TNF-α and another cytokine (IL-2, IFN-γ)
PRE = Pre-vaccination Dose 1 (Day 0)
PI(D21) = Post-vaccination Dose 1 (Day 21)

Secondary Outcome Variable (s):
Descriptive Statistics on the frequency cytokine-positive CD8 T-cells (per million CD8 T-cells) for each vaccine strain at Day 0 and Day 21 (ATP cohort for immunogenicity)

Test description	Measurement method used	Group	Timing	N	Nmiss	GM	SD	Median
CD8- ALL DOUBLES	A/New Caledonia	Flu YNG	PRE	54	1	5.25	351.78	1.00
			PI(D21)	53	2	2.63	752.09	1.00
		Flu ELD	PRE	43	2	6.01	147.35	1.00
			PI(D21)	44	1	6.62	212.39	1.00
		FluAS25	PRE	60	1	10.15	113.81	1.50
			PI(D21)	61	0	7.84	746.90	1.00
	A/Wisconsin	Flu YNG	PRE	53	2	2.05	64.82	1.00
			PI(D21)	52	3	2.48	261.81	1.00
		Flu ELD	PRE	44	1	4.00	60.77	1.00
			PI(D21)	44	1	4.09	74.91	1.00
		FluAS25	PRE	58	3	5.79	114.87	1.00
			PI(D21)	60	1	6.57	117.40	1.00
	B/Malaysia	Flu YNG	PRE	53	2	6.27	188.40	1.00
			PI(D21)	53	2	6.20	113.59	1.00
		Flu ELD	PRE	44	1	7.29	125.49	1.00
			PI(D21)	44	1	9.09	143.66	1.00
		FluAS25	PRE	58	3	12.95	196.58	36.50
			PI(D21)	61	0	8.35	137.67	1.00
CD8- IFNγ	A/New Caledonia	Flu YNG	PRE	54	1	3.92	301.04	1.00
			PI(D21)	53	2	3.23	748.10	1.00
		Flu ELD	PRE	43	2	4.32	124.85	1.00
			PI(D21)	44	1	6.18	193.41	1.00
		FluAS25	PRE	60	1	7.21	92.94	1.00
			PI(D21)	61	0	7.49	105.94	1.00
	A/Wisconsin	Flu YNG	PRE	53	2	1.96	44.45	1.00
			PI(D21)	52	3	2.02	223.96	1.00
		Flu ELD	PRE	44	1	1.77	23.00	1.00
			PI(D21)	44	1	1.77	22.80	1.00
		FluAS25	PRE	58	3	2.48	39.82	1.00
			PI(D21)	60	1	2.48	62.90	1.00
	B/Malaysia	Flu YNG	PRE	53	2	6.22	161.76	1.00
			PI(D21)	53	2	5.25	83.86	1.00
		Flu ELD	PRE	44	1	3.16	83.75	1.00
			PI(D21)	44	1	3.12	103.60	1.00
		FluAS25	PRE	58	3	6.61	64.04	1.00
			PI(D21)	61	0	3.37	85.99	1.00
CD8- IL2	A/New Caledonia	Flu YNG	PRE	54	1	3.74	217.83	1.00
			PI(D21)	53	2	2.21	550.49	1.00
		Flu ELD	PRE	43	2	3.87	90.57	1.00
			PI(D21)	44	1	4.84	112.23	1.00
		FluAS25	PRE	60	1	5.42	73.74	1.00
			PI(D21)	61	0	5.79	744.94	1.00
	A/Wisconsin	Flu YNG	PRE	53	2	1.84	53.38	1.00
			PI(D21)	52	3	2.66	215.93	1.00
		Flu ELD	PRE	44	1	3.80	48.73	1.00
			PI(D21)	44	1	3.94	60.48	1.00

		FluAS25	PRE	58	3	4.05	120.14	1.00
			PI(D21)	60	1	5.73	107.00	1.00
	B/Malaysia	Flu YNG	PRE	53	2	4.19	98.65	1.00
			PI(D21)	53	2	5.60	81.91	1.00
		Flu ELD	PRE	44	1	5.68	118.30	1.00
			PI(D21)	44	1	7.02	123.09	1.00
		FluAS25	PRE	58	3	8.64	205.32	1.00
			PI(D21)	61	0	6.58	121.18	1.00
CD8- TFNα	A/New Caledonia	Flu YNG	PRE	54	1	3.93	350.61	1.00
			PI(D21)	53	2	3.23	739.68	1.00
		Flu ELD	PRE	43	2	4.26	130.20	1.00
			PI(D21)	44	1	7.55	207.40	1.00
		FluAS25	PRE	60	1	8.02	105.89	1.00
			PI(D21)	61	0	6.60	735.42	1.00
	A/Wisconsin	Flu YNG	PRE	53	2	1.99	60.29	1.00
			PI(D21)	52	3	2.67	262.06	1.00
		Flu ELD	PRE	44	1	3.58	60.75	1.00
			PI(D21)	44	1	4.90	80.79	1.00
		FluAS25	PRE	58	3	4.56	104.79	1.00
			PI(D21)	60	1	5.81	115.46	1.00
	B/Malaysia	Flu YNG	PRE	53	2	3.75	176.47	1.00
			PI(D21)	53	2	6.09	101.62	1.00
		Flu ELD	PRE	44	1	7.32	106.09	1.00
			PI(D21)	44	1	8.54	129.43	1.00
		FluAS25	PRE	58	3	11.88	187.29	33.50
			PI(D21)	61	0	6.35	142.35	1.00

N = number of subjects with available results
Nmiss = number of subjects with missing results
GM= Geometric Mean
SD = Standard Deviation
All doubles: T-cells producing at least 2 cytokines (among IL-2, TNF-α, IFN-γ)
IFN-γ: T-cells producing at least IFN-γ and another cytokine (IL-2, TNF-α)
IL-2: T-cells producing at least IL-2 and another cytokine (TNF-α, IFN-γ)
TNF-α: T-cells producing at least TNF-α and another cytokine (IL-2, IFN-γ) PRE = Pre-vaccination Dose 1 (Day 0)
PI(D21) = Post-vaccination Dose 1 (Day 21)

Secondary Outcome Variable (s):
Descriptive statistics on the memory B-cells frequencies (per million B-cells) for each vaccine strain at Day 0 and Day 21 (B-cells subset from ATP cohort for immunogenicity)

Stimulating antigen	Group	Timing	N	Nmiss	GM	SD	Median
A/New Caledonia	Flu YNG	PRE	17	3	6912.30	4414.16	7149.0
		PI(D21)	18	2	10817.80	5006.09	10881.0
	Flu ELD	PRE	18	2	9100.26	6263.37	9380.0
		PI(D21)	18	2	9993.72	6992.62	8691.0
	FluAS25	PRE	17	3	6005.00	9403.08	7118.0
		PI(D21)	14	6	11330.92	12770.90	16630.0
A/Wisconsin	Flu YNG	PRE	17	3	2267.70	2332.10	2974.0
		PI(D21)	18	2	4202.20	3828.62	4146.0
	Flu ELD	PRE	18	2	2031.06	2044.10	1602.5
		PI(D21)	18	2	3872.58	6283.34	3999.0
	FluAS25	PRE	17	3	2214.48	2857.94	4088.0
		PI(D21)	14	6	10793.78	17959.93	10190.0
B/Malaysia	Flu YNG	PRE	17	3	2812.41	2470.83	3454.0
		PI(D21)	18	2	6700.10	5645.00	7621.5
	Flu ELD	PRE	18	2	1575.32	2315.88	1517.5
		PI(D21)	18	2	6300.71	7763.56	8215.0

	FluAS25	PRE	17	3	3072.14	3652.99	3492.0
		PI(D21)	14	6	8601.58	8901.94	12458.5

N = number of subjects with available results
Nmiss = number of subjects with missing results
GM= Geometric Mean
SD = Standard Deviation
PRE = Pre-vaccination Dose 1 (Day 0)
PI(D21) = Post-vaccination Dose 1 (Day 21)

Safety Results: Number (%) of subjects with unsolicited adverse events occurring during the 21-day post-vaccination period (Total Vaccinated Cohort)

Most frequent adverse events - On-Therapy (occurring within Days 0-20 following vaccination)	**Flu YNG Group N = 55**	**Flu ELD Group N = 45**	**FluAS25 Group N = 62**
Subjects with any AE(s), n (%)	23 (41.8)	10 (22.2)	15 (24.2)
Subjects with any Grade 3 AE(s), n (%)	5 (9.1)	1 (2.2)	1 (1.6)
Subjects with any related AE(s), n (%)	4 (7.3)	1 (2.2)	4 (6.5)
Nasopharyngitis	6 (10.9)	3 (6.7)	4 (6.5)
Headache	4 (7.3)	1 (2.2)	2 (3.2)
Nausea	3 (5.5)	-	-
Pharyngolaryngeal pain	3 (5.5)	-	-
Pyrexia	2 (3.6)	-	1 (1.6)
Upper respiratory tract infection	1 (1.8)	2 (4.4)	-
Vomiting	1 (1.8)	1 (2.2)	1 (1.6)
Diarrhoea	1 (1.8)	-	1 (1.6)
Injection site induration	-	1 (2.2)	1 (1.6)
Injection site pruritus	1 (1.8)	-	1 (1.6)
Injection site reaction	2 (3.6)	-	-
Toothache	2 (3.6)	-	-
Vertigo	-	-	2 (3.2)
Arthralgia	-	-	1 (1.6)
Cough	1 (1.8)	-	-
Disturbance in attention	-	-	1 (1.6)
Dizziness	-	-	1 (1.6)
Dyspnoea	-	-	1 (1.6)
Ear pain	1 (1.8)	-	-
Feeling hot	-	-	1 (1.6)
Gastroenteritis	-	1 (2.2)	-
Hangover	1 (1.8)	-	-
Insomnia	-	-	1 (1.6)
Musculoskeletal stiffness	-	-	1 (1.6)
Neck pain	-	1 (2.2)	-
Pain in extremity	-	-	1 (1.6)
Pharyngitis	1 (1.8)	-	-
Pyelonephritis	-	-	1 (1.6)
Rhinitis	1 (1.8)	-	-
Sinusitis	1 (1.8)	-	-
Sneezing	-	-	1 (1.6)

-: Adverse event absent
Grade 3 AE: AE that prevented normal activity
Related AE: AE considered by the investigator to be causally related to the study vaccination

Safety Results: Number (%) of subjects with Serious Adverse Events (SAEs) during the entire study period (Total Vaccinated Cohort)

Serious adverse event, n (%) [n considered by the investigator to be related to study medication]

All SAEs	**Flu YNG Group N = 55**	**Flu ELD Group N = 45**	**FluAS25 Group N = 62**
Subjects with any SAE(s), n (%) [n related]	0 (0.0) [0]	0 (0.0) [0]	0 (0.0) [0]
Fatal SAEs	**Flu YNG Group**	**Flu ELD Group**	**FluAS25 Group**

	N = 55	N = 45	N = 62
Subjects with fatal SAE(s), n (%) [n related]	0 (0.0) [0]	0 (0.0) [0]	0 (0.0) [0]

Conclusion: At Day 21, at least 93.4% and 88.9% of the subjects had seroprotection rates (HI antibody titres ≥ 1:40) for the A/New Caledonia strain and for the A/Wisconsin strain, respectively while all subjects had seroprotection rates for the B/Malaysia strain. Across groups, pain was the most frequently reported solicited local symptom. Headache and fatigue were the most frequently reported solicited general symptoms for the FluYNG and the FluELD groups. In the FluAS25 group, the most frequently reported solicited general symptom was fatigue, followed by shivering. Unsolicited AEs were reported by 23 (41.8%), 10 (22.2%) and 15 (24.2%) subjects from the Flu YNG, Flu ELD and FluAS25 group, respectively. Unsolicited AEs reported by 5 (9.1%), 1 (2.2%) and 1 (1.6%) subjects from the Flu YNG, Flu ELD and FluAS25 group, respectively, were graded severe (Grade 3), while unsolicited AEs reported by 4 (7.3%), 1 (2.2%) and 4 (6.5%) subjects from the Flu YNG, Flu ELD and FluAS25 group, respectively, were considered by the investigators to be related to the study vaccination. No SAEs were reported throughout this study.

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