Clinical Study Protocol: FLU-v 003 (WITH AMENDMENTS)

A randomised, double-blind, placebo-controlled, single-centre phase IIb trial as part of the EU-funded UNISEC project to assess the immunogenicity and safety of different formulations and dosing regimens of FLU-v vaccine administered subcutaneously in healthy adults aged 18-60 years.

STATISTICAL ANALYSIS PLAN

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Clinical Phase:	IIb
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Study Principal Investigator:	Paul Groeneveld Isala Hospitals Isala Academy, department of Innovation and Science Dokter van Deenweg 1 8052 BP Zwolle The Netherlands
Sponsor:	PepTcell Limited (trading as SEEK) 45 Beech Street London EC2Y 8AD UK
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Written by:	Md. Atiqul Islam, PhD/ E. Hak, PhD/O. Pleguezuelos, PhD
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Statistical Analysis Plan for Clinical Study Protocol:

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Approved by:

Coordinating investigator	**Prof. dr. Eelko Hak** **PharmacoTherapy, -Epidemiology & -** **Economics** **University of Groningen, The Netherlands**
Date: 16 February 2018	Signature _____
Sponsor's Representative	Dr. Olga Pleguezuelos CSO & Project Manager, SEEK 45 Beech Street, London, EC2Y 8AD, United Kingdom
Date: _____	Signature _____
Biostatistician	**Dr. Md. Atiqul Islam** PharmacoTherapy, -Epidemiology & - Economics University of Groningen, The Netherlands
Date _____	Signature _____

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List of Abbreviations

ADCC	Antibody-Dependent Cell-Mediated cytotoxicity
AE	Adverse Event
CA	Competent Authority
CI	Confidence Interval
CEF	Cytomegalovirus Epstein-Barr virus Flu virus
CMI	Cell Mediated Immunity
eCRF	electronic Case Report Form
CTL	Cytotoxic T Lymphocyte
CV	Curriculum Vitae
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked ImmunoSpot
EU	European Union
EudraCT	European Drug Regulatory Affairs Clinical Trials
FACS	Fluorescence-Activated Cell Sorting
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
IB	Investigator's Brochure
ICF	Informed consent form
IFN-γ	Interferon-gamma
IL2	Interleukin-2
IL-4	Interleukin-4
IMP	Investigational Medicinal Product
ITT	Intention to treat
MedDra	Medical Dictionary for Regulatory Activities
MFAS	Modified Full Analysis Set
LMM	Linear Mixed Models
NIPH	Norwegian Institute of Public Health
NP	Nucleoprotein
P1	Peptide pool 1
P2	Peptide pool 2
PBMC	Peripheral Blood Mononuclear Cells
PP	Per Protocol
RUG	University of Groningen
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SEB	Staphylococcus Enterotoxin B
SC	Subcutaneous
SUSAR	Suspected Unexpected Serious Adverse Reaction
SOC	System Organ Class
SOP	Standard Operating Procedure
TCC	Trial Coordination Center
Th	T helper
TNF-α	Tumor Necrosis Factor alpha
UMCG	University Medical Center Groningen
WFI	Water For Injection

1 Introduction

1.1 Scope

This document contains detailed information to aid the production of the statistical report and Clinical Study Report (CSR) including a description of the summary tables, figures and listings for study FLU-v 003. The SAP is developed according to the CONSORT guidelines for the conduct and report of trials. Any deviation from the original SAP will be described and justified in the study report.

1.2 Trial Study Design

This is a single centre, randomised (2:2:1:1), double-blind, parallel group, placebo-controlled phase IIb study of FLU-v vaccine over up to a maximum of one year (from screening to study completion) with an envisaged enrollment of approximately 222 healthy adults aged 18-60 years, and at least 180 participants are expected to complete the study (1). Randomization will be performed using a web-based system (ALEA) by the Trial Coordination Centre at the University Medical Centre Groningen. Randomization will be stratified by age group (18-40, 41-60 years) with the 2:2:1:1 treatment ratio being maintained in appropriately sized blocks within each stratum to ensure similar age distribution between all treatment groups. This is because older subjects may be more prone to infection than younger subjects though they are not expected to respond differently to the treatments.

After providing consent, subjects will enter a screening period of approximately 7 days to verify eligibility for the trial. Baseline information on demographics and medical history will be collected including 15 ml of blood to examine blood picture, pregnancy test and antibodies against circulating influenza viruses. All screening evaluations will be reviewed for each subject and once confirmed eligible, the subject will be randomised. Randomised subjects will enter a 21-day treatment period, followed by 2 follow-ups on day 42 and 180, for a maximum of one year study participation (from screening to study completion). Subjects will attend clinic visits as shown in the Flow Chart (Please see Annex-I).

There will be two treatment sessions (Treatment 1 and 2 at visits 2 and 3 respectively). The first vaccination day will be designated as Day 0.

The study follows a factorial design where the two factors are treatment (FLU-v/placebo) and formulation (unadjuvanted/suspension, adjuvanted/emulsion). Subjects will be randomised in two strata (age 18 to 40, age 41 to 60) to one of the following treatment regimens:

- Group 1 (n=74): FLU-v (unadjuvanted) as a suspension in pH neutral HCl/NaOH (0.5mL) on Day 0 and Day 21
- Group 2 (n=74): (0.5mL) ISA-51-adjuvanted FLU-v emulsified in water for injection (WFI) on Day 0, saline (0.5mL) on Day 21
- Group 3 (n=37): Saline solution (0.5mL) on Day 0 and Day 21
- Group 4 (n=37): WFI and ISA-51 emulsion (0.5mL) on Day 0, saline (0.5mL) on Day 21

Table 1: Vaccination schedule

Group	Admin 1 (day 0)	Admin 2 (day 21)
1	FLU-v in pH neutral HCl/NaOH suspension	FLU-v in pH neutral HCl/NaOH
2	FLU-v adjuvanted with ISA-51+WFI emulsion	Saline
3	Saline	Saline
4	WFI and ISA-51 emulsion	Saline

Administrations will be given 21 ± 3 days apart. All administrations will be given subcutaneously. Solicited adverse events (AEs) will be collected by AE questionnaire/diary card for 21 days after each immunisation. Unsolicited adverse events and serious adverse events (SAEs) will be collected throughout the whole study period. The treatments will be administered starting in third quarter of 2016 in order to provide protection for the subsequent influenza season starting in December 2016. Blood samples will be taken from all subjects on day 0 (before the first vaccination), day 42 (21 days after the second vaccination) and day 180 (159 days after the second vaccination) for the evaluation of FLU-v-specific cellular and humoral immune responses. Incidence of RT-PCR-confirmed influenza A and/or B infection and severity and duration of symptoms in confirmed infected subjects will be recorded during the influenza

season (December 2016 to March 2017) to evaluate the clinical efficacy of the tested vaccines. Serum samples collected at the start of the trial (screening: visit 1) will be evaluated with serology tests at the end of the trial to detect antibodies against the influenza virus strains that circulated whilst efficacy was monitored. This is to identify subjects who had pre-existing antibodies to the circulating strains prior to the trial start.

Subjects are free to withdraw from the study at any time for any reason. In addition, the Investigator may withdraw a subject from the study in order to protect the subject's health. The reasons for withdrawal will be recorded on the CRF and included in the final report.

1.3 Study Objectives

To evaluate the safety and immunogenicity of the influenza vaccine (FLU-v, as a suspension or adjuvanted as emulsion) in healthy adults, in particular to show that the TH1 cytokine response at 42 and 180 days after vaccination is greater in the adjuvanted FLU-v and unadjuvanted FLU-v than in the placebo.

1.3.1 Primary objectives

1.3.1.1 Th1 Cellular Immunogenicity

- To compare the TH1 cytokine response in the treatment arms compared to placebo from baseline to day 42 and day 180 following vaccination.

1.3.1.2 Safety

- To evaluate the incidence of solicited AEs in all groups for 21 days after each immunization.
- To evaluate the incidence and nature of unsolicited AEs and SAEs in all subjects during the whole study period.

1.3.2 Secondary objectives

1.3.2.1 Th2 cellular immunogenicity

- To compare the TH2 cytokine response in the treatment arms compared to placebo from baseline to day 42 and day 180 following vaccination.

1.3.2.2 IgM and IgG antibody responses

- To evaluate the FLU-v specific IgG and IgM antibody responses in all subjects from baseline at day 0 to day 42 and day 180 following vaccination in the treatment arms compared to placebo arms.

1.3.3 Exploratory objectives

1.3.3.1 Cellular immunogenicity

- To evaluate the change from baseline in the level of FLU-v specific cellular immune responses based on additional CMI assays such as ELISPOT (Enzyme-Linked ImmunoSpot) in the treatment arms compared to placebo at 42 and 180 days following vaccination, in a subset of subjects. The subset of subjects will be selected after unblinding and analysing the data from the immunogenicity primary endpoint. The analytical lab will remain blinded.

- To evaluate whether FLU-v specific cellular immune responses prevaccination compared to postvaccination are able to cross-recognise different influenza strains *in vitro*. Strain recognition will be measured by ELISPOT (Enzyme-Linked ImmunoSpot) in a subset of subjects. The subset of subjects will be selected after unblinding and analysing the data from the immunogenicity primary endpoint. The analytical lab will remain blinded.

- To evaluate the effect of previous influenza vaccines on the cellular immune responses to FLU-v in all subjects.

The results from exploratory immunogenicity will be reported as an addendum to the main CSR.

1.3.3.2 IgG subclasses

- To evaluate the FLU-v specific IgG1 and IgG3 antibody responses from baseline at day 0 to day 42 and day 180 following vaccination in the treatment

arms compared to placebo arms. IgG1 and IgG3 subclasses will be measured in those subjects that were responders for IgG.

The results from the IgG subclass analysis will be reported as an addendum to the main CSR.

1.3.3.3 Clinical Efficacy

- To evaluate the efficacy of FLU-v vaccine, whether given as suspension or as adjuvanted (ISA-51) emulsion compared to placebo, in the reduction of the incidence of RT-PCR confirmed influenza A and/or B infection in all subjects during the influenza season 2016-2017.
- To evaluate the efficacy of FLU-v vaccine whether given as suspension or as adjuvanted (ISA-51) emulsion, in the reduction of symptom scores among RT-PCR confirmed influenza A and/or B infection cases during the influenza season 2016-2017.
- To explore the relationship between clinical efficacy and cellular and humoral response.

1.4 Schedule of visits

Time		**Screening**	**Day 0**	**Day 21**	**Day 42**	**Day 180**	**Unscheduled**	
Contact	**Expected Number of subjects prior to study conduct**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit**	**Phone call**
I. Study enrollment								
Check inclusion criteria	300	√	√					
Check exclusion criteria	222	√	√					
General physical examination	222	√						
Medical and medication history	222	√						
History of influenza vaccination (within 6 months)	222	√						
Alcohol and drug usage history/habits	222	√						
Smoking history/habits	222	√						
Demographic data	222	√						
Informed consent	222	√						
Blood sampling (15 ml for baseline blood profile, for all participants)	222	√						
Blood pregnancy test (only for female) part of the 15 ml baseline sample	Depends[1]	√						
II. Study procedure								
Randomization	222		√					
Pre-vaccination body temperature (judged by clinical Investigator)	222		√	√				
Blood sampling (50 ml for CMI measurements and 10 ml for the antibody response analysis)	222		√		√	√		
Vaccination	222		√	√				
30-minute post-vaccination observation	222		√	√				
Issue symptom diary card/diary score				√				
Issue AE diary card/questionnaire	222		√	√				

III. Study follow-up								
Examination of AE diary card/questionnaire Reporting of severe/high-grade solicited AEs by the subjects	222			√	√			
Physical examination of severe/high-grade AEs	Depends[1]						√	
Evaluation of severe AEs (soon after reporting by the subject until resolution of the event)	Depends[1]							√
Reporting of unsolicited AEs and SAEs	222	**Reported throughout the entire study period**						
Reporting of diary card/symptom score	222	**Self-recording daily during the influenza period.** **Self-reporting when reached the symptom criteria for swab sampling.**						
ILI (nasal and tonsil swabs) sampling	Depends[1]						√	
ILI follow-up (till resolution of the event)	Depends[1]							√
Recording of (urgent) hospitalization/care visit of all cause	Depends[1]							√

Note:

1. The number of subject depends on the actual needs/reporting cases.

2 Statistical Methods

2.1 Data Management

The data management system is Oracle Clinical (Version 4.6.6). Staff from the trial team at Isala Hospital in Zwolle will be trained for data-entry into the eCRF system. The trial team is responsible for entering all study related information from randomised subjects in the eCRF system. Based on the Data management plan created by the Trial Coordination Center (TCC) in the UMCG and the University of Groningen (RUG), the eCRF system will create queries for the trial site which they have to resolve. The study monitor will verify the eCRF data with the source documents and can create queries for the trial site based on the assessment of the source documents. However, the study monitor will not update the information him/herself. Also the data manager will not update the eCRF but will communicate any data edits or errors with the site. The data manager will keep track of a Data management report which will include e.g. protocol deviations and violations and reported SAEs.

2.1.1 Coding

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 17.1 (or higher) terminology for System Organ Class (SOC) and Preferred Term (PT).

2.2 Statistical Analysis Software

Data manipulation, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations will be stored on the password protected RUG computer network. Applicable software applications are Microsoft Office, primarily using SAS® version 9.4 for Windows and MIMOSA, and package in R (version 3.4.2). If the use of other software is warranted, the final clinical study report will specify what software was used for what purposes. Quality assurance of the statistical analysis will be carried out according to the Standard Operational Procedure (SOP) Statistical Analysis and Reporting version 1.0, presented in Annex-3.

2.3 Data Collection and data sets

Data generated as per protocol will be entered onto the eCRF in accordance with the parameters described in ICH Topic E6 for GCP "Guidelines - Responsibilities of Sponsor, Clinical Study Monitor, and Investigator". A study monitor will verify the source documentation at several site visits and review the eCRF data. Linkage of datasets (see Table 2) will be done by randomization number and data lock will conclude the final linkage after resolving inconsistencies during linkage.

Table 2: Datasets and contents

Dataset	*Expected Approximate Number of participants prior to trial conduct*	*Institution*
eCRF	*222*	*UMCG*
Randomization	*222*	*UMCG*
Primary CMI (FACS)	*222*	*RKI*
Primary CMI (ELISA)	*222*	*RKI*
Exploratory CMI (ELISPOT and Multiplex ELISA)	*Subset of subjects (approximately 50)*	*NIPH*
Secondary FLU-v Antibody assays	*222*	*NIPH*
HAI at screening	*222*	*NIPH*
Influenza symptoms	*222*	*UMCG/Isala*
Influenza RT-PCR	*Only those that are swabbed*	*UMCG*

2.4 Handling of Missing Data

For the cellular and humoral responses (e.g. IFNγ data from ELISA and FACS data), missing values will be explained in individual data tables. No procedures for replacing missing values by estimates will be implemented, and complete case analysis will be conducted. Sensitivity analysis will be conducted, for example using multiple imputation (if needed), and from the PP analysis to see whether the conclusions are similar, and the results will be documented accordingly.

Note that every effort should be made to ensure that subjects are followed up and attend clinic visits in order to minimize the amount of missing data. For outliers, sensitivity analyses may be conducted excluding any values which appear to be outliers to investigate their influence on results. If there is scientific evidence or explanation for one or more

outliers, this will be presented and the results of the analyses will be discussed in the light of such explanations. However, the primary analysis will be based on all data in the Full Analysis Set (FAS) including all subjects with pre-vaccination data, two vaccinations and at least one set of post-vaccination data. Note that appropriate data transformation (e.g. logarithmic) may reduce the influence of outliers, and will be preferred over excluding cases.

All summary tables will show the number of cases with missing data. Any deviation from the original statistical analysis plan (SAP) will be described and justified in the study report.

2.5 General Statistical Methods

All measured variables and derived parameters will be listed individually by treatment group and subject number, and according to ITT, Full Analysis Set (FAS), Safety set, or Per Protocol (PP), see also 2.7. All key data will be summarized in tables using appropriate summary statistics by treatment group.

Continuous variables will be summarized as mean with standard deviation (SD) or median with interquartile range (IQR) and/or 95% Confidence Interval (CI) depending on the nature of the variables. Categorical variables will be presented as frequency counts (N) with percentages. For parametric distributed data, analysis of variance (ANOVA) will be used to compare means across treatment arms, and for non-parametric data Kruskal-Wallis test will be used to test whether samples of four arms originate from the same distribution, and will be followed by post-hoc tests to see the exact differences between two arms. For categorical data, Chi-square test or Fisher's exact test will be conducted to compare proportions across different treatment arms. All adverse events and concomitant medications will be recorded as frequencies (N) for the Safety Population.
For specifics see section 2.9.

2.6 Sample Size

Although a variety of immune parameters will be analyzed, the minimum sample size has been determined on the basis of the FLU-v-specific IFN-γ responses (assessed by ELISA)

recorded in an earlier Phase I trial, FLU-v-001 (2). IFN-γ is one of the most important markers of cell-mediated immunity for influenza protection. Taking the likely pattern of response and a loss to follow up (~20%) into account, it was estimated that 222 subjects were required in order to detect a 2.5-fold increase in the normalized value (change from baseline) for adjuvanted and unadjuvanted FLU-v relative to placebo.

The primary analysis objective of the study is to assess whether the active treatment (vaccine), in particular the adjuvanted version (administered as emulsion), produces a greater TH1 cytokine response than when given as a suspension (i.e. no adjuvant). It is therefore necessary to power the study to detect an interaction between the effects of treatment (i.e. vaccine versus placebo) and delivery method (adjuvanted or emulsified versus saline or solution). If there is such an interaction i.e. if the effect of the adjuvanted vaccine compared to placebo is greater than the effect of the vaccine when given as a solution compared to placebo then the study should be able to detect this. Conversely, if there is no such effect, then it is possible to test the overall effect of the vaccine relative to the control, irrespective of whether it is delivered as an emulsion (adjuvanted) or as a solution (non-adjuvanted). Another requirement for the study is to estimate the difference between the adjuvant and non-adjuvant administration of the vaccine.

The proposed plan is to do a weighted allocation of patients to treatments, with twice as many patients allocated to the active treated group than to the placebo. Since there are two factors namely treatment and formulation, the study will follow a factorial design, so there are effectively 4 groups of patients. Three scenarios were used to derive the sample size and to investigate the power, of which the first (see below) was used as the model to drive power calculations. All calculations assumed that the final data will be log-transformed since the data is likely to be very skewed with some high values. Furthermore, analyzing logged data allows estimates to be produced of fold-increases in cytokine responses, which are perhaps more readily interpretable. The assumptions are set out in Table 3.

Table 3 Scenario used for power calculations (Numbers in cells contain $\log_e$ response)

Scenario	Relevant tests	Treatment	Adjuvant therapy	
			No	Yes
2-fold increase Active non-adj. vs. placebo non-adj. 5-fold increase Active adj. vs. placebo adj.	Interaction Active vs placebo (adj.)	Placebo	2.5	2.5
2.5-fold Increase adj. vs. non-adj. (active)	Adj. vs non-adj. (active)	Active	3.2	4.1

The sample size calculations were conducted using PROC GLMPOWER in SAS® v9.4. This allows you to vary the input assumptions as well as investigating specific tests for the various treatment combinations. The analysis would be conducted using analysis of variance. The inclusion of baseline information may be performed using analysis of covariance.

A two-sided hypothesis test with Type I error (alpha) of 5% was assumed throughout. The target power was 80%. A standard deviation of 1 was assumed, and calculations have been done for equal allocation and also 2:1 allocation (active relative to placebo).

The most important test is that of the interaction between the effects of treatment and adding the adjuvant therapy. If this is non-significant then it is possible to conduct an overall test of the treatment effect. In particular, for Model 1, where there is an interaction, it is important to be able to detect this. Table 4 shows the total number of patients required for the study, in order to achieve 80% power for the various tests.

Table 4: Total sample size for study

Model	Test	SD=1.0	
		1:1 allocation	2:1 allocation
1	Interaction	160	180
2	Active adj. vs. placebo adj.	132	150
3	Adj. vs. non-adj. (active)	80	66

The sample sizes in Table 4 do not allow for loss to follow up, assay problems or any other major protocol deviations. If a withdrawal rate of 20% is assumed, the figures need to be divided by 0.80. So, for example, 200 patients would need to be recruited to achieve 160 evaluable patients.

The final choice of sample size needs to be made considering the relative importance of the various tests and the likelihood of the proposed treatment differences occurring. As a first step the interaction test is the most important: if there is no interaction the numbers required on each treatment to test for an overall treatment effect are relatively small. To test for interaction, equal allocation is more efficient than unequal allocation.

Based on all the information provided, a sample size of 222 subjects was chosen, assuming loss to follow up of approximately 20% to provide 80% power to test the interaction between formulation and treatment based on the assumptions using a 2:1 allocation and a SD = 1.

2.7 Study Populations

All analysis populations will be determined at the end of the study when the database is completed before the unblinded data is released to the Statistician and Sponsor in order to avoid any bias. Subjects will be assigned to the different populations based on the information provided in the data management report. Listings will be made of all visits with numbers of subjects according to ITT, FAS, PP and Safety population for each group but only the FAS and PP immunogenicity data and the safety data from the Safety population will be analysed and presented in tables and/or figures

Four analysis populations are defined as follows:

2.7.1 Intention to Treat

The Intention to treat (ITT) population comprises all subjects randomised to receive treatment, irrespective of whether they receive any injections.

2.7.2 Full Analysis Set

The Full Analysis Set (FAS) will be derived from the ITT population to include any subject that received vaccinations on day 0 and day 21, has pre-vaccination cellular immunological data and at least one set of post-vaccination data (i.e. at either Day 42 or Day 180). All primary and secondary immunogenicity endpoints will be analysed in the FAS population.
Exploratory immunogenicity analysis will be also performed in a subset of the FAS population determined based on the results of the primary and secondary immunogenicity results gathered after unblinding the study. The external laboratory performing the exploratory analysis will remain blinded.

Clinical efficacy exploratory analysis will be performed in the FAS population.

2.7.3 Per Protocol

The Per Protocol (PP) population comprises those subjects who receive both injections, as per the randomisation schedule who provide a blood sample for cellular immunogenicity determination on Day 0, Day 42 and Day 180, and who do not have any major protocol

violations regarding to eligibility criteria or study procedures. FAS analysis set subjects will be reviewed for their inclusion/exclusion to the PP analysis set. Analysis of all primary and secondary immunogenicity endpoints will be repeated in this population.
All exploratory clinical efficacy analysis will be repeated in this population.

2.7.4 Safety

The safety population comprises all subjects who received at least one injection. Safety analyses will be conducted using the safety population.

2.8 Level of Significance

Throughout the analysis, two-sided 5% level of significance will be used. In case of MIMOSA analysis of FACS data, False Discovery Rate (FDR)<0.05 will be conducted.

2.9 Statistical Methods

In general, analysis in the FAS and PP populations will be conducted to determine relative risk reductions/increases and absolute risk with their corresponding 95% confidence interval for the primary outcomes. Relative increase in positive response rates for any cellular markers at the patients level will also be expressed in percentage (Relative Risk-1 times 100) and corresponding 95% CIs. Differences between treatment arms will be tested using ANOVA or Kruskal-Wallis tests depending on the distribution of the variables, or Chi-square tests for categorical data.

2.9.1 Primary endpoints

2.9.1.1 Cellular immunogenicity

2.9.1.1.1 Th1 responses by Multiparametric Flow Cytometry

Cellular immune responses will be evaluated using FACS analysis on days 0, 42 (primary objective 1) and 180 (primary objective 2) in the FAS and PP populations. With FACS analysis it is possible to simultaneously measure CD3 (whole lymphocytes), CD3+CD4+ (helper T cells), CD3+CD8+ (cytotoxic T cells) and the functional markers response (i.e.,

IFN-γ, IL2, IL4, CD107a, TNF-α), where IFN-γ is considered one of the most important clinical markers of T helper 1 (Th1) responses and was used to calculate the sample size. IFN-γ, IL2, and TNF-α are known as Th1 cytokines which are important mediators of cellular immune responses to combat intracellular infections, and these markers will be tested in the primary endpoint. IL4 is known as Th2 cytokine associated with humoral immune responses, and will be analysed as a secondary endpoint (section 2.9.2.2).

The primary outcome measurement is based on Th1 cytokines produced by CD4+ and CD8+ T cells.

2.9.1.1.1.1 Data acceptance criteria <u>Multiparametric Flow Cytometry</u>

- Any set of data with less than 20,000 counts for CD3+ cells will be removed
- Any set of data with less than 3-fold difference between the number of positive cells for IFN -γ in the control and SEB will be removed.

Sensitivity analysis will be conducted with the subset of samples where:
Any data sets where the control has >10 more positive cells than peptide pool 1 (P1) or P2 or the control has 10% more positive cells than P1 or P2 will be removed.

2.9.1.1.1.2 Analyses with the accepted data sets

To assess the immunogenicity of FLU-v influenza vaccine, Mixture Models for Single-Cell Assays (MIMOSA) (3,4) analysis will be applied and based on a specific antigen or antigen pool, and then counting the number of CD4 or CD8 T-cells that produce each of the measured cytokines in response to stimulation.

Counts are produced for a negative control, two different peptide pools (high and low concentration) identical to the influenza antigens included in the vaccine FLU-v and two positive controls (SEB and CEF).

Briefly, MIMOSA is a two-component Beta-Binomial or Dirichlet-Multinomial mixture model to analyze a cytokine or group of cytokines and counts of cytokine positive and negative cells for antigen-stimulated and unstimulated samples from multiple individuals. Different antigens usually induce very different functional profiles, and many of the possible functional cell subsets are not expected to be associated with antigen specificity.

Our interest is in measuring how response changes over time (e.g. before and after vaccination). However, just measuring the stimulated sample is usually insufficient as there may be some background response even in the absence of stimulation. That is to say, there may be some T-cells that produce elevated cytokines even in the absence of stimulation. Therefore, we need to account for this background. To do so, we generally have two samples per subject, a stimulated sample and a non-stimulated sample and Fisher's Exact test can handle for a significant increase to subject's T-cells response to antigen stimulation. Also the distribution of responses in the stimulated and non-stimulated samples might be different. MIMOSA models the cell counts across all subjects as arising from a mixture of two processes, either from the non-responder distribution or from the responder distribution. The expected proportion of positive cells arising from the responder distribution should be greater than that arising from the background / non responder distribution.

MIMOSA will provide separate results on whether a subject had a positive response for a particular Th1 cell activation marker (IFNγ,TNF-α, IL-2, CD107a) to a specific stimulant (2 different peptide pools) at a specific time-point (day 0, day 42 and day 180).

In these analyses a positive responder for a specific marker under study at a certain time point (day 0, day 42 or day 180) is defined as a person who has a positive response for that marker to any of the two tested pools based on the FDR-derived p-value (<0.05) at day 0, day 42 or day 180. Rates of positive responders per marker per treatment group will be compared using Chi-square tests or Fisher's exact test depending on the cell counts.

The increase in response from prevaccination to postvaccination will be expressed as the mean of ratios per treatment group for day 42 and day 180 compared to day 0, and differences in mean ratio's will be compared between treatment arms. In addition, if the fold-change in ratio's is V2/V4 or V2/V5 >2 then, subjects will be designated responder. Chisquare tests will be applied to test for statistical differences among the treatment arms and positive responder for each visit. The mean increase in response among the treatment arm will be tested in ANOVA/Kruskal- Wallis test depending on the nature of outcomes. Later post-hoc test using Dunnet's method will be used to test which arm will be significantly different compared to placebo.

2.9.1.1.2 INF-γ by ELISA

2.9.1.1.2.1 Data acceptance criteria ELISA

Data will be rejected based on the following conditions:

1. SEB lower limit: if lower than 1,500pg/ml, the sample set will be rejected

Or

2. Control upper limit: control must never be higher than 500pg/ml, if it is, the sample set is rejected.

Or

3. When controls are higher than P1 or P2:

- If control is lower or equal to 50, sample sets will be rejected when the control is more than 200% of the highest value for P1 or P2, will be rejected.
- If control is higher than 50, samples set will be rejected when the difference between the control and the for P1 or P2 is more than 30% of the highest value from P1 or P2

If for a subject day 0 and day 42 are valid but not day 180, analysis for day 42 will still be made. When day 0 and day 180 are valid and day 42 is rejected, the analysis can be done for day 180. If day 0 is rejected, then no further analysis can be done.

2.9.1.1.2.2 Analysis with the accepted data sets

Cellular Immunogenicity data will also be assessed by measuring the amount of secreted IFN-γ using ELISA. The data will be analysed as follows: data for each time point will be normalised by subtracting the signal for non-stimulated cells (control) from the signal for FLU-v stimulated cells (P1 or P2 peptide pools). If the normalized data above is negative for day 42 or day 180, a value of zero will be allocated. This indicates no increase in response. If the normalized data for day 0 is negative, a value of one will be allocated. The positive control will only be used to determine that cells were viable and the assay was successful. After normalizing the data, we will then compare normalized data for a particular stimulant at time 0 to time 42 or to time 180. An increase of at least 2-fold is necessary to determine that the subject responded to that stimulant on that particular time point.

To determine that the subject responded to the vaccine in a particular time-point, there should be a positive response from at least one of the vaccine related stimulants (P1 or P2). Responder rates between arms will be compared by Chi-square tests for each time point. Finally, the fold increase in IFNγ response will be calculated for both time points day 42 and day 180. The mean increase in response among the treatment arm will be tested in ANOVA/Kruskal- Wallis test depending on the nature of outcomes. Later post-hoc test using Dunnet's method will be used to test which arm will be significantly different than placebo.

2.9.1.2 Safety

An AE is any untoward medical occurrence occurring after the subject has given informed consent, whether or not considered related to the investigational product

2.9.1.2.1 Solicited Adverse Events: Post-vaccination diary cards

Solicited AEs in all subjects will be allowed within the time frame of day 0 until 21 days after each vaccination. Solicited AEs include the AEs listed on the AE diary card. Subjects must return the diary cards on the scheduled visit. Any deviation beyond that time will be reported as a protocol deviation.

2.9.1.2.2 Unsolicited Adverse Events

Unsolicited AEs and SAEs will be collected during the entire period of time the subject is enrolled in the study.

2.9.1.2.3 Serious Adverse Events

SAEs are defined as any untoward medical occurrence that resulted in:

- Death
- Life threatening
- Hospitalization or prolongation of an existing hospitalization
- Disability/incapacity
- Congenital anomaly/birth defect

2.9.1.2.4 Analysis of AEs and SAEs

Adverse events will be coded according to coding dictionaries (MedDRA version 17.1 or higher) for System Organ Class (SOC) and Preferred Term (PT). The incidence rate (number and percentage of subjects) will be presented for each vaccine group:

(1) at least one AE (solicited and unsolicited)
(2) at least one systemic AE (solicited and unsolicited)
(3) with each individual solicited AE (local and systematic)
(4) any AE (by preferred term following MedDRA coding) will be reported.

All adverse events will be listed (solicited and unsolicited separated) with information on onset, duration, frequency, severity, seriousness, relationship to the study medication, outcome and action taken. Frequency tables for severity and relationship to study medication will be provided by treatment group. Treatment emergent adverse events will also be presented by treatment group.

For each vaccine group the incidence rate (number and percentage of subjects with at least one SAE will be reported. Relatedness to the study medication will also be reported. SAEs and withdrawals due to AEs will be described in detail.

Formal hypothesis testing will not be conducted on the adverse event data since the study is not powered to detect differences in adverse event incidence. The absence of statistically significant differences would not mean that there is no difference in the safety profile. Estimates and confidence intervals therefore provide a more useful means of evaluating any difference in the safety profile. Graphs will be used to complement tables where appropriate.

2.9.2 Secondary endpoints

2.9.2.1 Immunoglobulin G (IgG) and M (IgM) specific for FLU-v

For the humoral response, titers of IgG and IgM specific for FLU-v will be collected in every single subject at day 0, day 42 and day 180. An increase of at least 2-fold in the antibody titer from prevaccination to postvaccination is necessary to determine that the subject showed a positive antibody response to the antigens in FLU-v at a particular time point. The changes from baseline in humoral response over time (day 42 and day 180) will be analyzed by Linear Mixed Models (LMM). IgG and IgM specific for FLU-v will be analyzed separately using LMM. The change of IgG/IgM from baseline will be compared between the treatment groups using LMM taking into account the correlation between repeated observations made on the same individual. Depending on the data structure, the model may include subject effects as random and fixed effect terms for time (day 0, 42, 180), treatment (FLU-v or placebo), formulation (adjuvanted or non-adjuvanted) and the interaction between treatment and formulation. The terms comprising treatment formulation and their interaction together allow for the evaluation of the overall difference between the four treatments. The significance of the interaction effect tests whether the effect of FLU-v relative to placebo differs according to whether it is administered as a solution (non-adjuvanted) or as an emulsion (adjuvanted) on IgG and IgM separately. If IgG or IgM specific for FLU-v data seem to be skewed with some very high values, data will be transformed appropriately (i.e., log) before analysis.

The analysis will provide estimates of the difference between groups from day 0 to either day 42 or day 180 on the log scale (if outcome is log-transformed) which can be back-transformed to give ratios of geometric means. This therefore provides estimates of the fold-increases in response. The absolute means will give estimates of the ratio relative to baseline. All estimates of treatment differences will be accompanied by 95% confidence intervals. The frequency of responders per treatment group, the geometric mean titer per

treatment group and the average response increase per treatment group will be presented in tables and/or figures.

2.9.2.2 Th2 responses: IL-4

Th2 cellular responses characterized by production of the cytokine Interleukin-4 (IL-4) will be measured by multiparametric flow cytometry. Data will be accepted and analysed as explained in section 2.9.1.1.1 Th1 responses by Multiparametric Flow Cytometry FACS: MIMOSA

2.9.3 Exploratory Endpoints

2.9.3.1 IgG subclasses

Titers of IgG1 and IgG3 specific for FLU-v will be collected in every single subject at day 0, day 42 and day 180. The changes from baseline over time (day 42 and day 180) will be analyzed by Linear Mixed Models (LMM). IgG1 and IgG3 specific for FLU-v will be analyzed separately using LMM. The change of IgG1/IgG3 from baseline will be compared between the treatment groups using LMM taking into account the correlation between repeated observations made on the same individual. Depending on the data structure, the model may include subject effects as random and fixed effect terms for time (day 0, 42, 180), treatment (FLU-v or placebo), formulation (adjuvanted or non-adjuvanted) and the interaction between treatment and formulation. The terms comprising treatment formulation and their interaction together allow for the evaluation of the overall difference between the four treatments. The significance of the interaction effect tests whether the effect of FLU-v relative to placebo differs according to whether it is administered as a solution (non-adjuvanted) or as an emulsion (adjuvanted) on IgG1 and IgG3 separately. If IgG1 or IgG3 specific for FLU-v data seem to be skewed with some very high values, data will be transformed appropriately (i.e., log) before analysis.

The analysis will provide estimates of the difference between groups from day 0 to either day 42 or day 180 on the log scale (if outcome is log-transformed) which can be back-transformed to give ratios of geometric means. This therefore provides estimates of the fold-increases in response. The absolute means will give estimates of the ratio relative to baseline. All estimates of treatment differences will be accompanied by 95% confidence intervals. The geometric mean titer per treatment group and the average response increase per treatment group will be presented in tables and/or figures.

2.9.3.2 Influenza strain cross-recognition by ELISpot

The frequency of PBMCs producing IFNg or Granzyme B in response to inactivated influenza virus or FLU-v antigens will be measured by ELISPOT and represented as spot-forming units (SFUs)/mill cells (5). A subset of responder subjects based on the primary immunogenicity endpoints will be selected. The changes from prevaccination to postvaccination for all stimulants will be presented separately and will be analysed by Linear Mixed Models (LMM). The change in SFUs per stimulant will be compared between treatment groups using LMM, taking into account the correlation between repeated observations made on the same individual. Depending on the data structure, the model may include subject effects as random and fixed effect terms for time (day 0, 42, 180), treatment (FLU-v or placebo), formulation (adjuvanted or non-adjuvanted) and the interaction between treatment and formulation. The terms comprising treatment formulation and their interaction together allow for the evaluation of the overall difference between the four treatments. The significance of the interaction effect tests whether the effect of FLU-v relative to placebo differs according to whether it is administered as a solution (non-adjuvanted) or as an emulsion (adjuvanted). If the data seem to be skewed with some very high values, data will be transformed appropriately (i.e., log) before analysis.

The absolute SFUs/ mean or median (depending on the distribution of the variable) per stimulant, treatment group, and time point, as well as the average response increase per influenza strain per treatment group will be presented in tables and/or figures.

2.9.3.3 Clinical Efficacy

2.9.3.3.1Reduction in the number of influenza-confirmed infections

The numbers of influenza-confirmed subjects is expected to be small (approximately 10-20% of subjects) based on the influenza infection rates in healthy adult population. Strain-specific influenza will be categorized into RT-PCR confirmed influenza A, or B, or the combination, and differences in outcome rates will be tested by chi-square tests.

An exploratory analysis looking at the relationship between clinical efficacy (i.e. infection rates) and the cellular and humoral responses will be performed by correlation analyses, and depending on the data structure and numbers. This will include graphical summaries of the data. For each vaccine group, the incidence rate of subjects with each strain-specific RT-PCR confirmed influenza A and/or B infection will be presented and will be investigated by proportion test between treatment arms.

2.9.3.3.2 Reduction in the symptomatology in influenza-confirmed infections

The severity of the influenza symptoms in laboratory-confirmed influenza cases will be expressed as:

-Total Symptom score (upper and lower respiratory and systemic symptoms) experienced which is calculated as total sum of symptoms over the duration of illness.

-Symptom duration (days): Number of days from first symptom to last symptom. Intervening days with no symptoms or missing symptom data will be included in the duration.

-Total symptoms score peak (i.e. the highest level of the total sum of all symptoms recorded on any day). Each symptom is scored as 0 to 3 and all scores for all symptoms are added for a single day.

- Average Symptom severity score: Scores for each symptom are added from the day the first symptom appeared to the day the last symptom persisted. Each symptom is scored 0-3 depending on the severity: no symptoms=0, just noticeable=1, bothersome but can still so activities=2, bothersome and cannot do daily activities=3. The sum of all the scores constitutes the daily symptom score. The overall score over the duration of illness is then

divided by the total number of days symptoms were observed. The results will be summarized in tables of descriptive statistics, calculating the median and interquartile range and then performing a two-sided Wilcoxon Rank Sum Test at 0.05 level of significance.

2.9.3.4 Effect of previous vaccinations on immunogenicity

Effect of previous influenza vaccines on the primary and secondary endpoints of immunogenicity as well as the clinical exploratory endpoints will be assessed after stratifying the subjects' data as follows:

-never received influenza vaccination.

-received influenza vaccination in the previous 24 months

-received influenza vaccination more than 24 months

Subgroup analyses will be pre-planned for comparing the responses (either immunological or clinical) in those with and without previous exposure to influenza vaccines. The study was not powered to analyse stratified data and the results are likely to show trends rather than statistical significant differences.

2.9.4 Multiplicity

No formal adjustment of significance levels will be performed, and p-values derived from multiple tests will be interpreted in the light of multiplicity. Since there are several primary outcome variables, namely the three TH1 cytokines, there is a risk of inflation of the Type 1 error (i.e. obtaining false positive results). As all FACS derived endpoints will be analyzed by MIMOSA model, False Discovery Rate (FDR) Q-value derived from p-values will handle the risk of Type-I error for one marker.

2.9.5 Protocol deviation/violations

Protocol deviations/violations will be identified based on conditions related to the categories:

2.9.5.1 Protocol entry criteria

Deviations from any criteria after inclusion will be reported in the listing.

2.9.5.2 Rescheduled study visits

Vaccination will be given at visit 2 on day 0 and at day 21±3 onvisit 3, so for visit 3, a window of 18-24 days is allowed. For all other study visits, a window of ±3 days is allowed. Otherwise it will be reported as a protocol deviation.

2.9.5.3 Outlier detections

All outliers of primary, secondary and exploratory endpoints will be checked. Sensitivity analyses may be conducted excluding any values which appear to be outliers to investigate their influence on results. If there is scientific evidence or explanation for one or more outliers, this will be presented and the results of the analyses will be discussed in the light of explanations. Of note, transformation of certain values may be a more appropriate technique to control for outlier values.

Major protocol deviations will be identified before the study closure, and listed in the Data Management Report. Protocol violations e.g. failure to obtain valid informed consent, accidental distribution of incorrect study injection or dose, not following inclusion/exclusion criteria, use of prohibited concomitant medications will also be reported.

2.10 Post-hoc analyses

Where possible all analyses will be prospectively planned and carried out according to this SAP. However, to gain more insights into the action of the vaccine or in the event of any significantly anomalous data or events which make any data or conclusions unreliable or scientifically questionable, then further relevant post-hoc analyses may be carried out. For example, these may include:

1. Analysis of double (responder for two cytokines) or triple (responders for three cytokines) responders by flow cytometry;
2. Analysis of primary, secondary and exploratory endpoints comparing treatment groups to combined placebo as below:

- Adjuvanted FLU-v vs combined placebo
- Non-adjuvanted FLU-v vs combined placebo

3. Comparison of Adjuvanted FLU-v vs non-adjuvanted FLU-v for all primary, secondary and exploratory endpoints.

4. Primary immunogenicity analysis performed for IFNγ analysis may be done replacing original data with data from repeated test following the same analyses as with the original data.

5. Additional immunogenicity analysis may be performed to provide wider knowledge on the mode of action of the vaccine. For example, but not limited to, Antibody-Dependent Cell-Mediated cytotoxicity (ADCC), complement activation, multiplex ELISA.

6. Efficacy analysis may also be performed in a subpopulation of the FAS: the modified FAS (MFAS) based on the prevaccination levels of HAI titers to the different strains. The current HAI titer cut-off that defines a 50% protection in the overall population is 40. This cut off and additional cut offs based on the level of protection seen in our study may be used to select the subpopulation.

7. Primary immunogenicity (Flow cytometry and IFNγ ELISA) may be analysed defining a responder as a subject that is positive for at least one of the following 5 peptide pools: high or low FLU-v GMP peptides, non-acetate high or low FLU-v peptides, overlapping peptides covering the regions of FLU-v.

3 List of tables, listings and figures

3.1 List of Tables

- All tables will be presented by treatment group and visit (if appropriate), see also Appendix Tables.
- Descriptive Statistics include N (sample size), mean, median, standard deviation, minimum, maximum and 95% CI (if appropriate).
- Frequency counts include number of events and percent (denominator of percent is equal to the total number of subjects measured)
- Safety Tables will include the safety population

14.1.1	Number of subjects per treatment group for the different analysis populations
14.1.2	Demographics Data (Safety Population)
14.1.3	Demographics Data (FAS Population)
14.1.4	Summary of Solicited Adverse Events from Post-vaccination diary card 1 (Safety Population)
14.1.5	Summary of Solicited Adverse Events from Post-vaccination diary card 2 (Safety Population)
14.1.6	Solicited and unsolicited Adverse Event summary (overall) (Safety Population)
14.1.7	Treatment Emergent Adverse Events Analysis (Safety Population)
14.1.8	Adverse events reported as mild in intensity by Classification (Safety population)
14.1.9	Adverse events reported as moderate in intensity by Classification (Safety population)
14.1.10	Adverse events reported as severe in intensity by Classification (Safety population)
14.1.11a	Adverse events reported as related to vaccine reported as mild by classification (Safety population)
14.1.11b	Adverse events reported as related to vaccine reported as moderate by classification (Safety population)
14.1.11c	Adverse events reported as related to vaccine reported as severe by classification (Safety population)
14.1.12	Adverse events at injection site (Safety population)
14.1.13	Serious Adverse Events by classification (Safety Population)
14.1.14	Clinical Laboratory Safety - Overall Summary (Safety Population)
14.1.15	Previous influenza vaccinations (FAS population)
14.1.16	HAI titers at screening (Phuket, Brisbane, Michigan, Hong Kong strains)
14.1.17	Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 0 (FACS analysis)
14.1.18	Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 42 (FACS analysis)
14.1.19	Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 180 (FACS analysis)
14.1.20	Primary immunogenicity: Difference in response from day 0 to day 42 for IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population (FACS analysis)
14.1.21	Primary immunogenicity: Difference in response from day 0 to day 180 for IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population (FACS analysis)
14.1.22	Primary immunogenicity: Responders for IFNγ cytokine secretion by PBMCs T cells by ELISA on day 42 and day 180 compared to day 0 (FAS and PP population)

14.1.23	Primary immunogenicity: Average increase for IFNγ cytokine secretion by PBMCs T cells by ELISA on day 42 and day 180 compared to day 0 (FAS and PP population)
14.1.24	Secondary immunogenicity: Responders for producing IL-4 in CD4+ T cells and CD8+ T cells by FACS on day 42 and day 180 in FAS and PP population
14.1.25	Secondary immunogenicity: Number of IgM responders in all treatment groups in FAS and PP populations on day 42 and day 180.
14.1.26	Secondary immunogenicity: Number of IgG responders in all treatment groups in FAS and PP populations on day 0, day 42 and day 180.
14.1.27	Secondary immunogenicity , Mean FLU-v specific IgM titers (Geometric mean) in all treatment groups in FAS and PP populations on day 0, day 42 and day 180
14.1.28	Secondary immunogenicity ,Mean FLU-v specific IgG titers (Geometric mean) in all treatment groups in FAS and PP populations on day 0, day 42 and day 180
14.1.29	Influenza confirmed infection A or B (FAS and PP)
14.1.30	Duration of symptoms in influenza confirmed infections (FAS and PP)
14.1.31	Total symptom score in influenza confirmed infections (FAS and PP)
14.1.32	Total symptom peak in influenza confirmed infections (FAS and PP)
14.1.33	Average severity score in influenza confirmed infections (FAS and PP)

3.2 List of Listings

– All Listings will be sorted by treatment group, subject number, visit and date (if applicable).

Listing	Title
16.2.1	***Subject Disposition***
16.2.1-1	Subject Disposition
16.2.1-2	Randomization
16.2.1-3	Scheduled Visits per subject
16.2.1-4	Unscheduled visits per subject
16.2.1-5	Collection of nasal and tonsil Swabs
16.2.1-6	Informed consent
16.2.2	***Protocol Deviations***
16.2.2-1	Protocol Deviations by category
16.2.2-2	Eligibility check by visit
16.2.3	***Subjects excluded from the immunogenicity analysis***
16.2.4	***Demographic Data and Baseline Assessment***
16.2.4-1	Demographics
16.2.4-2	Drug history (alcohol/smoking/recreational drugs)
16.2.4-3	Medical History
16.2.4-4	Prior and Concomitant Medication
16.2.4-5	Vital signs at screening visit
16.2.4-6	Physical Exam
16.2.4-7	Previous Influenza vaccination history
16.2.4-8	Laboratory analysis-Biochemistry
16.2.4-9	Laboratory analysis-Haematology
16.2.4-10	Pregnancy test
16.2.4-11	Unscheduled / Repeated Safety Tests LOG
16.2.5	***Compliance/Exposure/Drug concentration data***
16.2.5-1	Vaccine Administration
16.2.6	***Adverse Events***
16.2.6-1	Listing of Solicited Adverse Events (post-vaccination diary card)
16.2.6-2	
16.2.6-3	Listing of Unsolicited adverse events
16.2.6-4	Severe Adverse events
16.2.6-5	Vaccination Site Reaction
16.2.7	***Listing of individual Laboratory immunological parameters***
16.2.7-1	IgM FLU-v specific titers (day 0, day 42 and day180)
16.2.7-2	IgG FLU-v specific titers (day 0, day 42 and day 180)
16.2.7-3	FLU-v specific CMI responses (FACS)(day 0, day 42 and day 180)

16.2.7-4	FLU-v specific CMI responses (ELISA) (day 0, day 42 and day 180)
16.2.7-5	HAI titers at screening (4 strains)
16.2.7-6	Influenza RT-PCR test of swabs
16.2.7-7	Influenza Symptom Questionnaire
16.2.7-8	Study Completion
16.2.7-9	Comments

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3.3 List of Figures

Examples of Figures for the primary and secondary figures are listed below:

14.2.1 Frequency of CD4+ and CD8+ IFNγ responders by FACS in each treatment group for the FAS and PP

14.2.2 Frequency of CD4+ and CD8+ TNFα responders by FACS in each treatment group for the FAS and PP

14.2.3 Frequency of CD4+ and CD8+ IL-2 responders by FACS in each treatment group for the FAS and PP

14.2.4 Frequency of CD4+ and CD8+ CD107 responders by FACS in each treatment group for the FAS and PP

14.2.5 Frequency of CD4+ and CD8+ IL-4 responders by FACS in each treatment group for the FAS and PP

14.2.6 Frequency of IFNγ responders by ELISA in each treatment group for the FAS and PP

14.2.7 Frequency of IgG responders in each treatment group for the FAS and PP

14.2.8 Frequency of IgM responders in each treatment group for the FAS and PP

14.2.9 Geometric mean titer of IgM antibodies per group per time point for the FAS and PP on day 0, day 42 and day 180

14.2.10 Geometric mean titer of IgG antibodies per group per time point for the FAS and PP on day 0, day 42 and day 180

14.2.11 Average fold increase FLU-v specific IgM titers in all treatment groups in FAS and PP populations from day 0 to day 42 and day 180

14.2.12 Average fold increase FLU-v specific IgG titers in all treatment groups in FAS and PP populations from day 0 to day 42 and day 180

4. References

1. Van Doorn E, Pleguezuelos O, Liu H, Fernandez A, Bannister R, Stoloff G, et al, Evaluation of the immunogenicity and safety of different doses and formulations of a broad spectrum influenza vaccine (FLU-v) developed by SEEK: study protocol for a single-center, randomized, double-blind and placebo-controlled clinical phase IIb trial, BMC Infectious Diseases. 2017;17(1):241

2. Pleguezuelos O, Robinson S, Stoloff GA, Caparros-Wanderly W. Synthetic Influenza vaccine (FLU-v) stimulates cell mediated immunity in a double- blind, randomized, placebo-controlled Phase I trial. Vaccine. 2012;30:4655–60.

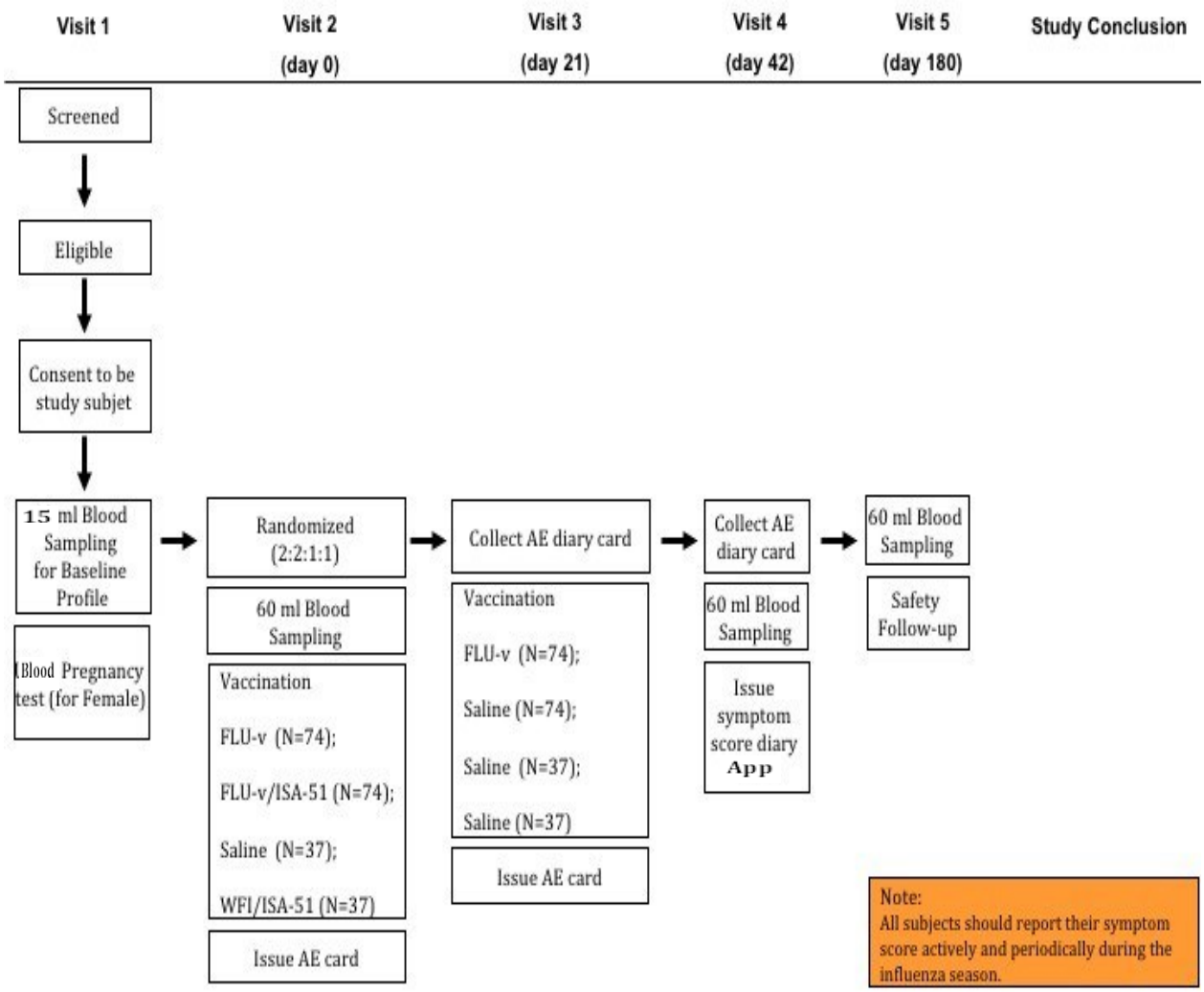
3. Finak G, MCDavid A, Chattopadhyay P, Dominguez M, DeRosa S, Roederer M, et al, Mixture models for single-cell assays with applications to vaccine studies, Biostatistics.2013;15(1):87-101

4. Lin L, Finak G,,Ushey K,,Seshadri C, Hawn TR, Frahm N, et al, COMPASS identifies T-cell subsets correlated with clinical outcomes, Nat Biotechnol. 2015;33(6):610-6.

5. Lindemann M, Witzke O, Lutkes P, Fiedler M, Kreuzfelder E, Philipp T, et al. ELISpot assay as a sensitive tool to detect cellular immunity following influenza vaccination in kidney transplant recipients. Clin Immunol. 2006;120(3):342–8.

5. Annexes

5.1 Annex 1: Flow chart of study design



5.2 Annex 2: Tables

14.1.1 Number of subjects per treatment group for the different analysis populations

	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)
ITT	N=XX (%)	N=XX (%)	N=XX (%)
FAS	N=XX (%)	N=XX (%)	N=XX (%)
PP	N=XX (%)	N=XX (%)	N=XX (%)
Safety	N=XX (%)	N=XX (%)	N=XX (%)

Note: abbreviations: ITT= Intention To Treat, FAS = Full Analysis Set, , PP = per protocol, N= Number

14.1.2 Demographic Data (Safety population)

	Treatment Group				
	Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total, n (%) (N=)
Age at screening (Mean /SD)	Xx/xx	Xx/xx	Xx/xx	Xx/xx	
Age at screening (median/ min-max)					
< 50 years old	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
≥ 50 years old	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Sex Male	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Female	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Ethnicity					
Asian	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Black or African descendants	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
White	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Other	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Unknown or not reported	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)

Note: abbreviations: min = minimum, max = maximum, N=number

14.1.3. Demographic Data (FAS population)

	Treatment Group				
	Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total, n (%) (N=)
Age (Mean/SD)	Xx/xx	Xx/xx	Xx/xx	Xx/xx	
Age at screening (median/ min-max)					
< 50 years old	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
≥ 50 years old	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Male	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Female	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Ethnicity					
Asian	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Black or African descendants	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
White	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Other	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)
Unknown or not reported	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)	N=XX (%)

Note: abbreviations: min = minimum, max = maximum, N = number, SD = standard deviations

Primary safety outcomes

14.1.4 Summary of Solicited Adverse Events from Post-vaccination diary card 1 (Safety population)

Subject Status	Relatedness	Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total (N=XX)
All adverse events	n/a	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
AE related to vaccine	Unrelated	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Unlikely	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Possibly related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Probably related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Definitely related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
AE severity	Mild	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Moderate	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Severe	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)

Note: abbreviations: n/a = not available, N = number, AE = Adverse Event

14.1.5 Summary of Solicited Adverse Events from Post-vaccination diary card 2 (Safety population)

Subject Status	Relatedness	Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total (N=XX)
All adverse events	n/a	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
AE related to vaccine	Unrelated	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Unlikely	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Possibly related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Probably related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Definitely related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
AE severity	Mild	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Moderate	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Severe	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)

Note: abbreviations: n/a = not available, N = number, AE =Adverse Event

14.1.6 . Solicited and Unsolicited Adverse Events Summary (Overall) (Safety population)

		Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total (N = XX)
Number of subjects with at least one solicited AE	n/a	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Solicited AE related to vaccine	Unrelated	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Unlikely	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Possibly related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Probably related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Definitely related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Solicited AE severity	Mild	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Moderate	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Severe	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Number of subjects with at least one unsolicited AE	n/a	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Unsolicited AE related to vaccine	Unrelated	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Unlikely	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Possibly related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Probably related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Definitely related	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Unsolicited AE severity		Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Moderate	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
	Severe	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
SAE		Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)

Note: abbreviations: n/a = not available, N = number, AE = Adverse Event

14.1.7 . Treatment Emergent Adverse Events Analysis (Safety Population)

	Treatment groups									
	Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total N = XX	
	sbj, n (%)	Events	sbj, n (%)	Events	sbj, n (%)	Events	sbj n (%)	Events	sbj, n (%)	Events
Number of Subjects with one or more TEAE	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx (%)	Xx (%)
Severe TEAEs	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx (%)	Xx (%)
TEAEs Definitely Related to Study Treatment	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx (%)	Xx (%)
TEAEs Leading to Early Termination	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx (%)	Xx (%)
Treatment-Emergent Serious Adverse Events	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx(%)	Xx (%)	Xx (%)
Deaths	Xx(%)	NA	Xx(%)	NA	Xx(%)	NA	Xx(%)	NA	Xx (%)	Xx (%)

Note: The column subjects refers to a patient that has at least one of the events. Events refers to the total number of events which may occur more than once in one patient.
Abbrevations: sbj =subjects, TEAEs = Treatment emergent adverse events, N = number

14.1.8 Adverse events reported as mild in intensity by Classification **(Safety population)**

System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										
	Macular degeneration										
Gastrointestinal disorders	Abdominal discomfort										
	Abdominal pain										
	Abdominal pain upper										

Note: Variables listed as examples. sbj =subjects N = number

14.1.9 Adverse events reported as moderate in intensity by Classification **(Safety population)**

System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										
	Macular degeneration										
Gastrointestinal disorders	Abdominal discomfort										
	Abdominal pain										
	Abdominal pain upper										

Note: Variables listed as examples. sbj =subjects N = number

14.1.10 Adverse events reported as severe in intensity by Classification**. (Safety population)**

System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										
	Macular degeneration										
Gastrointestinal disorders	Abdominal discomfort										
	Abdominal pain										
	Abdominal pain upper										

Note: Variables listed as examples. sbj =subjects N = number

14.1.11 a. Adverse events reported as related to vaccine reported as mild by classification **(Safety population)**

System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										
	Macular degeneration										
Gastrointestinal disorders	Abdominal discomfort										
	Abdominal pain										
	Abdominal pain upper										

Note Abbreviations: . sbj =subjects, N = number:

14.1.11 b Adverse events reported as related to vaccine reported as moderate by classification **(Safety population)**

System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										

Note: Variables listed as examples. sbj =subjects N = number

	Macular degeneration										
System Organ Class/ Preferred Term		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=xx)	
		N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N(% (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)	N (%) (Sbj)	N (%) (Events)
Blood and lymphatic system disorders	Lymphadenopathy										
Ear and labyrinth disorders	Vertigo										
Eye disorders	Eye oedema										
	Macular degeneration										
Gastrointestinal	Abdominal discomfort										
Gastrointestinal disorders	Abdominal pain										
	Abdominal pain upper										

14.1.11 c. Adverse events reported as related to vaccine reported as severe by classification **(Safety population)**

disorders	Abdominal pain										
	Abdominal pain upper										

Note: Variables listed as examples. sbj =subjects N = number

14.1.12 Adverse events at injection site (Safety population)

Adverse event		Two doses FLU-v non-adjuvanted (N=XX)		One FLU-v adjuvanted (N=XX)		Two doses non-adjuvanted placebo (N=XX)		One dose adjuvanted placebo (N=XX)		Total (N=XX)	
Administration site conditions		Subjects N/%	Events N/%	Subjects N/%	Events N/%	Subjects N/%	Events N/%	Subjects N/%	Events N/%	Subjects N/%	Events N/%
Hematoma injection site	Mild										
	Moderate										
Injection site erythema	Mild										
	Moderate										
Injection site induration	Mild										
	Moderate										
Injection site pain	Mild										
	Moderate										
Injection site pruritus	Mild										
	Moderate										
Injection site swelling	Mild										
	Moderate										
Injection site warmth	Mild										
	Moderate										

Note: Abbreviations: sbj =subjects, N= number

14.1.13. Serious Adverse Events by classification (Safety Population)

Treatment	System Organ Class	Preferred Term	Relatedness	Duration (Days)	Treatment	Outcome
One dose adjuvanted FLU-v (N=xx)						
Two doses non-adjuvanted FLU-v (N=xx)						

One dose adjuvanted placebo (N=xx)						
Two doses non-adjuvanted Placebo (N=xx)						

Note Abbreviations: N = number

14.1.14 Clinical Laboratory Safety - Overall Summary (Safety Population) Treatment		Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total (N=XX)
Hb	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Ht	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Leu	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
RBC	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Platelet	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
MCH	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
MCHC	Out of range					
	Clinically significant					

	Mean/SD					
	Median/Range					
MCV	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
TBil	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Uric acid	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Creatinine	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Tprot	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Albumin	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
AlkPhos	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Sodium	Out of range					
	Clinically significant					

	Mean/SD					
	Median/Range					
Potassium	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
AST	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
ALT	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
GGT	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Glucose	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Urea	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
LDH	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Calcium	Out of range					
	Clinically significant					

	Mean/SD					
	Median/Range					
Phosphate	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
HD-Cholesterol	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
LD-Cholesterol	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
Triglyceride	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					
CRP	Out of range					
	Clinically significant					
	Mean/SD					
	Median/Range					

Note Abbreviations: N = number, Hb =haemoglobin, Ht=haematocrit, Leu = leukocytes, RBC= red blood cells, MCH = mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, MCV= mean corpuscular volume, TBil = total bilirubin, Tprot = total protein, AlkPhos = alkaline phosphatase, AST = aspartate amino transferase, ALT = alanine amino transferase, GGT = gamma-glutamyl transferase, LDH = lactacte dehydrogenase, HD = High density, LD = low density , CRP= C-reactive protein

14.1.15 Previous Influenza Vaccinations (FAS population)

	Two doses FLU-v non-adjuvanted (N=XX)	One FLU-v adjuvanted (N=XX)	Two doses non-adjuvanted placebo (N=XX)	One dose adjuvanted placebo (N=XX)	Total (N=XX)
Never received influenza vaccination	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Received in the previous 2 years	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)
Received over 2 years ago	Xx (%)	Xx (%)	Xx (%)	Xx (%)	Xx (%)

Note: Abbreviations: N= number

14.1.16. HAI titers at screening (Phuket, Brisbane, Michigan, Hong Kong strains)

Number of subjects with HAI>40 at prescreening	**One dose adjuvanted FLU-v vs. adjuvanted Placebo**			**Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo**		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Phuket	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Brisbane	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Michigan	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Hong Kong	N(%)	N(%)	P-value	N (%)	N(%)	P-value
Any of above mentioned viruses	N(%)	N(%)	P-value	N (%)	N(%)	P-value

Note: Threshold for B strains is at >80, Full name of the strains: Phuket = B/Phuket/3073/2013, Brisbane = B/Brisbane/60/2008, Michigan = A/Michigan/45/15(H1N1)pdm09,Hong Kong = A/Hong Kong/5738/2014 (H3N2).
Abbreviations: N= number

Primary immunogenicity tables

14.1.17. Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 0 (FACS analysis)

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	Adjuvanted Placebo N=xx	P-value	Non-Adjuvanted FLU-v (2 doses) N=xx	Non-Adjuvanted Placebo N=xx	P-value
Positive for IFNγ producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for TNFα producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for IL-2 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for CD107a CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value
Positive for IFNγ producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for TNFα producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for IL-2 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for CD107a CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.18 Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 42 (FACS analysis)

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Positive for IFNγ producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for TNFα producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for IL-2 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for CD107a CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value
Positive for IFNγ producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for TNFα producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for IL-2 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for CD107a CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.19 Primary immunogenicity: Responders for producing IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population at day 180 (FACS analysis)

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	Adjuvanted Placebo N=xx	P-value	Non-Adjuvanted FLU-v (2 doses) N=xx	Non-Adjuvanted Placebo N=xx	P-value
Positive for IFNγ producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for TNFα producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for IL-2 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for CD107a CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value
Positive for IFNγ producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for TNFα producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for IL-2 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for CD107a CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.20 Primary immunogenicity: Difference in response from day 0 to day 42 for IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population (FACS analysis)

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v v Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	Adjuvanted Placebo N=xx	P-value	Non-Adjuvanted FLU-v (2 doses) N=xx	Non-Adjuvanted Placebo N=xx	
Positive for IFNγ producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for TNFα producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for IL-2 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for CD107a CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	
Positive for IFNγ producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for TNFα producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for IL-2 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for CD107a CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.21 Primary immunogenicity: Difference in response from day 0 to day 180 for IFNγ, TNFα, IL-2 and CD107a in CD4+ and CD8+ cells in FAS and PP population (FACS analysis)

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v v Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	Adjuvanted Placebo N=xx	P-value	Non-Adjuvanted FLU-v (2 doses) N=xx	Non-Adjuvanted Placebo N=xx	
Positive for IFNγ producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for TNFα producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for IL-2 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for CD107a CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	
Positive for IFNγ producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for TNFα producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for IL-2 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	
Positive for CD107a CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N(%)	

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.22. Primary immunogenicity: Responders for IFNγ cytokine secretion by PBMCs T cells by ELISA on day 42 and day 180 compared to day 0 (FAS and PP populations)

	One dose adjuvanted FLU-v vs. adjuvanted	Two doses non-adjuvanted FLU-v vs Non-

Number of responders	Placebo			Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 42 Positive for IFNγ producing Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for IFNγ producing Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Responders are defined based on having an increase response of at least 2-fold from day 0.

Main comparisons:

- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.23. Primary immunogenicity: Average increase for IFNγ cytokine secretion by PBMCs T cells by ELISA on day 42 and day 180 compared to day 0 (FAS and PP population)

Average increase	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 42 Positive for IFNγ producing Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for IFNγ producing Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

Secondary outcomes

14.1.24 Secondary immunogenicity**: Responders for producing IL-4 in CD4+ T cells and CD8+ T cells by FACS on day 42 and day 180 in FAS and PP population**

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 42 Positive for IL-4 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for IL-4 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Day 180 Positive for IL-4 producing CD4+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for IL-4 producing CD8+ Tcells	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number

Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.25 Secondary immunogenicity: Number of IgM responders in all treatment groups in FAS and PP populations on day 42 and day 180.

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 42 Positive for increased IgM titers	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for increased IgM titers	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number.
Responders are defined based on having an increase response of at least 2-fold from day 0.
Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.26. Secondary immunogenicity**:** Number of IgG responders in all treatment groups in FAS and PP populations on day 0, day 42 and day 180.

Number of responders	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 42 Positive for increased IgG titers	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Day 180 Positive for increased IgG titers	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number.
Responders are defined based on having an increase response of at least 2-fold from day 0.
Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.27 Secondary immunogenicity: **Mean FLU-v specific IgM titers (Geometric mean) in all treatment groups in FAS and PP populations on day 0, day 42 and day 180**

Geometric mean	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 0 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgM titers			P-value			P-value
Day 42 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgM titers			P-value			P-value
Day 180 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgM titers			P-value			P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number, SD = Standard Deviation, SE = Standard Error, Q = quartile
Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.28 Secondary immunogenicity**: Mean FLU-v specific IgG titers (Geometric mean) in all treatment groups in FAS and PP populations on day 0, day 42 and day 180**

Geometric mean	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Day 0 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgG titers			P-value			P-value
Day 42 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgG titers			P-value			P-value
Day 180 Geometric mean/ SD/ median/ SE/ (Q1,Q3) IgG titers			P-value			P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number, SD = Standard Deviation, SE = Standard Error, Q = quartile
Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

Exploratory outcomes

14.1.29. Influenza confirmed infection A or B (FAS and PP)

Test	One dose adjuvanted FLU-v vs. adjuvanted Placebo			Two doses non-adjuvanted FLU-v vs Non-Adjuvanted Placebo		
	Adjuvanted FLU-v (1 dose) N=xx	**Adjuvanted Placebo N=xx**	**P-value**	**Non-Adjuvanted FLU-v (2 doses) N=xx**	**Non-Adjuvanted Placebo N=xx**	**P-value**
Positive for influenza A	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for influenza B	N (%)	N (%)	P-value	N (%)	N (%)	P-value)
Positive for influenza A (H3N2)	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for influenza A (H1N1)	N (%)	N (%)	P-value	N (%)	N (%)	P-value
Positive for Influenza) any strain)	N (%)	N (%)	P-value	N (%)	N (%)	P-value

Note: * = P values based on Chi-square test , ** = P values based on Fishers exact test , Abbreviations: N = number. H3N2 = A/Hong Kong/5738/2014 , H1N1 = A/Michigan/45/15(H1N1)pdm09,
Main comparisons:
- Adjuvanted FLU-v vs adjuvanted placebo
- Non adjuvanted FLU-v vs non-adjuvanted placebo

14.1.30 Duration of symptoms in influenza confirmed infections (FAS and PP)

	One dose Adjuvanted FLU-v N=xx	**Two Doses Non-Adjuvanted FLU-v N=xx**	**One dose Adjuvanted Placebo N=xx**	**Two dose non-adjuvanted Placebo N=xx**
N	Xx	xx	xx	xx
Mean (days)	xx.xx	xx.xx	xx.xx	xx.xx
SD	xx.xx	xx.xx	xx.xx	xx.xx
SE	xx.xx	xx.xx	xx.xx	xx.xx
Median	xx.xx	xx.xx	xx.xx	xx.xx
Q1	xx.xx	xx.xx	xx.xx	xx.xx
Q3	xx.xx	xx.xx	xx.xx	xx.xx
Minimum	xx.xx	xx.xx	xx.xx	xx.xx
Maximum	xx.xx	xx.xx	xx.xx	xx.xx
P-value [Adj FLU-v one-dose vs. Adj Placebo] = x.xxx				
P-value [non-Adj FLU-v two-doses vs. nonAdj Placebo 2 doses] = x.xxx				

Note: * = P-values based on t-test ** = P-values based on M-U test, *** = P-values based on ANOVA **** = P-values based on Kruskal Wallis. Abbreviations: Adj = Adjuvanted, N= Number, SD = Standard Deviation, SE= Standard Error, Q= Quartile

14.1.31: Total symptom score in influenza confirmed infections (FAS and PP)

	One dose Adjuvanted FLU-v N=xx	Two Doses Non-Adjuvanted FLU-v N=xx	One dose Adjuvanted Placebo N=xx	Two dose non-adjuvanted Placebo N=xx
N	Xx	xx	xx	xx
Mean (symptom score)	xx.xx	xx.xx	xx.xx	xx.xx
SD	xx.xx	xx.xx	xx.xx	xx.xx
SE	xx.xx	xx.xx	xx.xx	xx.xx
Median	xx.xx	xx.xx	xx.xx	xx.xx
Q1	xx.xx	xx.xx	xx.xx	xx.xx
Q3	xx.xx	xx.xx	xx.xx	xx.xx
Minimum	xx.xx	xx.xx	xx.xx	xx.xx
Maximum	xx.xx	xx.xx	xx.xx	xx.xx
P-value [Adj FLU-v one-dose vs. Adj Placebo] = x.xxx				
P-value [non-Adj FLU-v two-doses vs. nonAdj Placebo 2 doses] = x.xxx				

Note: * = P-values based on t-test ** = P-values based on M-U test, *** = P-values based on ANOVA **** = P-values based on Kruskal Wallis. Abbreviations: Adj = Adjuvanted, N= Number, SD = Standard Deviation, SE= Standard Error, Q= Quartile

14.1.32: Total symptom peak in influenza confirmed infections (FAS and PP)

	One dose Adjuvanted FLU-v N=xx	Two Doses Non-Adjuvanted FLU-v N=xx	One dose Adjuvanted Placebo N=xx	Two dose non-adjuvanted Placebo N=xx
N	Xx	xx	xx	xx
Symptom peak	xx.xx	xx.xx	xx.xx	xx.xx
SD	xx.xx	xx.xx	xx.xx	xx.xx
SE	xx.xx	xx.xx	xx.xx	xx.xx
Median	xx.xx	xx.xx	xx.xx	xx.xx
Q1	xx.xx	xx.xx	xx.xx	xx.xx
Q3	xx.xx	xx.xx	xx.xx	xx.xx
Minimum	xx.xx	xx.xx	xx.xx	xx.xx
Maximum	xx.xx	xx.xx	xx.xx	xx.xx
P-value [Adj FLU-v one-dose vs. Adj Placebo] = x.xxx				
P-value [non-Adj FLU-v two-doses vs. nonAdj Placebo 2 doses] = x.xxx				

Note: Symptom peak is defined as highest number of symptoms recorded in a single day.
* = P-values based on t-test ** = P-values based on M-U test, *** = P-values based on ANOVA **** = P-values based on Kruskal Wallis. Abbreviations: Adj = Adjuvanted, N= Number, SD = Standard Deviation, SE= Standard Error, Q= Quartile

14.1.33 Average severity score in influenza confirmed infections (FAS and PP)

	One dose Adjuvanted FLU-v N=xx	**Two Doses Non-Adjuvanted FLU-v N=xx**	**One dose Adjuvanted Placebo N=xx**	**Two dose non-adjuvanted Placebo N=xx**
N	Xx	xx	xx	xx
Average severity score	xx.xx	xx.xx	xx.xx	xx.xx
SD	xx.xx	xx.xx	xx.xx	xx.xx
SE	xx.xx	xx.xx	xx.xx	xx.xx
Median	xx.xx	xx.xx	xx.xx	xx.xx
Q1	xx.xx	xx.xx	xx.xx	xx.xx
Q3	xx.xx	xx.xx	xx.xx	xx.xx
Minimum	xx.xx	xx.xx	xx.xx	xx.xx
Maximum	xx.xx	xx.xx	xx.xx	xx.xx
P-value [Adj FLU-v one-dose vs. Adj Placebo] = x.xxx				
P-value [non-Adj FLU-v two-doses vs. nonAdj Placebo 2 doses] = x.xxx				

Note: * = P-values based on t-test ** = P-values based on M-U test, *** = P-values based on ANOVA **** = P-values based on Kruskal Wallis. Abbreviations: Adj = Adjuvanted, N= Number, SD = Standard Deviation, SE= Standard Error, Q= Quartile

5.3 Annex 3: SOP statistical analysis and reporting

	SOP Statistics
FTEE 08-02-2018	Statistical Analysis and Reporting

1. Product name:

Statistical Analysis and Reporting

2. Purpose:

- To report the outcome of the statistical analysis.

3. Contents:

- Necessary information:
  - Protocol
  - SAP
  - Data management report (including classification)
  - Randomisation list (if applicable)
  - Locked data sets

- The statistical analysis will be performed according to the latest version of the SAP. Analysis will be performed using the locked dataset(s). Every program will start with a standard header stating the name of the program, the statistical software package, the study name, a short study title, the date, the researcher who wrote the program and the person who tested the program. Example:

```
/*=============================================================*/
/* PROGRAM: NAME.R  or NAME.sas          */
/* STUDY:                                                      */
/* STUDY TITLE:                                                */
/* SPONSOR:                                                    */
/* NOTES:                                                      */
/* PROGRAM BY: Name, dd-mm-yyyy                                */
/* TEST BY: Name, dd-mm-yyyy                                   */
/*=============================================================*/
```

Testing (test A) of the statistical analysis consists of:

A1) Validation of the analysis program. The named statistician in the SAP and a second statistician must perform this test.
A2) Test of statistical analysis on adherence to the statistical analysis plan performed by the named statistician in the SAP and a second statistician.

The outcome for A1 and A2 must be documented and signed by both statisticians. If in disagreement, a third statistician must repeat steps A1 and A2 and the process must also be documented and signed.

After test A1 of the statistical analysis has been successfully performed, the locked data is ready to be analysed as per SAP. Locked data must be analysed by the named statistician in the SAP and a second qualified statistician to corroborate the results. This step must be documented and signed (Appendix 1). Once all parties involved have agreed the resulting data, the output will be available for reporting.

- (Parts) of the output from the statistical report will be used for the clinical study report which appropriately integrates the statistical work with clinical and other material. Statistical output will be attached to the CSR for reference.

Authorised by:	Prof. dr. E. Hak	**Process owner:**	Dr. M. A. Islam	
Signature:		**Release date:** 08-02-2018	**Version** 0.2	1 / 2

	SOP Statistics
FTEE 08-02-2018	Statistical Analysis and Reporting

Annex 1. Results of Test A

Test	Result	Name	Qualification	Signature
A1		Dr. Atique Islam	Statistician	
A1		Dr. Edwin Martens	Statistician	
A2		Dr. Atique Islam	Statistician	
A2		Dr. Edwin Martens	Statistician	