CCI

Final Analysis
Statistical Analysis Plan (SAP)

medicago

Protocol Title:	A Randomized, Observer-blind, Placebo-controlled, Multicenter, Phase 3 Study to Assess the Efficacy, Safety, and Immunogenicity of a Plant-Derived Quadrivalent VLP Influenza Vaccine in Adults 18-64 Years of Age
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REVISION HISTORY

Version/Date	Version name	Section	Changes implemented
30-AUG-2017	Draft Version 1.0	Not Applicable	Not Applicable
07-SEP-2017	Draft Version 2.0	Multiple	Updated per client comments.
11-SEP-2017	Draft Version 3.0	Section 8.10	Added the following verbiage per client request: The unblinded study team is different from the independent biostatistical team (Section 3.2.2) who review the number of influenza cases and consists of approximately five people from the CRO and from Medicago.
07-DEC-2017	Draft Version 4.0	Multiple	Updated per CBER comments.
15-JUN-2018	Draft Version 5.0	Multiple	Updated SC rate for MN assay, the calculation of age and method of CI for efficacy endpoints (section 8.6.2.2.1) .
26-JUN-2018	Final Version 1.0	Multiple	Updated Medicago Reviewer to Annie Séguin. Changed version number and date.

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LIST OF ABBREVIATIONS

The following abbreviations will be used within this SAP.

Abbreviations	
AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ARU	Attack Rate in Unvaccinated Subjects
ARV	Attack Rate in Vaccinated Subjects
ATC	Anatomical Therapeutic Chemical
BLQ	Below Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
CMI	Cell-mediated Immune (response)
CRO	Contract Research Organisation
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
eDiary	Electronic Diary
FAS	Full Analysis Set
FDA	Food and Drug Administration
GMA	Geometric Mean Area
GMFR	Geometric Mean Fold Rise or Seroconversion Factor
GMT	Geometric Mean Titer
HI	Hemagglutination Inhibition
HR	Heart Rate
IA	Interim Analysis
ICH	International Conference on Harmonisation
ILI	Influenza-like Illness
IM	Intramuscular
IPP	Immunogenecity Per Protocol

LB	Lower Bound
MedDRA®	Medical Dictionary for Regulatory Activities®
MN	Microneutralization
NOCD	New Onset of Chronic Disease
OC	Observed Cases
OT	Oral Temperature
PBMC	Peripheral Blood Mononuclear Cell
PP	Per Protocol
PT	Preferred Term
RR	Relative Risk
RCDC	Reverse Cumulative Distribution Curve
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SAS®	Statistical Analysis System®
SD	Standard Deviation
SOC	System Organ Class
SC	Seroconversion
SP	Seroprotection
SRH	Single Radial Hemolysis
TEAE	Treatment-emergent Adverse Event
TFLs	Tables, Figures and Listings
VE	Vaccine Efficacy
VLP	Virus-like Particle
WHO	World Health Organization

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide detailed descriptions of the statistical methods, data derivations and data displays for study protocol CP-PRO-QVLP-012 Final Version 3.0 "A Randomized, Observer-blind, Placebo-controlled, Multicenter, Phase 3 Study to Assess the Efficacy, Safety, and Immunogenicity of a Plant-Derived Quadrivalent Virus-like particles (VLP) Influenza Vaccine in Adults 18-64 Years of Age dated 26 July 2017 for the final analysis. The table of contents and templates for the Tables, Figures and Listings (TFLs) will be produced in a separate document.

Any deviations from this SAP will be described and justified in the Clinical Study Report (CSR).

The preparation of this SAP has been based on International Conference on Harmonisation (ICH) E9 guidelines.

All data analyses and generation of TFLs will be performed using Statistical Analysis System® (SAS®) software (version 9.3 or higher).

2 STUDY OBJECTIVES

2.1 Primary objective(s)

- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, against protocol-defined respiratory illness due to laboratory-confirmed influenza caused by vaccine matched strains.

2.2 Secondary objective(s)

Efficacy:

- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, against protocol-defined respiratory illness due to any laboratory-confirmed influenza strains;
- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, against laboratory-confirmed protocol-defined influenza-like illness (ILI) caused by vaccine-matched strains;
- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, against laboratory-confirmed protocol-defined ILI caused by any influenza viral types/subtypes (matched, mismatched, and un-typed).
- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, as measured by the incidence of subjects presenting with symptoms of protocol-defined ILI, regardless of laboratory results.

Safety:

- To assess the safety and tolerability, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain.

Immunogenicity:

- To assess, in a subset of 400 subjects, the immunogenicity of a single dose of Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain or placebo, as measured by hemagglutination inhibition (HI) assay, microneutralization (MN) assay, and single radial hemolysis (SRH) assay against homologous and heterologous (HI only) influenza strains.

2.3 Exploratory objective(s)

Efficacy:

- To evaluate per age stratum, the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, against laboratory-confirmed influenza illness caused by vaccine-matched strains;
- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain against mismatched influenza strains;

- To evaluate the efficacy, relative to placebo, of a single dose of the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, as measured by the incidence of subjects presenting with symptoms of protocol-defined respiratory illness, regardless of laboratory results.

Immunogenicity:

- To assess the cell-mediated immune (CMI) response against homologous and heterologous strains on Days 0 and 21 in a subset of 400 subjects (same subset as the immunogenicity subset).

Safety:

- To evaluate respiratory illness outcome, occurrences of pneumonia, new onset or exacerbations of cardio-respiratory conditions, and health care utilization of subjects administered the Quadrivalent VLP Influenza Vaccine given at a dose of 30 µg/strain, relative to subjects administered the placebo.

3 STUDY DESIGN

3.1 General study design

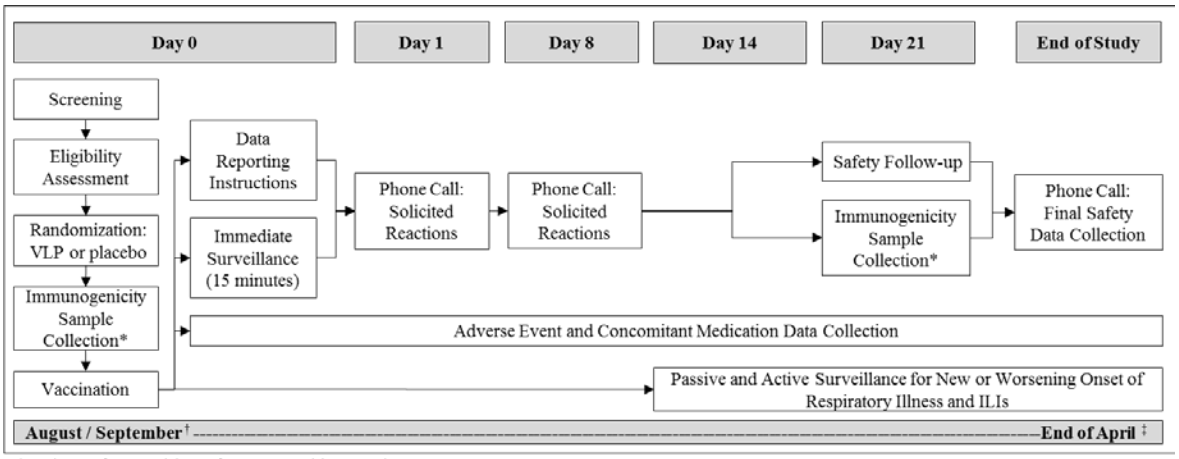
This is a Phase III, Randomized, Observer-blind, Placebo-controlled, Multicenter, Study to Assess the Efficacy, Safety, and Immunogenicity of a Plant-Derived Quadrivalent VLP Influenza Vaccine in Adults 18-64 Years of Age, consisting of phone calls and study visits:

- Screening / Vaccination period (Day 0)
- Survellance period (Day 14 post-vaccination to approximately April 30, 2018) with case detection and safety follow up.
- Safety (Day0 to Day 8, Day 21, Survellance period)
- Immunogenicity Sample collection period/Safety Follow up (Day 0 and Day 21) for a subset of subjects

The influenza strain composition of the Quadrivalent VLP Influenza Vaccine will be based on the 2017-2018 recommended World Health Organization (WHO) strains for vaccination. The composition of the Quadrivalent VLP Influenza Vaccine to be used in this study includes a mix of recombinant A/H1, A/H3, and two B hemagglutinin proteins expressed as VLPs for the 2017-2018 influenza virus strains.

Approximately 10,000 healthy male and female subjects aged 18 to 64 years will be randomized in a 1:1 ratio into one of two parallel treatment groups, such that approximately 5,000 subjects will receive the Quadrivalent VLP Influenza Vaccine at a dose of 30 µg/strain and approximately 5,000 subjects will receive the placebo. Within the two treatment groups, subjects will be stratified by site and two age groups (18-49 years of age and 50-64 years of age in a 1:1 ratio).

A basic outline of the study design is presented in Figure 1.



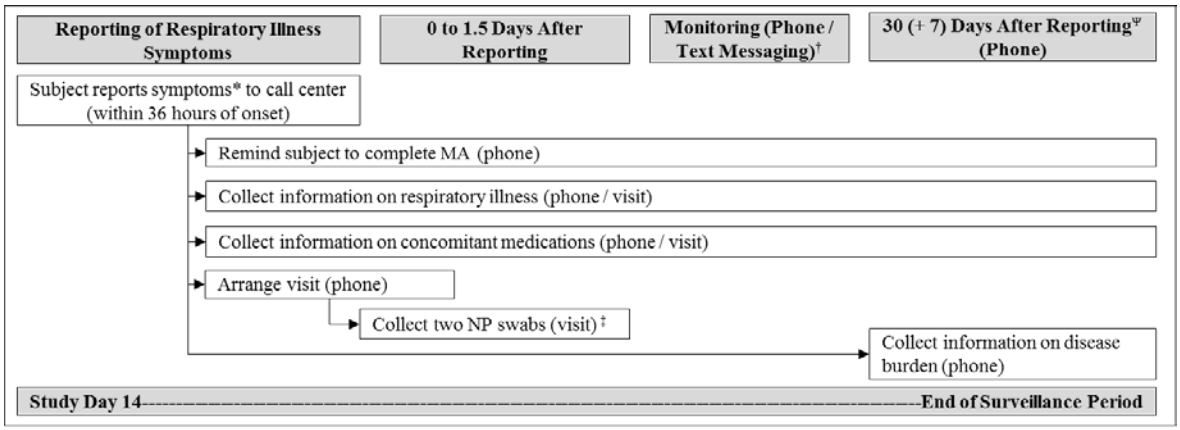
* Subset of 400 subjects for CMI and humoral assessments
† August / September for North America and Europe; approximately early November for Asia
‡ The duration of the surveillance period may be adjusted, based on the observed epidemiology during the season.

Figure 1: Flow Chart of Study Procedures

Subjects will participate in this study for approximately eight to ten months, during which a first visit will be scheduled on Day 0 for screening and vaccine administration. Subjects will be instructed on how to record reactions, adverse events (AEs), concomitant medication use, and respiratory illness symptoms in their memory aid and eDiary (electric data capture system), as applicable. A phone contact will be made on Days 1 and 8, specifically for review of the subject's safety and concomitant medication data. A visit will occur on Day 21 for a subset of 400 subjects for serology sampling and immunogenicity assessments (CMI, HI, MN, and SRH). From Day 14 until the end of the surveillance period, subjects will be instructed to report symptoms meeting the definition of a new or worsening respiratory illness and ILI (passive surveillance). In addition, active surveillance will be performed during this same period: symptoms of a new or worsening respiratory illness and ILI will be collected a minimum of three times per week (at least one of these contacts each week will be through a scripted telephone call, with the remaining contacts via text messaging). A final phone contact will be made at the end of the surveillance period (approximately 30 April 2018; however, the duration of surveillance period may be adjusted, based on the observed epidemiology during the season in participating countries) for a safety assessment.

Reports of respiratory illness within the specified window will be followed up for the collection of information regarding symptoms, concomitant medication use, and disease burden and the collection of two nasopharyngeal swabs.

Figure 2 outlines the process to be followed for reports of respiratory illness.



* One or more of the following symptoms that persist(s) for or reoccur(s) after a period of at least 12 hours: sneezing, stuffy or runny nose (nasal congestion), sore throat, cough, sputum production, wheezing, and difficulty breathing
† After NP swab collection and until the final 30 (+ 7) day follow-up, the planned active surveillance (phone and text messaging) will continue and will include questions on the respiratory illness progression
¥ Data past Day 30 of onset of respiratory illness is not to be collected
‡ Swabs must be collected within 36 hours (preferably 24 hours) after reporting of symptoms and prior to the use of influenza antiviral treatment medication

Figure 2 Flowchart of Process of Respiratory Illness Report

Throughout the influenza season, the number of laboratory-confirmed influenza cases will be reviewed in a blinded manner on a regular basis by selected members of Medicago and of the Contract research organisation (CRO) not directly involved in the trial conduct. In the event of an insufficient number of reported cases, the extension of enrollment or the extension of the study will be addressed in a protocol amendment.

3.2 Randomization and blinding

3.2.1 Randomization

Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (e.g. demographic and baseline characteristics) are evenly balanced across treatment groups and age strata, and to enhance the validity of statistical comparisons across treatment groups.

Approximately 10,000 subjects are planned for randomization in a 1:1 ratio to receive the Quadrivalent VLP Influenza Vaccine at a dose of 30 µg/strain or the placebo. Randomization will be stratified by site and two age groups (18 to 49 years of age and 50 to 64 years of age in a 1:1 ratio).

Individuals will be randomly assigned to a vaccine group by use of an interactive randomization system.

Potential study subjects will be screened and assigned a subject number. Once all screening procedures, including Day 0 pre-randomization procedures, have been completed and the study eligibility is confirmed by the Investigator, the randomization numbers will be allocated to subjects within the appropriate treatment group by the randomization system.

The randomization number and treatment will be recorded along with the six-digit number for each subject in the investigational product accountability log. The Investigator will not be provided with randomization codes, but it will be possible to unblind the treatment in an emergency situation.

3.2.2 Observer-blinding

Observer-blinded treatment will be used to reduce potential bias during data collection and evaluation of the clinical endpoints.

This is an observer-blind study: the subjects, the Investigators, and those responsible for study endpoint evaluations or review or analysis of the study data will not have access to the randomization codes. Any code break will be documented and reported to Medicago (or its designee) in a timely manner. In a medical emergency, the Investigator may unblind the treatment for that subject without prior consultation with the Sponsor. In such an event, the Investigator will need to contact the responsible Medical Officer as soon as possible after the unblinding to discuss the case.

Although there may be some visual differences in the VLP vaccine and placebo preparations, the site staff involved in the preparation and administration of the treatments will not be involved in any activity that could introduce a bias, such as the evaluations of respiratory symptoms, ILI, AEs, or reactogenicity of the subjects following vaccination.

This study is blinded through to the end of the surveillance period of the last subject. The RT-PCR

samples corresponding to influenza cases that might lead to the unblinding of the randomization code, will not be available during the course of the study to any investigator or any person directly involved in the clinical conduct of the study (including data cleaning and data analysis) except the independent biostatistical team from the CRO and selected individuals from Medicago and the CRO. The selected individuals will review the number of influenza cases to allow for discussion of the clinical data and critical business decisions (e.g. extension of recruitment for another season) prior to study completion. It is anticipated that approximately five people from Medicago and from the CRO will have access to the number of cases in a blinded manner. Also, the central laboratories and the staff at the clinical site (except the staff involved in the preparation/administration of study vaccine, the quality assurance auditor, and the quality control reviewers) will remain blinded throughout the entire study duration.

Blinding measures will be applied to maintain the observer-blindness of the blinded staff and to allow identification of the study treatment only by staff involved in the preparation/administration of the study vaccine/placebo.

3.2.3 Age Group Stratification

Subjects under each treatment group will be stratified by site and two age groups (18 to 49 years and 50 to 64 years in a 1:1 ratio). The use of a 1:1 ratio in this study provides equal weight to both age groups to facilitate a better comparison between the two strata.

3.3 Study treatments and assessments

The duration of this study will be approximately eight to ten months post-vaccination for all subjects.

On Day 0, subjects will receive one intramuscular (IM) injection into the deltoid region of the non-dominant arm (if possible), of their assigned treatment (30 µg/strain of Quadrivalent VLP Influenza Vaccine or placebo) based on randomization.

The volume of injection will be 0.5 mL for both the vaccine and placebo.

A detailed description of procedures and assessments to be conducted during this study is summarized in Table 1 and Table 2 below.

Table 1: Schedule of Study Assessments

Visit Type	Screening/ Vaccination	Post-vaccination Visits/Contacts			End of Surveillance[1]
Study Day	Day 0	Day 1 (+ 1)	Day 8 (- 1/+ 1)	Day 21 (- 2/+ 3)	(± 3)
Visit Number	1	Phone	Phone	2 (Visit or Phone)	Phone
Informed consent	X				
Demographics	X				
Medical history/prior medication	X				
Vaccination history[2]	X				
Inclusion/exclusion criteria	X				
Randomization	X				
Vaccine administration	X				
Immediate surveillance (15 minutes)	X				
Provide eDiary instructions to subjects	X				
Serology for HI, MN, and SRH titers	X[3]			X[3]	
CMI (peripheral blood mononuclear cell [PBMC]assay)	X[3]			X[3]	
Vital Signs (blood pressure [BP], heart rate [HR], oral temperature [OT])	X				
Height, weight, and body mass index (BMI)	X				
History/symptom-directed physical examination	X				
Urine (dipstick) pregnancy test	X				
Oral digital thermometer and instructions on reactions and respiratory illness symptoms[4]	X				
Collection of solicited local/ systemic reactions	X	X	X	X	
Concomitant medications	At any time during the study period				
Collection of respiratory illness symptoms through passive and active surveillance	Passive Surveillance: Subjects will be instructed to contact the study site if they experience symptoms of respiratory illness from Day 14 until the end of the surveillance period[1]. Active Surveillance: Between Day 14 and the end of the surveillance period[1], the subjects will be contacted a minimum of three times per week. At least one of these contacts each week will be through a scripted telephone call, with the remaining contacts via text messaging; a higher proportion of telephone contacts may be used if deemed appropriate by the clinic site (e.g. difficulty in obtaining responses via text messaging).				
Collection of NP swabs for laboratory confirmation of influenza[5]	From Day 14 until the end of the influenza surveillance period[1], NP swabs will be collected from subjects who report a new or a worsening respiratory illness (as defined in the protocol). Swabs will be collected within 36 hours (preferably within 24 hours) after reporting of the qualifying respiratory illness				

	symptoms.	
Collection of disease burden and health care information[6]	For each case of respiratory illness (as defined in the protocol), a questionnaire on disease burden will be completed by telephone at the end of the 30-day follow up period for the illness, regardless of whether or not a swab is obtained. Subjects will be provided with a memory aid to facilitate accurate reporting.	
AEs, Serious Adverse Events (SAEs), and NOCDs[7]	At any time during the study period	
Termination record		X

[1] The end of the surveillance period is targeted as approximately 30 April 2018; however, the duration of surveillance period may be adjusted, based on the observed epidemiology during the season in participating countries.

[2] Information on past influenza vaccinations for 24 months prior to study entry

[3] Only a subset of 400 subjects will have a blood draw for CMI and humoral immunogenicity testing. This subset will be comprised of the first subjects enrolled in pre-defined sites in North America; a representative age distribution will be targeted.

[4] After vaccination, subjects will be instructed on the memory aid and eDiary provided for their use for recording reactions, AEs, concomitant medication use, and respiratory illness symptoms. They will be reminded of the reportable respiratory illness symptoms that will trigger the need for NP swabbing, as well as of the overall active and passive surveillance process.

[5] Swabs are to be collected from any subject with a respiratory illness from Day 14 to the end of the surveillance period of the study. If the respiratory illness starts prior to Day 14, swabs are not to be collected, even if symptoms persist beyond Day 14.

[6] A questionnaire for the collection of disease burden (please refer to protocol) and includes occurrences of any of the following in association with any respiratory illness with onset from Day 14 to the end of the surveillance period: pneumonia (clinical diagnosis), new onset or exacerbations of pre-existing cardio-respiratory conditions, hospitalizations, emergency room visits, and non-routine medical office visits, as well as any additional diagnoses associated with the illness.

[7] Adverse events will be collected up to Day 21; SAEs, AEs leading to withdrawal, and NOCDs will be collected through to the end of the study. Specific contacts for the collection of information regarding all of these events will occur on Day 21 (in the surveillance telephone call on or shortly after Day 21 or during the Day 21 visit [for the subset of subjects participating CMI and humoral immunogenicity testing]) and during the end of surveillance telephone contact for SAEs, AEs leading to withdrawal, and NOCDs.

Table 2 Time and Events Schedule: Respiratory Illness Onset and Follow Up

Days after Reporting of Respiratory Illness	0-1.5 Days	0-1.5 Days[1]	Monitoring[2]	30 (+ 7) Days[3]
Contact Type	**Phone**	**Visit**	**Phone / Text Messaging**	**Phone**
Verify information on respiratory illnesses and schedule appointment (at clinic or home) for two NP swabs within 36 hours (preferably within 24 hours) of the reporting of a respiratory illness	X			
Remind subject to continue to record data and in a timely manner	X			
Collection of the two NP swabs[1]		X		
Collection of reportable concomitant medications	X	X	X	X
Collection of information on respiratory illness symptoms [4]	X	X	X	X
Collection of disease burden and health care information				X

[1] Swabs are to be collected within 36 hours after reporting of the respiratory illness (preferably within 24 hours) and prior to the use of influenza antiviral treatment medication (e.g. oseltamivir, zanamivir, rapivab).
[2] After NP swab collection and until the final 30 (+ 7) day follow-up, the planned active surveillance (phone and text messaging) will continue and will include questions on the respiratory illness progression.
[3] The seven-day window allows provision to complete the telephone call. Information from more than 30 days from respiratory illness onset does not need to be collected.
[4] During collection of information on respiratory illness symptoms, the presence or absence of concurrent systemic symptoms (i.e. fever, feverishness [feeling of warmth], chills [shivering], tiredness [fatigue], headache, myalgia [muscle aches], nausea, vomiting, or diarrhea) will also be collected.

4 STUDY ENDPOINTS

4.1 Efficacy Endpoints:

4.1.1 Primary efficacy endpoint

- Occurrences of protocol-defined respiratory illness due to laboratory-confirmed influenza (≥ 14 days post-vaccination) caused by influenza viral types/subtypes that are matched (and/or antigenically similar) to the strains covered in the vaccine formulation.

4.1.2 Secondary efficacy endpoints

The secondary efficacy endpoints of this study are:

- Occurrences of protocol-defined respiratory illness due to laboratory-confirmed influenza (≥ 14 days post-vaccination) caused by any influenza viral types/subtypes (matched, mismatched, and un-typed).
- Occurrences of laboratory-confirmed influenza (according to protocol defined ILI) illnesses (≥ 14 days post-vaccination) caused by influenza viral types/subtypes that are matched (and/or antigenically similar) to the strains covered in the vaccine formulation.
- Occurrences of laboratory-confirmed influenza (according to protocol defined ILI) illnesses (≥ 14 days post-vaccination) caused by any influenza viral types/subtypes (matched, mismatched, and un-typed).
- Occurrences of protocol-defined ILI ≥ 14 days post-vaccination (confirmed or not).

4.1.3 Exploratory endpoints

The exploratory endpoints of this study are:

- Occurrences, per age stratum, of laboratory-confirmed influenza (≥ 14 days post-vaccination) caused by influenza viral types/subtypes that are matched (and/or antigenically similar) to the strains covered in the vaccine formulations, according to protocol-defined respiratory illness;
- Occurrences of laboratory-confirmed influenza illnesses (≥ 14 days post-vaccination) caused by mismatched influenza viral strains, according to protocol-defined respiratory illness;
- Occurrences of respiratory illness ≥ 14 days post-vaccination (confirmed or not).

4.2 Safety endpoints

4.2.1 Secondary safety endpoints

The safety endpoints of this study are:

- Percentage, intensity, and relationship to vaccination of immediate complaints (15 minutes post-vaccination);

- Percentage, intensity, and relationship to vaccination of solicited local and systemic signs and symptoms (for seven days following study vaccine administration);
- Percentage, intensity, and relationship of TEAEs for 21 days following study vaccine administration;
- Occurrences of deaths, SAEs, AEs leading to withdrawal, and NOCDs up to the end of the surveillance period.

4.2.2 Exploratory endpoints

- Information on respiratory illness outcome, occurrences of pneumonia, new onset or exacerbations of cardio-respiratory conditions, and health care utilization during the entire trial follow up period. These results will be summarized separately and not be included in the CSR.

4.3 Immunogenicity endpoints (subset of subjects)

4.3.1 Secondary endpoints

- HI antibody response induced by the Quadrivalent VLP Influenza Vaccine against the homologous and heterologous influenza strains on Days 0 and 21 in a subset of 400 subjects (300 who received the VLP vaccine and 100 who received the placebo). HI antibody titers will be analyzed as follows:
  - Geometric Mean Titers (GMT) of HI antibody on Days 0 and 21;
  - Seroconversion (SC) rate: the proportion of subjects in a given treatment group with either a ≥ 4-fold increase in reciprocal HI titers between Day 0 and Day 21 or a rise of undetectable HI titer (i.e. < 10) pre-vaccination (Day 0) to an HI titer of ≥ 40 on Day 21;
  - Seroprotection (SP) rate: the proportion of subjects in a given treatment group attaining a reciprocal HI titer of ≥ 40 on Day 21 (the percentage of vaccine recipients with a serum HI titer of at least 1:40 following vaccination);
  - Geometric Mean Fold Rise (GMFR): the geometric mean of the ratio of GMTs (Day 21/Day 0).
- MN antibody response induced by the Quadrivalent VLP Influenza Vaccine against the homologous influenza strains on Days 0 and 21, in the subset of 400 subjects, will be analysed as follows:
  - GMTs of MN antibody on Days 0 and 21;
  - SC rate: the proportion of subjects in a given treatment group with either a ≥ 4-fold increase in reciprocal MN titers between Day 0 and Day 21 or a rise of undetectable MN titer (i.e. < 14.1) pre-vaccination (Day 0) to an MN titer of ≥ 56.4 at Day 21 post-vaccination ;GMFR (Day 21/Day 0).

  Note: A number of studies show the MN assay to have a two-fold or higher sensitivity than the HI assay. However, there is still limited data available on the comparative

sensitivities of these two assays and the data that is available and is largely focused on H1N1 and H3N2. As a result, since this is a secondary analysis with a relatively small sample size, a conservative approach was taken by using the HI assay correlates of protection for the MN assay.

- SRH antibody response induced by the Quadrivalent VLP Influenza Vaccine against the homologous strains on Days 0 and 21 in a subset of 400 subjects will be analysed as follows:
    - Geometric Mean Areas (GMA) of SRH antibody on Days 0 and 21;
    - SC rate: proportion of subjects in a given treatment group showing at least 50 % increase in GMA between Days 0 and 21;
    - SP rate: the proportion of subjects in a given treatment group attaining an area ≥ 25 mm^2 following vaccination (Day 21);
    - GMFR: the geometric mean of the ratio of GMAs (Day 21/Day 0).

4.3.2 Exploratory endpoints

- CMI response induced by the Quadrivalent VLP Influenza Vaccine against homologous and heterologous strains on Day 21 (in the subset of 400 subjects).

CMI responses against homologous and heterologous strains will be evaluated by measuring the % response in CD4+ Cells for parameters as below:

1. IFN-γ + IL-2+ TNF-α+
2. IFN-γ - IL-2+ TNF-α +
3. IFN-γ + IL-2- TNF-α +
4. IFN-γ + IL-2+ TNF-α -
5. IFN-γ - IL-2- TNF-α +
6. IFN-γ - IL-2+ TNF-α -
7. IFN-γ + IL-2- TNF-α -
8. SUM of IFN-γ+ (the sum of all IFN-γ+ responses; i.e. 1+3+4+7, from the list above)
9. SUM of IL-2+ (the sum of all IL-2+ responses; i.e. 1+2+4+6, from the list above)
10. SUM of TNF-α+ (the sum of all TNF-α+ responses; i.e. 1+2+3+5, from the list above)
11. SUM of total responsive (CD4+ expressing one cytokine; i.e. sum of all elements from the list above).

5 SAMPLE SIZE AND POWER

The sample size of approximately 10,000 subjects (5,000 subjects per treatment group) was selected based on the assumption that the Quadrivalent VLP Influenza Vaccine would have a VE of at least 70% and that the attack rate in unvaccinated subjects (ARU) would be 2% or greater for laboratory-confirmed influenza cases caused by vaccine-matched strains. The sample size was chosen to have 90% power to determine whether the lower bound (LB) of the two-sided 95% CI for the primary endpoint would be greater than 40%, assuming a 10% attrition rate.

If the ARU is lower than 2%, but greater than or equal to 1.5%, this sample size will have an 80% power to determine whether the LB of the two-sided 95% CI for the primary endpoint (VE) would be greater than 40%, assuming a 10% attrition rate.

A protocol amendment will be issued to address changes to the enrollment plan or to the study extension into another season to enrol more subjects in the event of insufficient ARU.

Table 3 shows the sample size simulations result. The simulation was repeated 1000 times in order to calculate the power.

Table 3 Sample Size Simulation Result

Placebo Control AR (ARU)	VE (vs placebo)	LB of 95% CI	Power	Sample Size per Group
2%	70%	40%	90%	4430
1.50%	70%	40%	80%	4490

[Note] Under the assumptions of 70% vaccine efficacy (VE) and 2% influenza attack rate (Placebo), we expect a total of 116 to 130 cases of influenza (89 cases for Placebo and 27 cases for VLP in case of 4430 per group considering 10% dropout rate, 100 cases for Placebo and 30 cases for 5000 per group).
If the influenza attack rate is lower than 2%, but greater than or equal to 1.5%, we expect a minimum of 89 total cases.

Immunogenicity Subset

A sample size of 300 subjects (for the VLP vaccine group) was selected to ensure that the LBs of the 95% CI for the co-primary endpoints (SC and SP rates) for each strain meet CBER's criteria with 80% power (individual power is 97.4%).

6 ANALYSIS POPULATIONS

6.1 Safety Analysis Set (SAS)

The SAS is defined as all subjects who received either the Quadrivalent VLP Influenza Vaccine or the placebo. All safety analysis will be performed using the SAS, according to the treatment the subjects actually received.

6.2 Full Analysis Set (FAS)

The FAS will consist of the subset of subjects in the SAS who were successfully contacted at least once during the surveillance period. Subjects who received the wrong treatment will be analyzed as randomized.

6.3 Per-Protocol (PP) set

The PP set will consist of the subjects who participated in the study until at least the end of the peak period (approximately the end of February for Europe and North America) or for at least five months or until the end of the surveillance period (other countries); had no major protocol deviations related to subject eligibility, the ability to develop a valid immune response, prohibited medication use, or the efficacy analyses; and who received the vaccine or placebo.

Subjects who received the wrong treatment, but for whom the treatment received can be unequivocally confirmed, will be analyzed as treated, provided they have no other deviations that compromise their data.

The PP set will be the primary analysis population for the efficacy and immunogenicity endpoints. The analysis of efficacy and immunogenicity analysis will be repeated using the FAS as the sensitivity analysis.

Note:

Protocol deviations and the definition of the peak influenza period will be reviewed and documented during a blinded data review prior to database lock and confirmed at the time of database lock. Since peak influenza period in each region can vary from year to year, the exact period for individual regions will be defined at the end of the season, based on reported positive influenza tests (regional surveillance reporting). The handling of missing data and whether the potential impact of any protocol deviations require the exclusion of a subject from analyses will also be considered during the blinded data review

6.4 Immunogenicity PP (IPP) Set

The IPP set will consist of the subset of subjects who participated in the immunogenicity portion of the study, who had a Day 21 immunogenicity sample collection; who had no major deviations related to subject eligibility, the ability to develop a valid immune response, prohibited medication use, or the immunogenicity analyses; and who received the vaccine or placebo. Subjects who received the wrong treatment, but for whom the treatment received can be unequivocally confirmed, will be analyzed as treated, provided they have no other deviations that compromise their data.

The analyses of all immunogenicity endpoints will be performed using the IPP set.

6.5 Protocol deviations/violations and exclusions from analysis sets

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. Major and minor protocol deviations will be identified and documented during a blinded data review prior to database lock. Subjects will be excluded from analyses if the deviation is considered to impact the analyses.

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of adverse events (including death) is not considered as a protocol deviation. The missing assessments that should have otherwise been collected for that subject later in the study are also not considered as a protocol deviation.

Protocol deviations will be reviewed by Medicago (or its designee) to identify any non-compliances likely to have a significant effect on the safety and rights of a subject or the reliability and robustness of the data generated. These deviations will be included in the clinical study report.

7 STATISTICAL CONSIDERATIONS AND ANALYSIS

7.1 Derived Variables

7.1.1 Demographics

Age collected in CRF will be used on tables.

BMI (kg/m^2) is calculated using the following formula:

BMI=Weight (kg) / Height^2 (m^2)

7.1.2 Immunogenicity

Values below the HI antibody responses below the lower limit of quantification (Below limit of Quantification (BLQ)<10, recorded as "< 10") will be attributed a value of 5. Values below MN antibody responses below the lower limit of quantification (Below limit of Quantification (BLQ)<14.1, recorded as "< 14.1") will be attributed a value of 7.1. All BLQ responses for the SRH antibody (recorded as 4 mm^2) will be attributed a value of 2 mm^2.

For HI assay:

Seroconversion rate is defined as the proportion of subjects in a given treatment group with either a ≥ 4-fold increase in reciprocal HI titers between Day 0 and Day 21 or a rise of undetectable HI titer (i.e. < 10) pre-vaccination (Day 0) to an HI titer of ≥ 40 on Day 21.

Seroprotected rate is defined as the proportion of subjects in a given treatment group attaining a reciprocal HI titer of ≥ 40 on Day 21 (the percentage of vaccine recipients with a serum HI titer of at least 1:40 following vaccination).

For MN assay:

Seroconversion rate is defined as the proportion of subjects in a given treatment group with either a ≥ 4-fold increase in reciprocal MN titers between Day 0 and Day 21 or a rise of undetectable MN titer (i.e. <14.1) pre-vaccination (Day 0) to an MN titer of ≥ 56.4 on Day 21.

For SRH assay:

Seroconversion rate is defined as the proportion of subjects in a given treatment group showing at least 50 % increase in GMA between Days 0 and 21.

Seroprotected rate is defined as the proportion of subjects in a given treatment group attaining an area ≥ 25 mm^2 following vaccination (Day 21).

GMT calculation

The GMT calculations are performed by taking the anti-log of the mean of the log titer transformations. The back-transformation for mean GMT will be calculated with two-sided, 95% confidence limits, based on the ANOVA model.

GMFR calculation

The GMFR will be derived by using Analysis of Covariance (ANCOVA) to model the difference in the log of the titer values between Day 21 and Day 0, with treatment group as main effect and baseline titer as covariate. The GMFR will be compared between the treatment groups. Pair-wise comparisons between the treatments groups will be performed using two-sided, 95% confidence, based on the least-squares mean differences from the ANCOVA model.

7.2 Handling of missing data and outliers

For a given subject and a given efficacy/immunogenicity measurement, missing measurements will not be replaced. Therefore, an analysis will exclude subjects with missing values.

For the analysis of solicited symptoms, systemic reactions and respiratory illness symptoms, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the Safety cohort will include only subjects/doses with documented safety data (i.e. eDiary).

7.3 Data presentation description

The following decimal description will be used for the demography, efficacy, reactogenicity and immunogenicity analyses.

Category	Parameters	Number of decimal digits
Demographic characteristics	Mean, Median	1 (One more decimal place than the raw data)
	SD	2 (Two more decimal place than the raw data)
	Min, Max	0 (Same number of decimal places as the raw data)
	% of count	1
Efficacy	VE & 95% CI	1
Humoral and Cellular Immunogenicity evaluation	GMTs including LL & UL of 95% CI	1
	GMFR including LL & UL of 95% CI	2
	SC & SP rate including LL & UL of 95% CI	1
	CMI mean, median	1
	CMI SD,	2
	P-Value	3 (0.xxx format)
Safety	% of count	1

For some parameters (% of count, 95% CI and VE), careful consideration may be required in some instances (e.g. very low-incidence) and in such situations, details will be described in footnotes or programming notes.

7.4 Group description

The following groups will be used for the statistical analyses.

Group order in tables	Group label in tables	Group definition for footnote
1	30 µg VLP vaccine	Subjects who have received one dose of 30 µg/strain of Quadrivalent VLP Influenza Vaccine
2	Placebo	Subjects who have received one dose of placebo.

7.4.1 Missing data analysis methods

Not applicable.

7.4.2 Handling of missing or incomplete dates

7.4.2.1 Missing or Partial AE Start Date

If only Day of AE start date is missing:

If the AE start year and month are the same as that for the first vaccination dose date, then:

- If the full (or partial) AE end date is NOT before the first dose date or AE end date is missing, then impute the AE start day as the day of first dose date; otherwise, impute the AE start day as 1.
- Otherwise, impute the AE start day as 1.

Compare the imputed AE start date with TE period to determine whether the AE is medical history or TEAE.

If Day and Month of AE start date are missing:

If AE start year = first dose year, then:

- If the full (or partial) AE end date is NOT before the first dose date or AE end date is missing, then impute the AE start Month and Day as the Month and Day of first dose date; otherwise, impute the AE start Month as January and the Day as 1.

Compare the imputed AE start date with TE period to determine whether the AE is is medical history or TEAE.

If Year of AE start date is missing:

If the year of AE start is missing or AE start date is completely missing then query site with no imputation. Also compare the full (or partial) AE end date to the first dose date. If the AE end date is before the first dose date then the AE should be considered as a medical history. Otherwise, the AE will be considered as TEAE.

7.4.2.2 Missing AE Severity or Relationship

There is no imputation in cases where severity or relationship information is missing for AEs.

7.4.2.3 Missing or Partial Concomitant Medication Start/Stop Dates

Missing or partial concomitant medication start date:

- If only DAY is missing and the day , use the first day of the month.
- If DAY and Month are both missing, use the first day of the year.

Missing or partial concomitant medication stop date:

- If only DAY is missing, use the last day of the month.
- If DAY and Month are both missing, use the last day of the year.
- If DAY, Month and year are all missing, assign ‘continuing’ status to stop date

8 STATISTICAL METHODS

8.1 General statistical conventions

All statistical procedures will be completed using (SAS®) version 9.3 or higher.

Unless otherwise stated, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05. Two-sided 95% confidence intervals (CI) will be provided when relevant.

Continuous variables will be summarized using descriptive statistics, including number of subjects (n), mean, median, standard deviation (SD), minimum and maximum.

For categorical variables, summaries will include counts of subjects and percentages. Percentages will be rounded to one decimal place.

For summary purposes, baseline will be defined as the last nonmissing value on or before vaccination. All summaries will be presented by treatment group and age group, unless otherwise specified.

All subject data, including those derived, will be presented in individual subject data listings. All listings will be sorted by investigational site, subject number, date/time and visit. The vaccine group (VLP and Placebo) as well as subject's age stratum will be stated on each listing.

8.2 Subject disposition

All subjects who provide informed consent will be accounted for in this study. Subject disposition information will be summarized by treatment group and overall. Screened subjects will be presented for the overall population. The frequencies and percentages of subjects who are vaccinated in each analysis set, completed through day 21, completed the study and early study withdrawals along with primary reasons for withdrawal will be presented. The same information will be repeated for the immunogenicity subset.

The number of subjects vaccinated will be used as the denominator for the percentage calculation.

Subject disposition will be listed.

In addition, a listing will be provided to include population sets, completion of the study and withdrawal information by subject and age stratum.

Screen failures will also be listed with reason for not being randomized.

8.3 Protocol deviations

The protocol deviations by category will be summarized by treatment group and overall.

Population membership details will be listed, including reason for exclusion from each population (on randomized patients).

A listing will include the protocol deviation identified based on data recorded on the eCRF and/or protocol deviation logs from CCI (on randomized patients) and presented along with the date the deviation occurred relative to study day, deviation category and the classification of major/minor.

8.4 Demographics and baseline characteristics

8.4.1 Demographics

Age, height, weight and BMI at baseline will be summarized descriptively. Age category (18-49 years and 50-64 years), sex, race, ethnicity and influenza immunization history will be summarized using the SAS, PP and IPP.

The same information will be listed by subject and age stratum.

8.4.2 Baseline and disease characteristics

Continuous baseline variables such as OT (°C), systolic BP (mmHg), diastolic BP (mmHg), and HR (breaths/min) will be summarised by descriptive statistics in the same way as continuous demographic variables for the SAS, PP and IPP.

8.4.3 Medical history

The frequencies and percentages of subjects with medical history findings will be presented by system organ class (SOC) using the Medical Dictionary for Regulatory Affairs® (MedDRA) Version 18.1 or higher by treatment group and overall. Medical history data will be tabulated for the SAS following the order mentioned in Appendix A section 11.

The details of medical history findings will be listed by subject and age stratum.

In addition, another listing will be added to include details of influenza immunization history by subject and age stratum

8.4.4 Prior and concomitant medications

Medications used in this study will be coded by using the latest available version of the WHO Drug Dictionary.

Prior medications are medications used only before the first study vaccination (medication end date < first study vaccination date).

Concomitant medications: are defined as those medications with a start date on or after the vaccination dose of study drug or the medications started before the dose and continued during the vaccination period.

Prior medications and concomitant medications will be summarized descriptively using frequency tables by Anatomical Therapeutic Chemical (ATC) class and preferred term (or ATC Level 3) by treatment group. Details for imputing missing or partial start and/or stop dates of medication are

described in Section 7.4.2.

In addition, prior medications and concomitant medications will be listed with ATC classification and preferred term (or ATC Level 3) by subject and age stratum.

8.5 Extent of exposure

Since the subjects will receive only one dose of study vaccination, duration will not be calculated.

An individual listing will be provided to include details of vaccine administration by subject and age stratum.

8.5.1 Treatment duration

Since the subjects will receive only one dose of study vaccination, duration will not be calculated.

8.5.2 Treatment compliance

Treatment compliance is expected to be 100%, since the study treatments will be administered IM at each Investigator site by site staff. In addition, the Investigator or designated study center personnel will maintain a log of all study treatments dispensed and returned during the study.

Compliance information will not be summarized.

8.6 Efficacy analyses

8.6.1 Analysis methods

The first analysis of efficacy will be based on the occurrence of the primary endpoint (i.e., first episodes of protocol-defined respiratory illness due to laboratory-confirmed influenza) from Day 14 until the end of the surveillance period (passive and active surveillance).

Following randomization and vaccination, subjects will be instructed to report respiratory symptoms and symptoms meeting the definition of ILI from Day 14 until the end of the surveillance period (passive surveillance). The subjects will be given a reminder aid listing the symptoms of respiratory illness and ILI and contact information for the study site. During this same period, an active surveillance will also be performed via regular phone calls and occurrence of respiratory illness symptoms will be solicited a minimum of three times per week. At least one of these contacts each week will be through a scripted telephone call, with the remaining contacts via text messaging; a higher proportion of telephone contacts may be used if deemed appropriate by the clinical site (e.g. difficulty in obtaining responses via text messaging).

A respiratory illness will be defined as the occurrence of a new onset of one or more of the following symptoms that persist(s) for or reoccur(s) after a period of at least 12 hours:

- Sneezing;
- Stuffy or runny nose (nasal congestion);
- Sore throat;

- Cough;
- Sputum production;
- Wheezing;
- Difficulty breathing.

An episode is considered to extend from the first day of the first symptom to the last day of the last symptom. In most instances a new episode can only begin after resolution of all symptoms of the previous episode, with a separation of a seven-day symptom-free interval between episodes.

Within 36 hours (preferably within 24 hours) after the reporting of a respiratory illness, the clinic site will collect nasopharyngeal (NP) swabs from the subject (two per subject per event). The swabs must be collected prior to the use of any influenza antiviral treatment medication (e.g. oseltamivir, zanamivir, rapivab). If the respiratory illness starts prior to Day 14, swabs are not to be collected, even if symptoms persist beyond Day 14. One swab will be submitted for analysis by multiplex reverse transcription polymerase chain reaction (RT-PCR). In the event of a positive RT-PCR result (positive for A or B strains), the second swab will be used to attempt to isolate the virus or for additional cell-culture testing (typing, subtyping, and strain identification and genetic sequencing using HI assay against a panel of known standard ferret reference antisera to different viral strains) to determine if the virus detected is matched or similar to any of the strains covered in the vaccine formulation for the respective season.

A positive RT-PCR result will be considered a laboratory-confirmed case of influenza illness. Any respiratory illness must be followed up for 30 days following the start date; this follow up will be conducted via the planned active surveillance contacts (telephone and text messaging), using a phone script. At the end of the 30-day follow up, a questionnaire regarding disease burden due to the respiratory illness will be completed; subjects will be provided with a memory aid for use over the 30 days, to facilitate accurate reporting at the end of the follow-up period.

ILI will also be monitored during this study. A subject will be considered to have protocol-defined ILI if the following two conditions are both met between Day 14 and the end of the surveillance period:

- He/she has at least one of the following pre-defined respiratory symptoms:
  - Sore throat;
  - Cough;
  - Sputum production;
  - Wheezing; or
  - Difficulty breathing;

AND

- He/she has at least one of the following systemic symptoms:

- Fever (defined as a temperature > 37.2 °C or > 99.0 °F);
- Chills;
- Tiredness;
- Headache; or
- Myalgia.

8.6.1.1 Treatment by center interaction analysis (multi-center study)

There is no center-interaction analysis, as randomization will be done using center as stratification factor.

8.6.2 Analysis of primary efficacy endpoint

The primary efficacy endpoint defined in section 4.1.1 will be analysed using the PP set as outlined below:

For the primary endpoint, VE will be evaluated as 1 minus the relative risk (RR) of vaccinated (30 µg/strain VLP vaccine) and unvaccinated (placebo) subjects to become infected with protocol-defined respiratory illness caused by one or more vaccine-matched strains in a timeframe of 14 or more days following vaccination (i.e. the proportion of subjects with one or more episodes of respiratory illness shown to be caused by viral types/subtypes that are matched and/or antigenically similar to the strains covered in the vaccine formulation).

VE will be calculated using the following formula:

$$VE=(1-RR)=(1-ARV/ARU)*100\ \%$$

Where:

RR = relative risk

ARV = attack rate in vaccinated subjects; and

ARU = attack rate in unvaccinated subjects.

The VE success criterion is defined as a > 40% lower limit of the two-sided 95 % CI.

The analyses of the primary efficacy endpoint will be repeated for the FAS.

The primary endpoint will be summarized descriptively with VE and its 95% CI. Sub-analyses (VE and its 95% CI) by each strain (homologous A/Michigan/45/2015 [H1N1], homologous A/Hong Kong/4801/2014 [H3N2], homologous B/Brisbane/60/2008 and homologous B/Phuket/3073/2013) and by age group (18-49 and 50-64) will be summarized.

8.6.2.1 Multiplicity

Multiplicity adjustment will not be done for the primary endpoint because there is only one.

8.6.2.2 Methodology for computing CI

8.6.2.2.1 Exact 95% CIs

The 95% CI around RR will be calculated based on score confidence limits.

SAS® Code: Reference (4,5)

```
Proc freq data= ADZ (with the appropriate analysis dataset);
table group*resp/relrisk (cl=SCORE) ;
run ;

Note: LB=1-95% Upper Confidence Limit
```

8.6.3 Analysis of secondary efficacy endpoints

The secondary efficacy endpoints which are defined in 4.1.2 will be analysed in the following manner:

VE and 95% CI will be calculated for the secondary efficacy endpoints using the same formula indicated for the primary efficacy analysis. For secondary efficacy endpoints, the sub-analyses (VE and its 95% CI) by each strain (where applicable) and age group will be summarized.

An overall summary of subjects with ILI and summaries of subjects of each ILI symptom will be presented by treatment group.

All the secondary efficacy analyses will be based on the PP and will be repeated for the FAS as well.

8.6.3.1 Multiplicity

Multiplicity adjustment will not be done for the secondary efficacy endpoints.

8.6.3.2 Methodology for computing CI

8.6.3.2.1 Exact 95% CIs

The 95% CIs will be calculated in the same way as for the primary analysis mentioned above (Section 8.6.2).

8.6.4 Analysis of exploratory endpoint(s)

The exploratory efficacy endpoints which are defined in Section 4.1.3 will be analysed using the same formula indicated for the primary efficacy analysis. The sub-analyses (VE and its 95% CI) by age group and by strain (where applicable) will be summarized.

And overall summaries of subjects with respiratory illness symptoms and subjects with systemic symptoms , and sumaries of subjects with each respiratory illness and systemic symptom will be presented by treatment group and age group.

In addition, an individual listing will be included to present details of the results of RT-PCR and typing (laboratory-confirmed influenza vaccine matched, mismatched strains, or un-typed strains, respiratory illness symptoms, and ILI by subject and age stratum.

All the exploratory efficacy analyses will be based on the PP and will be repeated for the FAS as well.

8.7 Analysis of immunogenicity

8.7.1 Immunogenicity Evaluations

Immunogenicity will be evaluated by the humoral immune response (HI, MN, and SRH assays) and the CMI response induced in subjects on Days 0 and 21 in a subset of 400 subjects (300 from the VLP vaccine group and 100 from the placebo group) from selected sites. The North American sites selected for this subset of 400 subjects will be pre-defined prior to study start and will target providing a representative age distribution.The subjects identified for the immunogenicity analyses will also be stratified by age group (1:1 ratio).

For the immunogenicity analysis of the HI assays against the homologous strains, the point estimates and the corresponding two-sided 95% CI will be calculated to determine if the CBER criteria for licensure for influenza vaccine are met. The following analyses for the HI assay (homologous strains) will be performed on the IPP:

8.7.2 Analysis of Secondary Endpoints

The secondary immunogenicity endpoints are defined in section 4.3.1.

HI antibody titers will be analyzed and evaluated according to the CBER criteria, using a stepwise closed testing procedure as follows:

- GMTs of HI antibody: The point estimates and the corresponding two sided 95% CI by treatment group and strain will be calculated as the antilog of the mean and 95% CI of log transformed titer values on Days 0 and 21;
- SC rate: The point estimates and the corresponding two-sided 95% CI for subjects achieving SC by treatment group and strain will be calculated to determine whether the lower bound of the CI will meet or exceed 40%;
- SP rate: The point estimates and the corresponding two-sided 95% CI for subjects achieving SP by treatment group and strain will be calculated to determine whether the lower bound of the CI will meet or exceed 70%.
- GMFR: the geometric mean of the ratio of GMTs (Day 21/Day 0).

  For SC and SP rates, the following stepwise closed testing procedure will be applied while evaluating the results against the CBER criteria (for SC rate, the lower bound of the 95% CI will meet or exceed 40%; for SP rate, the lower bound of the 95% CI will meet or

exceed 70%). The stepwise closed testing procedure will be continued until the first rejection of the CBER criteria occurs.

Step (1) A/H1N1:SC rate→SP rate →Step (2) A/H3H2:SC rate→SP rate→ Step (3) B/Brisbane:SC rate→SP rate → Step (4) B/Phuket:SC rate→SP rate

MN antibody response against the homologous influenza strains on Days 0 and 21will be analysed as follows:

- GMTs of MN antibody on Days 0 and 21(as defined above for HI);
- SC rate: the proportion of subjects in a given treatment group with either a ≥ 4-fold increase in reciprocal MN titers between Day 0 and Day 21 or a rise of undetectable MN titer (i.e. < 14.1) pre-vaccination (Day 0) to an MN titer of ≥ 56.4 at Day 21 post-vaccination.GMFR: the geometric mean of the ratio of GMTs (Day 21/Day 0).

SRH antibody response against the homologous influenza strains will be analysed as follows:

- GMAs of SRH antibody on Days 0 and 21;
- SC rate: proportion of subjects in a given treatment group showing at least 50% increase in GMA between Days 0 and 21;
- SP rate: the proportion of subjects in a given treatment group attaining an area ≥ 25 mm2 following vaccination (Day 21);
- GMFR: the geometric mean of the ratio of GMAs (Day 21/Day 0).

The GMFR will be derived by using ANCOVA to model the difference in the log of the titer values between Day 21 and Day 0, with treatment group as main effect and baseline titer as covariate.

The GMTs, GMFRs, SP and SC rates of the HI and MN antibody assay and the GMAs, GMFRs, SC rates, and SP rates for the SRH antibody assay will be compared between the treatment groups using descriptive statistics and 95% CI. For SC rate and SP rate, Fisher's exact tests will be used. GMT for HI, MN and GMA for SRH will be compared between treatment groups using analysis of variance (ANOVA) model. GMFR for HI, MN and SRH will be compared using analysis of covariance (ANCOVA) model.

A sub-analyses stratified by age group for the secondary immunogenicity endpoints will performed. The analyses of the secondary immunogenicity endpoints will be repeated for the FAS.

An individual listing will be provided to include all assay results (HI, MN and SRH) and whether a particular subject has seroconverted/seroprotected based on IPP set.

In addition, the below listed figures will be generated:

- Reverse Cumulative Distribution Curves (RCDC) for Homologous Strains Measured and Heterologous Strains Measured by HI based on IPP.
- RCDC for Homologous Strains Measured by HI based on FAS.
- RCDC for Homologous Strains Measured by MN based on IPP.
- RCDC for Homologous Strains Measured by SRH based on IPP.

8.7.2.1 Multiplicity

A multiplicity adjustment was done at the time of sample size determination (N=300) as described below:

The lower bound (LB) of the 95% CI for the percentage of subjects achieving SC and SP for the four homologous antigens will be compared to CBER's criteria as co-primary endpoints where a stepwise closed testing approach will be applied for multiplicity of hypothesis tests. The stepwise closed testing procedure will be stopped as soon as the LB of 95% CI for SC or SP of one strain does not meet CBER's criteria.

8.7.2.2 Methodology for computing CI

8.7.2.2.1 95% CIs

The 95% CI for GMTs will be obtained within each group separately. The 95% CI for the mean of log-transformed titer/concentration will be first obtained assuming that log-transformed concentrations/titers are normally distributed with an unknown variance. The 95% CI for GMTs will be then obtained by exponential-transformation (anti-log with power 10) of the 95% CI for the mean of log-transformed titers.

SAS® Code for comparing GMTs:

```
PROC MIXED DATA=<Data>; * specify and sub-select data set as applicable;
  *BY Agegrp;  * as applicable;
CLASS trtpn; * other factors may be added, if applicable;
MODEL AVAL = trtpn;   * AVAL=logtiter
  * add factors and options (e.g. ALPHA=.. Solutions) as applicable;
 LSMEANS trtpn / CL ALPHA=0.05 STDERR; * modify/adapt as applicable
Ods output LSMEANS =_outmix;
  RUN;
  QUIT;
Data _outmix;
Set _outmix;
GMT= 10**(Estimate);
GMT_LCL = 10**(Lower);
GMT_UCL = 10**(Upper);
RUN;
```

SAS® Code for comparing GMFR:

```
PROC MIXED DATA=<Data> (analysis dataset);
class trtpn;
model diff= trtpn Base; * diff = log10Day21 – log10Day0.Base=log10Day0
Lsmeans trtpn/cl alpha=0.05;
Ods output LSMEANS =_outmix;
run;
Quit;
Data _outmix;
Set _outmix;
GMFR = 10**(Estimate);
GMFR_LCL = 10**(Lower);
GMFR_UCL = 10**(Upper);
Run;
```

8.7.2.2.2 Exact 95% CIs

The exact 95% CIs (Clopper-Pearson method) will be used for proportion of SC and SP subjects within a group.

SAS® Code for SC and SP (Exact 95% CIs):

```
PROC FREQ data=<Data> ;
      BY ANTIGEN trtpn;
      TABLE SC/out=CNT binomial;  *SC or SP
      EXACT binomial;
      ODS OUTPUT binomialprop=bin (where=(name1 in ('XL_BIN', 'XU_BIN')));
run;
```

8.7.3 Analysis of Exploratory Endpoint

The exploratory immunogenicity endpoint is defined in 4.3.2

The CMI response induced by the Quadrivalent VLP Influenza Vaccine against homologous and heterologous strains on Day 21 (in the subset of 400 subjects) will be compared between the treatment groups using non-parametric (Wilcoxon) models for each strain and parameter by age group and overall.

For CMI response CD4+ cells results will be presented along with available subjects (n), in terms of mean, median, SD, min and max. For the p-value calculation between the treatment groups for individual parameters, Wilcoxon rank-sum test will be used for Day 21, whereas the p-value will be calculated using Wilcoxon signed rank test for the difference between Day 0 and Day 21 values.

In addition, an individual listing will be provided to include CMI CD4+ cells response for each parameter with % response CD4+cells by subject and age stratum. The responses of each parameter will be illustrated using plots such as box plots by treatment group with parameters as X-axis and percentage of CD4+ T cells (%) as Y-axis.

8.8 Safety analyses

8.8.1 Evaluations

Safety and tolerability will be evaluated by solicited local and systemic reactions (immediate complaints within 15 minutes post-vaccination and solicited reactions up to seven days post-vaccination), unsolicited AEs up to 21 days post-vaccination, and SAEs and NOCDs up to the end of the surveillance period.

Subjects will be monitored for both solicited local reactions (erythema, swelling, and pain at the injection site) and solicited systemic reactions (fever, headache, fatigue, muscle aches, joint aches, chills, a feeling of general discomfort, swelling in the axilla, and swelling in the neck) from the time of vaccination through Day 7. While the subjects remain in the clinic following vaccine administration, staff will monitor them for immediate local and systemic reactions; after release from the clinic facility, from the evening of Day 0 to the evening of Day 7, subjects will measure and record their local and systemic reactions in their eDiary.

The intensity of the solicited local and systemic reactions will be graded as: mild (1), moderate (2), severe (3) or potentially life threatening (4). Their causal relationship with the study vaccine will be assessed by the Investigator (definitely not related, probably not related, possibly related, probably related or definitely related).

Table 4 shows severity grades for solicited local and systemic reactions.

Table 4 Severity Grades for Solicited Local and Systemic Reactions

Symptoms	Severity				
	None	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially life-threatening)
Injection Site Reactions (Local Reactions)					
Erythema (redness)	< 25 mm	25 - 50 mm	51 - 100 mm	> 100 mm	Necrosis or exfoliative dermatitis
Swelling	< 25 mm	25 - 50 mm and does not interfere with activity	51 – 100 mm or interferes with activity	> 100 mm or prevents daily activity	Necrosis
Pain	None	Does not interfere with activity	Repeated use of non-narcotic pain reliever for more than 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Solicited Systemic Reactions					
Fever (°C or °F)	< 38.0 °C < 100.4 °F	38.0 - 38.4 °C 100.4 - 101.1 °F	38.5 - 38.9 °C 101.2 - 102.0 °F	39.0 - 40.0 °C 102.1 - 104.0 °F	> 40.0 °C > 104.0 °F
Headache	None	No interference with activity	Repeated use of non-narcotic pain reliever for more than 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Fatigue	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Muscle aches	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Results in a visit to emergency room (ER) or hospitalization
Joint aches, chills, feeling of general discomfort or uneasiness (malaise), swelling in the axilla, swelling in the neck	None	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Results in a visit to emergency room (ER) or hospitalization

8.8.2 Adverse events

All Adverse events (AEs) will be classified by Primary SOC and PT according to the Medical Dictionary for Regulatory Activities® (MedDRA®) Version 19.0 or higher.

All AEs occurring within 21 days after vaccination will be reported in the "Adverse Event" screen in the subject's eCRF, irrespective of intensity or whether or not they are considered to be vaccination-related. Thereafter, from Day 22 to the end of the surveillance period, SAEs, AEs leading to withdrawal, and NOCDs will be monitored and reported in the eCRF.

The intensity of AEs will be graded as: mild (1), moderate (2), severe (3) or potentially life threatening (4), according to the Food and Drug Administration (FDA) Guidance for Industry (FDA, 2007). Their causal relationship with the study vaccine will be assessed by the Investigator (definitely not related, probably not related, possibly related, probably related or definitely related); see Section 13.1.9 in protocol for a definition of these causal relationships.

8.8.3 Secondary Endpoints

The secondary endpoints of safety that are mentioned in section 4.2.1 will be analysed as follows: All safety analyses will be based on the SAS.

Safety and tolerability endpoints (immediate complaints, solicited local and systemic reactions, and TEAEs, deaths, SAEs, AEs leading to subject withdrawal, and NOCDs) will be summarized by treatment group and by age group using descriptive statistics.

The original terms used in the eCRFs by Investigators to identify AEs will be coded using the MedDRA®. All eCRF reported AEs with onset post-vaccination will be included in the safety analyses.

Special attention will be given to those subjects who die, who discontinue from the study due to an AE, who experience an SAE (e.g. summaries, listings, and narrative preparation will be provided, as appropriate), or who experience an allergic or allergic-like reaction (hypersensitivity cases).

Overall summaries of subjects with solicited reactions and Unsolicited Treatment Emergent Adverse Events (TEAE) will be prepared as follows:

- Subjects with any solicited reactions or unsolicited TEAEs
- Any solicited local and systemic reactions occurring 15 minutes after vaccination (immediate complains)
- Solicited reactions including immediate complains
- Severe and potentially life-threatening solicited reactions
- Severe and potentially life-threatening related solicited reactions
- Solicited local reactions
- Solicited systemic reactions
- Unsolicited TEAEs (Day 0 to Day 21)
- Related unsolicited TEAEs

- Severe and potentially life-threatening unsolicited TEAEs
- Severe and potentially life-threatening related unsolicited TEAEs
- Serious TEAEs (Day 0 to Day 21)
- Serious TEAES (Day 0 to End of study)

The percentage of subjects with at least one immediate complaint (local or systemic reactions) and the percent of subjects with at least one immediate complaint of each of the individual local and systemic reactions will be summarized by treatment group and by age group.

In addition, the same summary will be tabulated by maximum severity grading and by causality for each treatment group overall and by age group.

The maximum event severity will be considered to be the greatest severity associated with each reaction according to the following order: mild < moderate < severe <potentially life-threatening. Causality will be presented as “Unrelated” (definitely not related and probably not related) and “Related” (possibly related, probably related and definitely related).

Similar summaries will be presented for solicited local and systemic reactions collected from Day 0 to Day 7 and unsolicited TEAEs collected up to Day 21. In addition, summaries of severe and potentially life-threatening solicited local and systemic reactions and unsolicited TEAEs will also be provided.

The analysis of unsolicited adverse events comprises the following categories:

- Occurrence of any unsolicited TEAE by system organ class (SOC) and PT from the administration of the vaccine dose upto day 21 (day 0 to day 21)
- Unsolicited TEAEs by SOC, PT and Maximum severity (Day 0 to 21)
- Unsolicited TEAEs by SOC, PT and relationship status (Day 0 to 21)
- Grade 3 and Grade 4 related unsolicited TEAEs by SOC and PT (Day 0 to Day 21)

AE summary tables will be presented for the below categories as well:

- AEs leading to death (Day 0 to End of study)
- Serious TEAEs (Day 0 to Day 21) by SOC and PT
- Serious TEAEs (Day 0 to End of study) by SOC and PT
- AEs leading to withdrawal (Day 0 to End of study)
- Occurrence of New Onset of a Chronic Disease (NOCD)s (Day 0 to Day 21) by SOC and PT
- Occurrence of NOCDs (Day 0 to End of study) by SOC and PT

- Occurrence of Hypersensitivity Cases (Day 0 to Day 21) by SOC and PT

Events that are considered to be a new onset of chronic disease (NOCD), as defined in the study protocol, will be collected until the end of surveillance. A summary of the occurrence of NOCDs, by SOC and PT, will be provided for Day 0 to Day 21 and from Day 0 to the end of surveillance.

Hypersensitivity cases will be identified using a standardized MedDRA® query (SMQ) for hypersensitivity. A summary of the occurrence of hypersensitivity events, by SOC and PT, will be provided for Day 0 to Day 21 and from Day 0 to the end of surveillance.

In summaries by SOC and PT, events will be sorted by internationally order (please refer Appendix A).

Where the same reaction or same adverse event, based on preferred terminology, is reported multiple times for the same subject in a treatment period, the subject will only be counted once in the preferred terminology level in summary frequency tables.

Individual listings will be provided for the categories below:

- Solicited local and systemic reactions Day 0 to Day 7
- Unsolicited AEs (Day 0 to Day 21)
- Serious TEAEs (Day 0 to end of surveillance)
- NOCD (Day 0 to End of Study)
- Hypersensitivity Cases (Day 0 to End of study)

8.8.4 Analysis of Exploratory Endpoints

The exploratory safety endpoints defined in 4.2.2 will be analysed as follows:

The occurrences of non-routine medical visits, emergency room visits, hospitalizations, pneumonia (clinical diagnosis), new onset or exacerbations of pre-existing cardio-respiratory conditions, and number of workdays missed will be summarized by treatment using descriptive statistics and percentages (surveillance period). These analysis will be done separately and not be included in the CSR.

8.8.5 Clinical laboratory evaluations

As part of screening procedures, a urine sample will be collected for female subjects of childbearing potential for a urine dipstick pregnancy test that will be performed during the eligibility assessment on Day 0.

An individual listing will be provided for the Urine Pregnancy Test Results.

8.8.6 Vital signs

Vital signs measurements (resting BP, HR, and OT) will be performed as part of screening procedures (during eligibility assessment on Day 0) and after the 15-minute post-vaccination

surveillance period.

An individual listing will be provided for vital signs measurements by subject and age stratum.

8.8.7 Physical examinations

A history- or symptom-directed physical examination will be performed by the Investigator as part of screening procedures (during the eligibility assessment on Day 0).

An individual listing will be provided for Physical Examination results by subject and age stratum.

8.8.8 Electrocardiograms

Electrocardiograms are not planned for this study.

8.8.9 Other safety assessments

No other safety assessments are planned for this study.

8.9 Other analysis

Not planned.

8.9.1 Subgroup analysis

The following subgroup analysis will be performed for the primary efficacy endpoint.

- Age group
- Laboratory-confirmed matched influenza strain
- Sex
- Race
- Ethnicity

8.10 Intermediate analysis

The purpose and analyses performed for intermmediate analysis will be included in a separate charter.

9 CHANGES TO PLANNED ANALYSIS FROM STUDY PROTOCOL

There are no changes in the analysis methods other than planned in protocol.

10 REFERENCES

1. ICH Topic E3: Structure and Content of Clinical Study Reports (CPMP/ICH/137/95-adopted December 1995).
2. ICH Topic E9: Statistical Principles for Clinical Trials (CPMP/ICH/363/96 – adopted March 1998).
3. FDA. (2007). Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.
4. SAS® Institute Inc. SAS 9.4 Help and Documentation
5. Walter A.Orenstein et al (1985),.Field evaluation of vaccine efficacy. the World Health Organization, 63 (6): 1055-1068.
6. Chan & zhang 1999 Chan, I.S.F., Zhang, Z., 1999. Test-based exact confidence intervals for the difference of two binomial proportions. Biometrics 55, 1201–1209.

11 APPENDICES

Appendix A – Order of SOC (Medical History, Unsolicited Treatment-Emergent AE)

SOC *Infections and infestations*
SOC *Neoplasms benign, malignant and unspecified (incl cysts and polyps)*
SOC *Blood and lymphatic system disorders*
SOC *Immune system disorders*
SOC *Endocrine disorders*
SOC *Metabolism and nutrition disorders*
SOC Psychiatric disorders
SOC *Nervous system disorders*
SOC *Eye disorders*
SOC *Ear and labyrinth disorders*
SOC *Cardiac disorders*
SOC *Vascular disorders*
SOC *Respiratory, thoracic and mediastinal disorders*
SOC *Gastrointestinal disorders*
SOC *Hepatobiliary disorders*
SOC *Skin and subcutaneous tissue disorders*
SOC *Musculoskeletal and connective tissue disorders*
SOC *Renal and urinary disorders*
SOC *Pregnancy, puerperium and perinatal conditions*
SOC *Reproductive system and breast disorders*
SOC *Congenital, familial and genetic disorders*
SOC *General disorders and administration site conditions*
SOC *Investigations*
SOC *Injury, poisoning and procedural complications*
SOC *Surgical and medical procedures*
SOC *Social circumstances*
SOC *Product issues*

Table 3-2. The MedDRA Terminology SOC List – Internationally Agreed Order