CLINICAL STUDY PROTOCOL

A Phase I/II, randomised, placebo-controlled, partially-blinded, parallel-group study to assess the safety, tolerability and immune response following vaccination with Immunose™ FLU

Protocol Number:	EURO 15-11
Protocol Version:	Final 2016-06-30
Protocol Status:	Final
Product:	Immunose™ FLU: Endocine™ + quadrivalent inactivated influenza antigens
Phase	I/II
EudraCT No.:	2016-001918-18
Coordinating Investigator:	Cornelia Lif-Tiberg, MD CTC Clinical Trial Consultants Dag Hammarskjölds väg 13 752 37 Uppsala, Sweden
Sponsor:	Eurocine Vaccines AB Fogdevreten 2 Karolinska Institutet Science Park 171 65 Solna, Sweden
Study Management:	PCG Clinical Services AB Kungsängsvägen 19 753 26 Uppsala, Sweden Phone: +46 18 430 3100
Planned clinical start:	Q3 2016
Planned clinical completion:	Q2 2017

1. SIGNATURES

1.1 INVESTIGATOR STATEMENT

I have read and understood this clinical study protocol (CSP) and agree to conduct the study accordingly. I have read and agree to comply with the Investigator obligations stated in this CSP.

I understand that deviations from the CSP are to be made in form of amendments, which must have the prior written approval by myself, Eurocine Vaccines AB and PCG Clinical Services AB (PCG).

I agree to ensure that all personnel that assist me in the conduct of the study are aware of their obligations. I agree to use the study material, including medication, only as specified in the CSP.

I am thoroughly familiar with the appropriate use of the study drug, as described in this CSP and any other information provided by Eurocine Vaccines AB including, but not limited to, the current Investigator's Brochure (IB).

I agree to report any serious adverse event (SAE) as described in this CSP.

I am aware of, and will comply with good clinical practice (GCP) and all applicable regulatory requirements.

The signature below constitutes the approval of this CSP and appendices, and provides the necessary assurances that this study will be conducted accordingly.

Principal Investigator:

_____ _____

Signature Date

_____

Printed Name

_____

Site

1.2 SIGNATURE PAGE

Sponsor's Representatives:

Hans Arwidsson, PhD
CEO Eurocine Vaccines AB

Date

Anna-Karin Maltais, PhD
CSO Eurocine Vaccines AB

Date

Markus Jerling, MD, PhD
Medical Monitor

Date

Coordinating Principal Investigator:

Cornelia Lif-Tiberg, MD
CTC Clinical Trial Consultants AB

Date

Biostatistician:

Nils Adriansson, BSc
PCG Clinical Services AB

Date

2. CLINICAL STUDY PROTOCOL SYNOPSIS

Study Title A Phase I/II, randomised, placebo-controlled, partially-blinded, parallel-group study to assess the safety, tolerability and immune response following vaccination with Immunose™ FLU
Protocol Number/Study Code EURO 15-11
EudraCT Number 2016-001918-18
Phase of Development Phase I/II
Name and Address of Sponsor Eurocine Vaccines AB Fogdevreten 2 Karolinska Institutet Science Park 171 65 Solna, Sweden
Planned Study Time Estimated first subject randomized: Q4 2016 Estimated last subject randomized: Q4 2016 Estimated last subject out: Q2 2017
Coordinating Investigator, site 1: Cornelia Lif-Tiberg, MD CTC Clinical Trial Consultants Dag Hammarskjölds väg 13 752 37 Uppsala, Sweden **Principal Investigator, site 2:** Daniel Wilhelms, MD CTC Clinical Trial Consultants AB Brigadgatan 26 587 58 Linköping, Sweden
Test products and placebo **Immunose™ FLU 1%:** Quadrivalent influenza vaccine (QIV), based on split, inactivated virus, containing 15 µg haemagglutinin (HA)/strain and 1% Endocine™ for intranasal administration at 2 vaccination occasions 3 weeks apart. **Immunose™ FLU 2%:** QIV, based on split, inactivated virus, containing 15 µg HA/strain and 2% Endocine™ for intranasal administration at 2 vaccination occasions 3 weeks apart. **Influenza antigen**: QIV, based on split, inactivated virus, containing 15 µg HA/strain for intranasal administration at 2 vaccination occasions 3 weeks apart. **Placebo:** Saline (NaCl) for intranasal administration at 2 dosing occasions 3 weeks apart.
Comparators [redacted]: Trivalent influenza vaccine (TIV) based on split, inactivated virus, containing 15 µg HA/strain for intramuscular administration at a single vaccination occasion. [redacted]: Quadrivalent live attenuated influenza vaccine (LAIV) containing $10^{7.0\pm0.5}$ fluorescent focus units (FFUs) for intranasal administration at a single vaccination occasion.
Indication Influenza
Diagnosis and main criteria for inclusion Healthy male and female volunteers aged 18 to 39 (both inclusive) with no serious illness who have provided written informed consent will be considered for participation in the study. Females must agree to use a highly efficient method of contraception. Subjects with a diagnosis of laboratory-confirmed influenza in the 2015/2016 season or who have received an influenza vaccine within 6 months of screening will not be eligible to participate in the study.

Study Design

This is a Phase I/II, randomised, partially double-blind (group 1 to 4), parallel-group study designed to evaluate the safety, tolerability and immune response following Immunose™ FLU vaccination at 2 sites in Sweden.

Consenting subjects will be assessed for eligibility at the screening visit (Visit 1) within 6 weeks prior to randomisation. Eligibility will be re-confirmed prior to each dose.

A total of 162 subjects will be randomised to one of 6 treatment groups in a 2:2:2:1:1:1 ratio on Day 0 (Visit 2) as follows:

- **Group 1:** Immunose™ FLU 1%, containing 15 µg HA/strain and 1% Endocine™ for intranasal administration (n=36)
- **Group 2:** Immunose™ FLU 2%, containing 15 µg HA/strain and 2% Endocine™ for intranasal administration (n=36)
- **Group 3:** Influenza antigen, containing 15 µg HA/strain for intranasal administration (n=36)
- **Group 4:** Saline (NaCl) for intranasal administration (n=18)
- **Group 5:** [redacted], containing 15 µg HA/strain for intramuscular administration (n=18)
- **Group 6:** [redacted], containing $10^{7.0\pm0.5}$ FFUs for intranasal administration (n=18)

Subjects in group 1 to 4 will receive treatment in a partially double-blinded fashion via intranasal administration on Day 0 (Visit 2) and on Day 21+7 (Visit 3). Since the visual appearance of the study drugs may differ, there is a risk that the administrating nurse will be unblinded. Therefore, the pre- and post-dose inspection of the administration site will be performed by an independent evaluator. The same independent evaluator will perform both pre- and post-dose assessments.

For subjects in group 1 to 4 there will be a total of 4 visits to the clinic; Visit 1 (screening), Visit 2 (Day 0), Visit 3 (Day 21+7) and Visit 4 (Day 42+7). Telephone contacts will be taken approximately every 8th week after the last visit to the clinic (visit 4) for collection of any adverse events (AEs) of special interest or serious AEs (SAEs) that may have occurred after Visit 4 during a 6 months safety follow-up period starting from the day of the last vaccination.

Subjects in group 5 and 6 will receive treatment in an unblinded fashion via a single intramuscular (group 5) or single intranasal (group 6) administration on Day 0 (Visit 2). No treatment will be given on Day 21+7 (Visit 3).

For subjects in group 5 and 6 there will be a total of 3 visits to the clinic; Visit 1 (screening), Visit 2 (Day 0), and Visit 3 (Day 21+7). Telephone contacts will be taken approximately every 8th week after the last visit to the clinic (Visit 3) for collection of any AEs of special interest or SAEs that may have occurred after Visit 3 during a 6 month safety follow-up period starting from the day of the last vaccination.

In all groups safety and tolerability, including local tolerability, will be assessed. Local tolerability (swelling and redness [assessed by an independent evaluator] and pain and pruritus [assessed by the subject]) will be assessed before and after each treatment (Visit 2 and 3 for group 1 to 4 and Visit 2 for group 5 to 6). Safety and tolerability will be assessed by vital signs (blood pressure and heart rate), physical examination, 12-lead electrocardiogram (ECG), collection of AEs and laboratory tests (haematology, clinical chemistry and urinalysis). Subjects with suspected influenza will be tested to confirm presence and strain of the influenza virus.

Local tolerability will also be assessed by the subjects' recording of the presence or absence of local administration site reactions (pain and pruritus) and solicited AEs (runny nose, nasal congestion, sneezing, fever [temperature >38°C], chills, sweating, headache, tiredness, muscle aches, joint pain, cough, sore throat, nausea) in an electronic diary for 10 days after each dosing occasion, starting in the evening the day after treatment.

Immune responses against the vaccine antigens will be evaluated in both nasal and blood samples. Influenza-specific immune responses will be evaluated in nasal samples (IgA) and in serum (HI and VN) and PMBC (T cells) from blood samples. Blood samples for peripheral blood mononuclear cell (PBMC) preparation and subsequent T-cell analysis will be taken from 10 subjects per group.

The total study duration for the subjects in the study will be up to 36 weeks (group 1 to 4) and 33 weeks (group 5 and 6) including a 6 week screening period and a 6 months follow-up period after the last dose.

Number of Patients

Approximately 243 subjects will be screened in order to randomise 162 subjects to 6 treatment groups in a ratio of 2:2:2:1.1:1.

Objectives

Primary Objective

To evaluate the safety and tolerability of Immunose™ FLU based on Endocine™ and quadrivalent influenza antigen.

Secondary Objective

To evaluate the immune response to Immunose™ FLU based on Endocine™ and quadrivalent influenza antigen by measurement of haemagglutination-inhibition (HI) and virus neutralization (VN) titres.

Exploratory Objective
To evaluate the immune response to Immunose™ FLU based on Endocine™ and quadrivalent influenza antigen by measurement of immunoglobulin A (IgA) in nasal secretions.

Primary Endpoints

- Type and incidence of AEs and SAEs from the time of first study drug administration (Visit 2) until the last visit to the clinic (Visit 4 for group 1 to 4 and Visit 3 for group 5 to 6).
- Type and incidence of SAEs and AEs of special interest during the 6 months safety follow-up.
- Frequency and severity of administration-site reactions as assessed by an independent evaluator with help from the study subject before study drug administration and 15 minutes and 120 minutes after study drug administration. Administration-site reactions to be recorded are swelling, redness, pain and pruritus in the administration area (nose or arm). Reactions are classified as none, mild, moderate or severe. The administration-site reactions pain and pruritus will also be recorded by the subject in an electronic diary for 10 days starting in the evening the day after each treatment and classified as none, mild, moderate or severe.
- Frequency of solicited AEs. The subjects will record the occurrence of solicited AEs in an electronic diary for 10 days after study drug administration. Solicited AE include runny nose, nasal congestion, sneezing, fever (i.e., temperature >38°C), chills, sweating, headache, tiredness, muscle aches, joint pain, cough, sore throat, nausea.
- Frequency of clinically significant changes in ECG, vital signs, physical examination findings and laboratory variables from baseline to the last visit to the clinic.

Secondary Endpoints

- Measurement of HI in blood, including:
  - Geometric mean titres (GMTs) and pre-/post-treatment ratios (GMRs)
  - Percentage of subjects with seroprotection (i.e., an HI titre ≥40) at Day 21 and for group 1 to 4 also at Day 42.
  - Percentage of subjects with seroconvertion (i.e., either a pre-treatment HI titre <10 and a post-treatment titre ≥40 or a pre-treatment HI titre ≥10 and a fourfold increase in titre) at Day 21 and for group 1 to 4 also at Day 42.
- Measurement of VN titres in blood (pre-treatment and 21 days after each treatment)
  - Geometric mean titres (GMTs) and pre-/post-treatment ratios (GMRs)

Exploratory endpoints

- Measurement of influenza-specific IgA in nasal secretions (pre-treatment and 21 days after each treatment)

Post-hoc exploratory endpoints
The following may be analysed if considered valuable based on the results of the secondary analyses:

- Influenza-specific T cell responses
- Single radial haemolysis (SRH) analysis
- Functional analysis of IgA in nasal swab
- Measurement of neuraminidase (NA) specific antibodies

Exploratory analyses may only be performed on selected subjects, time points and virus strains but will always be performed both pre-and post-treatment. Results from the analyses of exploratory endpoints may be presented separate from the clinical study report (CSR).

Statistical Methods:

The primary endpoints as defined in the primary objective will be presented using descriptive statistics. The secondary and exploratory endpoints will be analysed descriptively and, in addition, statistical analysis using analysis of covariance (ANCOVA) will be performed to evaluate the adjuvant effect of Endocine™ compared to the unadjuvanted group, and to evaluate the immune responses induced by Immunose™ FLU compared to placebo and the comparators.

Study Reporting:

After completion of the clinical phase of the study, an ICH-E3 compliant CSR will be prepared. Data collected during the follow-up phase will be added as an addendum to the CSR.

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APPENDICES

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4. Questions for the 6 months safety follow-up

4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation, acronym or specialist term	Explanation
AE	Adverse event
ALT	Alanine aminotransferase
AMP	Amphetamine
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomic therapeutic chemical classification system
BMI	Body mass index
BUP	Buprenorphine
BZO	Benzodiazepines
CDC	Centres for disease protection and control
CIOMS	Council for international organisations of medical sciences
COC	Cocaine
CPK	Creatinine phosphokinase
CRP	C-reactive protein
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
DMP	Data management plan
eCRF	Electronic case report form
ECG	Electrocardiogram
EEA	European economic area
EMA	European medicines agency
FAS	Full analysis set
FFU	Fluorescent focus unit
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GMR	Geometric mean ratio
GMT	Geometric mean titre
HA	Hemagglutinin
HI	Hemagglutination-inhibition
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form

ICH	International conference on harmonization
IEC	Independent ethics committee
IgA	Immunoglobulin A
IMP	Investigational medical product
ITT	Intention to treat
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LAIV	Live attenuated influenza vaccine
MedDRA	Medical dictionary for regulatory activities
MOP	Morphine
MPA	Medical products agency
MTD	Methadone
NA	Neuraminidase
OTC	Over-the counter
PBMC	Peripheral blood mononuclear cell
PBS	Phospate-buffered saline
PCG	PCG Clinical Services AB
pIMD	Potential immune-mediated disease
PPAS	Per protocol analysis set
PT	Preferred term
QIV	Quadrivalent influenza vaccine
RBC	Red blood cell count
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SDV	Source data verification
SmPC	Summary of product characteristics
SRH	Single radial haemolysis
SOC	System organ class
SUSAR	Suspected unexpected serious adverse reaction
THC	Marijuana
TIV	Trivalent influenza vaccine
VN	Virus-neutralization
WBC	White blood cell count
WHO	World health organisation
WMA	World medical association

5. STATEMENT OF COMPLIANCE

The study will be carried out in accordance with:

- The guidelines of the world medical association (WMA) declaration of Helsinki (as amended by the 64th WMA General Assembly, Seoul, October 2008) (attached as Appendix 1)
- The guidelines of good clinical practice (GCP) (CPMP/ICH/135/95)
- Explanatory note and comments to the above, issued as CPMP/768/9.
- EU Directive (2005/28/EG, April 2005)
- LVFS 2011:19 (Läkemedelsverkets Författningssamling, 2011-11-29)
- Demands of national drug and data protection laws and other applicable regulatory requirements

The following guidelines serve as a basis for the Immunose™ FLU development program:

- European medicines agency (EMA) guideline on influenza vaccines (EMA/CHMP/VWP/457259/2014)
- World health organisation (WHO) guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (2013)

6. ETHICAL CONSIDERATIONS

This study will be performed in accordance with the ethical principles that have their origin in the declaration of Helsinki and are consistent with guidelines of the international conference on harmonisation (ICH), GCP and applicable regulatory requirements.

The Sponsor or designee will obtain approval to conduct the study from the Swedish medical products agency (Läkemedelsverket) prior to the start of the study in accordance with Swedish regulatory requirements.

It is the responsibility of the coordinating Investigator to submit the clinical study protocol (CSP), the subject information sheet and informed consent form (ICF), subject recruitment procedures, the investigator's brochure (IB), information on payments and compensation available to subjects, Investigator remuneration and other study specific documentation as applicable to the independent ethics committee (IEC). Written approval must be obtained from the IEC prior to the start of the study.

The Sponsor will compensate the study site for their work in the study. However, the compensation will not be affected by the outcome of the study. Subjects will receive compensation for study participation and will be reimbursed for any reasonable travel cost as a result of participating in the study.

6.1 SUBJECT INFORMATION AND INFORMED CONSENT

The subject information and consent process is outlined in detail in Section 12.2. The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

6.2 SUBJECT DATA PROTECTION

The subject ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the EU Data Protection Directive (95/46/EC), the data will not identify any persons taking part in the study.

The potential study subject must be informed that by signing the ICF he/she approves that authorised representatives from Sponsor and PCG, the concerned IEC and Competent Authority have direct access to his/her medical records for verification of clinical study procedures. This agreement is to be substantiated in a separate document, according to local requirements.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete.

The Investigator must file a subject identification list which includes sufficient information to link records, i.e. the electronic case report form (eCRF) and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

6.3 SUBJECT INFORMATION CARD

All study subjects will be provided with a subject information card to be worn easily accessible during the study. The card will include the following information:

- That the subject is participating in a study
- That the subject is treated with any of the 6 study treatments
- Subject study ID
- The name and telephone number of the Investigator
- The name and address of the Sponsor

7. CONFIDENTIALITY STATEMENT

This CSP contains information that is confidential and proprietary to Eurocine Vaccines AB. This information is being provided to you for the purpose of conducting a clinical study for Eurocine Vaccines AB. You may disclose the contents of this CSP to study personnel under your supervision who need to know the contents for this purpose, as well as to your IEC. The contents of this CSP may not be disclosed to any other person or entity without the prior written permission of Eurocine Vaccines AB and may not be used for any other purpose than the conduct of this study. The foregoing shall not apply to disclosure required by governmental regulations or laws; however, you will give prompt notice to Eurocine Vaccines AB of any such disclosure.

Any supplemental information that may be added to this document is also confidential and proprietary to Eurocine Vaccines AB and must be kept in confidence in the same manner as the contents of this CSP.

Any person who receives this CSP without due authorisation from Eurocine Vaccines AB is requested to return it to Eurocine Vaccines AB or to promptly destroy it.

8. STUDY ADMINISTRATION

Study Role	Name, title and address	Telephone and e-mail
Sponsor	Eurocine Vaccines AB Fogdevreten 2 Karolinska Institutet Science Park 171 65 Solna, Sweden	
Sponsor's CEO	Hans Arwidsson, PhD Eurocine Vaccines AB	Telephone: [redacted] E-mail: hans.arwidsson@eurocine-vaccines.com
Sponsor's CSO	Anna-Karin Maltais, PhD Eurocine Vaccines AB	Telephone: [redacted] E-mail: annakarin.maltais@eurocine-vaccines.com
Sponsor's Project Manager	Marie Olliver, PhD Eurocine Vaccines AB	Telephone [redacted] E-mail: marie.olliver@eurocine-vaccines.com
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Coordinating Investigator, site 1	Cornelia Lif-Tiberg, MD	Telephone: [redacted] E-mail: [redacted]
Clinical Conduct Site 2	CTC Clinical Trial Consultants AB Brigadgatan 26 587 58 Linköping, Sweden	
Principal Investigator, site 2	Daniel Wilhelms, MD	Telephone: [redacted] E-mail: [redacted]
CRO	PCG Clinical Services AB Kungsängsvägen 19 753 23 Uppsala, Sweden	
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CRO Medical writer	Sandra Kuusk, PhD PCG Clinical Services AB	Telephone: [redacted] E-mail: Sandra.kuusk@pharmaconsultinggroup.com

Manufacturing of Immunose FLU and influenza antigen, packaging and labelling	[redacted]	
Manufacturing [redacted]	[redacted]	[redacted]
Manufacturing [redacted]	[redacted]	
Analytical laboratory (immunology)	Vismederi Srl Strada del Petriccio e Belriguardo, 35 53100 Siena, Italy	Chiara Centi Telephone: [redacted] E-mail: centi@vismederi.com
Process laboratory (PBMC)	The Rudbeck laboratory 751 85 Uppsala, Sweden	
Laboratory for influenza test	Klinisk mikrobiologi Dag Hammarskjölds väg 17 752 37 Uppsala	

CEO: chief executive officer, CRO: clinical research organisation, CSO: chief scientific officer

9. BACKGROUND INFORMATION

9.1 INTRODUCTION AND STUDY RATIONALE

Influenza is an infectious disease of birds and mammals caused by an RNA virus of the family Orthomyxoviridae. Typically, the influenza virus is transmitted from infected mammals through the air by coughs or sneezes, creating aerosols containing the virus that infects the upper respiratory tract causing fever, sore throat, muscle pain, headache, coughing, and weakness.

The influenza virus spreads around the world in seasonal epidemics, killing millions of people in pandemic years and hundreds of thousands in non-pandemic years.

Influenza virus is generally grouped into Influenza type A, B, and C where type A and B give rise to most influenza infections in humans. Influenza type A is further divided into subgroups, or strains, based on the appearance of the viral surface antigens haemagglutinin (HA) and neuraminidase (NA).

Continuous mutations of the influenza virus genome result in new variations of HA and NA. There are two mechanisms that contribute to these variations; antigenic shift and antigenic drift. Antigenic shift may occur through genetic reassortment between human influenza virions and virus particles from birds or other mammals resulting in virus particles with new combinations of HA and NA. If able to infect humans, these new strains may cause pandemic outbreaks. Antigenic drift gives rise to minor changes and occurs by accumulation of genomic point mutations. Eventually a variant protein, i.e. antigen, appears that no longer can be recognized by the antibody to the original antigen. This constant antigenic change means that a vaccine formulated for one year may be ineffective in the following year. Hence new vaccines have to be made on a regular basis.

The incidence and severity of influenza varies from year to year and it is estimated that typically about 3 to 5 million cases of severe illness and about 250,000 to 500,000 deaths occur every season. People at high risk of developing flu-related complications are children less than 5 years of age, adults 65 years of age and older, pregnant women, individuals with medical conditions including asthma, cardiac disease, uncontrolled diabetes, impaired immune response, chronic liver or kidney disease, and severe obesity with body mass index (BMI) >40.

Children younger than 2 years old are at especially high risk of developing severe influenza complications, emphasizing the need for improved influenza prevention efforts in this risk group. The Centres for Disease Protection and Control (CDC) in the U.S. recommends annual influenza vaccination for everyone 6 months of age and older, and the world health organisation (WHO) recommends influenza vaccination for children between 6 months and 5 years. Several other countries including Canada and the UK recommend annual influenza vaccination of children. The injected influenza vaccines currently approved for children have limited efficacy (around 60%, but often much lower) and are approved for use in children from 6 months. The nasal-spray vaccine Fluenz (also known as live attenuated influenza vaccine, or LAIV) has shown protective efficacy of 82% but is not approved for children under 2 years and children with severe asthma, two of the most vulnerable groups for severe influenza infection. The side effects are associated with the nature of the vaccine – that it is a live, replicating virus.

The most common theories why the nasal vaccine Fluenz Tetra evokes superior protection in children compared to injected influenza vaccines are that a live attenuated virus evokes a better T-cell response and/or that the nasal route of administration induces a more protective immune response in children than injected inactivated influenza antigen. The importance of the mode of administration could be explained by activation of the adenoid tonsil, an organ present in the nasal cavity in children, which is a mucosal inductive site for humoral and cellular immune responses. This organ normally disappears in early adolescence.

Eurocine Vaccines plans to develop Immunose™ FLU, a nasal, inactivated influenza vaccine candidate. Immunose FLU contains inactivated influenza antigens and the adjuvant Endocine™. Endocine

consists of the lipids [redacted] and [redacted]. The hypothesis is that Immunose FLU will confer a similar protection as Fluenz Tetra in children but with a safety profile allowing use in children from 6 months and in children with severe asthma. Endocine, the adjuvant component of Immunose FLU, supports transport of the antigen through the mucosal layer and can take advantage of nasal administration without the need for replication, which limits the use of Fluenz Tetra in young children.

Immunose FLU has been shown to induce complete protection in influenza-naïve ferrets challenged with influenza virus. Virus neutralising titres were similar to what has been shown with Fluenz in the ferret model and support the paediatric profiling of Immunose FLU. Immunose FLU also induces high haemagglutination inhibition (HI) antibody titres in influenza-naïve ferrets already after one vaccine dose.

Nasal vaccination has the potential to elicit both systemic and mucosal immunity against pathogens. However, split and subunit vaccines lack potency to stimulate mucosal immunity, and a nasal adjuvant is indispensable for eliciting a potent mucosal immune response to nasal vaccines. Adjuvants are substances that are added to a vaccine antigen in order to enhance the immunological response. Immunose FLU is composed of inactivated split influenza antigen combined with Eurocine Vaccines' proprietary adjuvant Endocine. [redacted] The two lipids in Endocine are naturally present in human adipose tissue. Both [redacted] and [redacted] are approved for pharmaceutical use and are described in the European and US Pharmacopoeia.

In the present study, the safety and tolerability and the immune response to intranasally administered Immunose FLU based on Endocine in 2 doses (1% and 2%) and quadrivalent influenza antigens will be evaluated in adults. One group receiving influenza antigen without Endocine and one group receiving saline alone, both for intranasal administration, are included to enable evaluation of the Endocine effect on the immune response and the safety and tolerability of Endocine compared to influenza antigen alone and to placebo. In addition, one group will receive [redacted] and one group will receive [redacted], for a general comparison of the safety and tolerability and the immune response of Immunose FLU with that of these marketed products.

9.2 TOXICOLOGY STUDIES WITH IMMUNOSE FLU

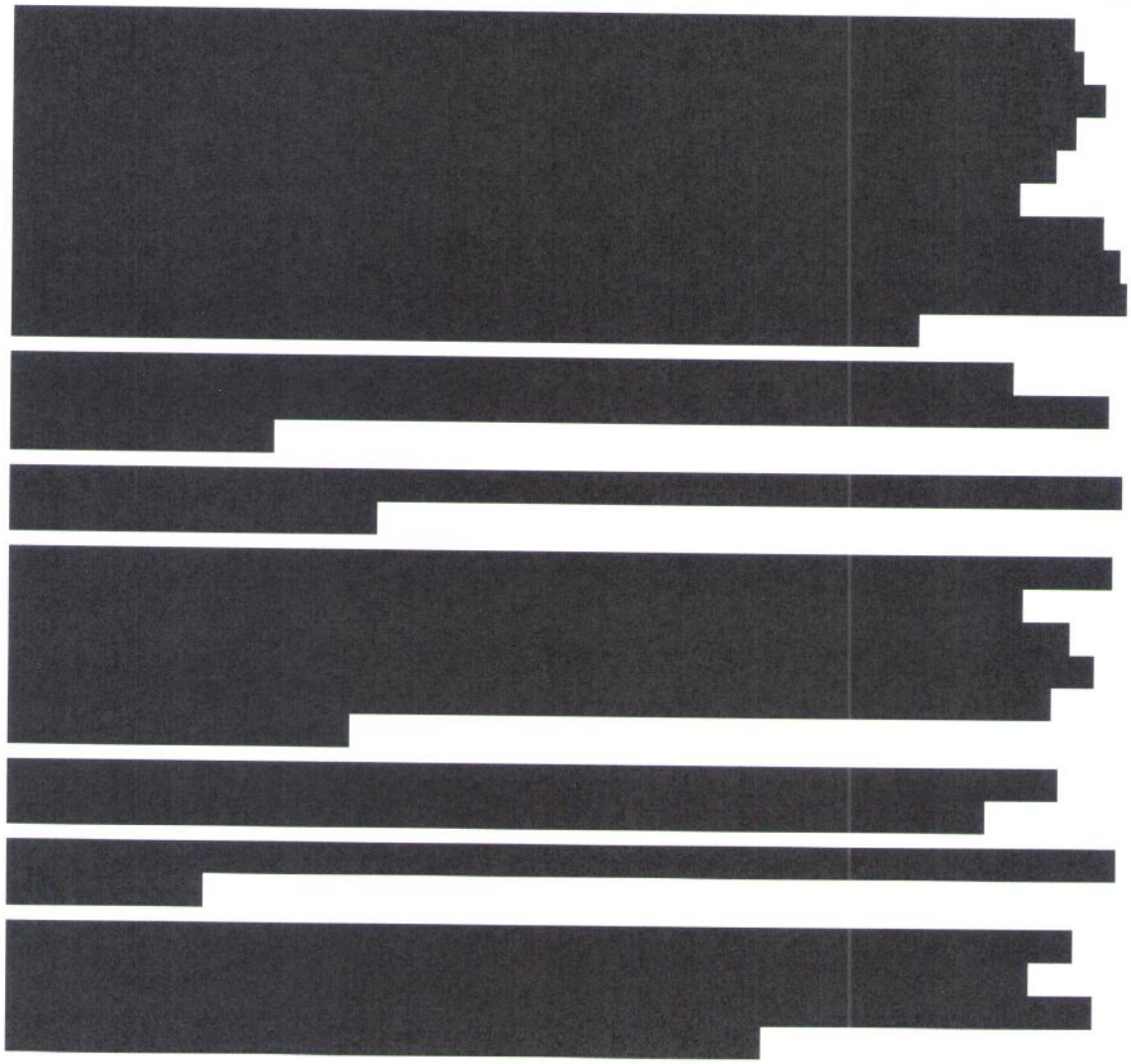
9.2.1 Repeat dose toxicity study of Immunose™ FLU based on split antigen in rat

[redacted]

[redacted]

9.2.2 Single dose study evaluating local irritation after vaccination with Immunose FLU based on split antigen in mice

[redacted]



9.3 SUMMARY OF FINDINGS FROM PRE-CLINICAL STUDIES

Endocine and Immunose FLU have been extensively studied and characterised in mice, rats, and ferrets. Endocine significantly increases both local and systemic immune responses after intranasal immunisation (Falkeborn et al., 2013). Endocine has been shown to potentiate the immune response to several different antigens and the results from studies with Immunose FLU show a broad immune response and protection from influenza disease (Maltais et al., 2014). For further details, refer to the IB.

9.4 SUMMARY OF FINDINGS FROM CLINICAL STUDIES

There is no clinical experience with Immunose FLU based on influenza split antigens and Endocine. However, there is clinical experience with Endocine using diphtheria toxoid as an antigen (dnr 151:2002/38324), human immune deficiency virus (HIV) as an antigen (CTN-BI/Vacc-4x/L3-2011/1) and most relevant using a whole inactivated influenza virus as an antigen (EUDRA CT 2007-005005-22). In total, 174 human subjects have been exposed to Endocine and the safety data support continued development. For further details, refer to the IB.

9.5 RATIONALE FOR THE STUDY DESIGN, DOSES AND CONTROL GROUPS

Immunose FLU with 2 different concentrations of Endocine will be investigated to assess the influence of adjuvant concentration on the response parameters, whereas the antigen dose will be kept constant according to standard practice for influenza vaccinations. The inactive control group (saline) is included for an enhanced interpretation of safety data through control for placebo effects, and for the analysis of immune responses through control for background antibody levels. The comparators to Immunose FLU; [redacted] for intranasal administration and [redacted] for intramuscular injection of antigen have been selected to compare the safety and tolerability and the overall immune response.

Immunose FLU will be administered at 2 occasions with a 3-week interval to maximize the magnitude of the immune response as indicated by non-clinical data. Influenza antigen without adjuvant and saline alone will also be administered nasally at two occasions. [redacted] and [redacted] will be administered at one occasion according to the prescribing information.

Endocine will be tested at the concentrations 1% and 2% in two different treatment groups. In a previous study with nasal influenza vaccination, Endocine was evaluated with concentrations ranging from 0.5% to 2% (report EV 2011-08, EUR-001). Local tolerability symptoms were more commonly reported following vaccination with Endocine compared to vaccination without Endocine with a slightly higher incidence of reactions following administration of 2% compared to 0.5% and 1%. Symptoms were rapidly reversible and there were no safety concerns for any treatment group. In the present study the dose-response for Endocine versus safety variables and immune response will be evaluated, and the dose steps 1% and 2% are considered adequate.

The immunology variables to be investigated in the study have been selected to represent both systemic and local immune responses, which both might be indicative of the protective efficacy in later studies.

Administration site reactions will be collected at the clinic during 2 hours of observation after each study drug administration. In previous studies with Endocine products such reactions, if occurring, were transient and in the vast majority of cases disappeared within this time period. Possible later local and systemic reactions will be collected over 10 days using a diary. In accordance with the guidelines on influenza vaccines and adjuvanted vaccines a long-term safety follow-up for 6 months after the last vaccination is included to detect any potential late occurring immune-mediated diseases (IMDs) that may be associated with the vaccination.

9.5.1 Choice of comparators

[redacted] is considered the best injectable comparator available for this study although it only contains three influenza strains while the other test products (including [redacted]) contain four strains. The three strains included in [redacted] are the same as in the test products (group 1-3) and the antigens have been manufactured at the same site ([redacted]).

The difference between trivalent influenza vaccines (TIV) and QIV is that the latter contains one extra influenza strain (an additional influenza B-strain). This extra strain is, as all other strains, chosen yearly by the WHO.

Several clinical studies (Cadorna-Carlos et al., 2015, Kieninger et al., 2013) have been performed showing that safety including solicited reactions, unsolicited adverse events (AEs) and serious AEs (SAEs) are similar for TIV and QIV. QIV vs TIV has shown superior immunogenicity for the additional B strain without interfering with immune responses to shared strains.

[redacted] is considered the best intranasal comparator for this study as it will allow comparison of local tolerability at the administration site and local immune responses (nasal IgA).

Several studies have shown that [redacted] may have some efficacy in adults (see Appendix 3). However, a conclusion on clinical benefit of this vaccine in adults could not be made, given that results observed in some studies versus injectable influenza vaccines were suggestive of a lower efficacy of [redacted]. For this reason, [redacted] is only indicated for children from 24 months to less than 18 years of age according to the EMA. However, [redacted] is not contraindicated in healthy adults and the same vaccine is sold under the trade name [redacted] in the United States is approved by the FDA for use in persons 2 to 49 years.

9.6 RISK-BENEFIT ASSESSMENT

Vaccination to protect against influenza has been used extensively over many decades and is recommended by the WHO to pregnant women, healthcare workers, children 6 to 59 months, the elderly and those with high-risk conditions. Subjects with known severe allergies to any of the vaccine products or a history of Guillain-Barré Syndrome within 6 weeks of previous influenza vaccination will be excluded from the present study. Nasal administration of LAIV is not recommended for pregnant women or immunosuppressed adults and these groups will therefore not be enrolled in the study.

Endocine has been investigated in 3 previous clinical studies with antigens from diphtheria, influenza and HIV and has been administered to a total of 174 subjects. Adverse drug reactions have been restricted to transient local reactions at the administration site. No safety concerns have been identified in these studies.

An increased risk of narcolepsy was found following vaccination with the pandemic influenza vaccine Pandemrix that was specifically produced for pandemic 2009 H1N1 influenza. The increased incidence of narcolepsy was the highest in children and adolescents (20 years and younger) and declined gradually with age up to approximately 30 years of age. No increase in narcolepsy incidence has been observed in conjunction with seasonal influenza vaccination in Sweden 2010-2011 as reported by the medical products agency (MPA). The Immunose FLU vaccines in the present study contain inactivated split influenza antigens that are active ingredients in a seasonal influenza vaccine [redacted]. The prescribing information of [redacted] and of [redacted] have no mentions of narcolepsy as a reported adverse reaction. Based on available information and recommendations it is assessed that there is no increased risk of developing narcolepsy when participating in the present study.

Subjects will remain at the clinic for at least 2 hours after administration for observation of local and systemic reactions by medical staff. The Investigator will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study. Study specific assessments and sampling procedures like blood pressure measurements and frequent blood sampling can cause transient discomfort but the risk is deemed to be low and ethically justifiable.

The design of the study will ensure that any aforementioned risk factor is minimised whilst the scientific and medical goals for the study will be fulfilled so that the inclusion of healthy volunteers in the study is ethically justifiable.

Possible benefits for the subjects include a thorough health evaluation and an influenza vaccination for subjects randomised to a treatment group where influenza vaccine is given. The treatments will be performed before the expected outbreak of influenza in the 2016/2017 season and may provide protection against the infection.

10. OBJECTIVES

10.1 PRIMARY OBJECTIVE

To evaluate the safety and tolerability of Immunose FLU based on Endocine and quadrivalent influenza antigen.

10.1.1 Primary endpoints

- Type and incidence of AEs and SAEs from the time of first study drug administration (Visit 2) until the last visit to the clinic (Visit 4 for group 1 to 4 and Visit 3 for group 5 to 6).
- Type and incidence of SAEs and AEs of special interest during the 6 months safety follow-up.
- Frequency and severity of administration-site reactions as assessed by an independent evaluator with help from study subject before study drug administration and 15 minutes and 120 minutes after study drug administration. Administration-site reactions to be recorded are swelling, redness, pain and pruritus in the administration area (nose or arm). Reactions are classified as none, mild, moderate or severe. The administration-site reactions pain and pruritus will also be recorded by the subject in an electronic diary for 10 days starting in the evening the day after each treatment and classified as none, mild, moderate or severe.
- Frequency of solicited AEs. The subjects will record the occurrence of solicited AEs in an electronic diary for 10 days after study drug administration. Solicited AE include runny nose, nasal congestion, sneezing, fever (i.e., temperature >38°C), chills, sweating, headache, tiredness, muscle aches, joint pain, cough, sore throat, nausea.
- Frequency of clinically significant changes in ECG, vital signs, physical examination findings and laboratory variables from baseline to the last visit to the clinic.

10.2 SECONDARY OBJECTIVE

To evaluate the immune response to Immunose FLU based on Endocine and quadrivalent influenza antigen by measurement of HI and VN titres.

10.2.1 Secondary endpoints

- Measurement of haemagglutination-inhibition (HI) in blood, including:
  - Geometric mean titres (GMTs) and pre-/post-treatment ratios (GMRs)
  - Percentage of subjects with seroprotection (i.e., an HI titre ≥40) at Day 21 and for group 1-4 also at Day 42.
  - Percentage of subjects with seroconvertion (i.e., either a pre-treatment HI titre <10 and a post-treatment titre ≥40 or a pre-treatment HI titre ≥10 and a fourfold increase in titre) at Day 21 and for group 1-4 also at Day 42.
- Measurement of virus neutralization (VN) titres in blood (pre-treatment and 21 days after each treatment)
  - GMTs and pre-/post-treatment GMRs

10.3 EXPLORATORY OBJECTIVE

To evaluate the immune response to Immunose FLU based on Endocine and quadrivalent influenza antigen by measurement of immunoglobulin A (IgA) in nasal secretions.

10.3.1 Exploratory endpoints

- Measurement of influenza-specific immunoglobulin A (IgA) in nasal secretions (pre-treatment and 21 days after each treatment)

10.3.1.1 Post-hoc exploratory endpoints

The following may be analysed if considered valuable based on the results of the secondary analyses:

- Influenza-specific T cell responses
- Single radial haemolysis (SRH) analysis
- Functional analysis of IgA in nasal swab
- Measurement of neuraminidase (NA) specific antibodies

Exploratory analyses may only be performed on selected subjects, time points and virus strains but will always be performed both pre-and post-treatment. Results from the analyses of exploratory endpoints may be presented separate from the clinical study report (CSR).

11. STUDY DESIGN

This is a Phase I/II, randomised, partially double-blind (group 1 to 4), parallel-group study designed to evaluate the safety, tolerability and immune response following Immunose FLU vaccination at 2 sites in Sweden.

Consenting subjects will be assessed for eligibility at the screening visit (Visit 1) within 6 weeks prior to randomisation. Eligibility will be re-confirmed prior to each dose.

A total of 162 subjects will be randomised to one of 6 treatment groups in a 2:2:2:1:1:1 ratio on Day 0 (Visit 2) as follows:

- **Group 1:** Immunose FLU 1%, containing 15 µg HA/strain and 1% Endocine for intranasal administration (n=36)
- **Group 2:** Immunose FLU 2%, containing 15 µg HA/strain and 2% Endocine for intranasal administration (n=36)
- **Group 3:** Influenza antigen, containing 15 µg HA/strain for intranasal administration (n=36)
- **Group 4:** Saline (NaCl) for intranasal administration (n=18)
- **Group 5:** [redacted], containing 15 µg HA/strain for intramuscular administration (n=18)
- **Group 6:** [redacted], containing $10^{7.0\pm0.5}$ FFUs for intranasal administration (n=18)

Subjects in group 1 to 4 will receive treatment in a partially double-blinded fashion via intranasal administration on Day 0 (Visit 2) and on Day 21+7 (Visit 3), see Table 1. Since the visual appearance of the study drugs may differ, there is a risk that the administrating nurse will be unblinded. Therefore the pre- and post-treatment inspection of the administration site will be performed by an independent evaluator. The same independent evaluator will perform both pre- and post-dose assessments. For further details on blinding, refer to Section 13.10.

For subjects in group 1 to 4 there will be a total of 4 visits to the clinic; Visit 1 (screening), Visit 2 (Day 0), Visit 3 (Day 21+7) and Visit 4 (Day 42+7). Telephone contacts will be taken approximately every 8th week after the last visit to the clinic (Visit 4) for collection of any AEs of special interest or SAEs that may have occurred after Visit 4 during a 6 months safety follow-up period starting from the day of the last vaccination. For details on the follow-up phase, refer to Section 15.3.11.

Subjects in group 5 and 6 will receive treatment in an unblinded fashion via a single intramuscular (group 5) or single intranasal (group 6) administration on Day 0 (Visit 2), see Table 3. No treatment will be given on Day 21+7 (Visit 3).

For subjects in group 5 and 6 there will be a total of 3 visits to the clinic; Visit 1 (screening), Visit 2 (Day 0), and Visit 3 (Day 21+7). Telephone contacts will be taken approximately every 8th week after the last visit to the clinic (Visit 3) for collection of any AEs of special interest or SAEs that may have

occurred after Visit 3 during a 6 months safety follow-up period starting from the day of the last vaccination.

In all groups, safety and tolerability, including local tolerability, will be assessed. Local tolerability (swelling and redness [assessed by the independent evaluator] and pain and pruritus [assessed by the subject]) will be assessed before and after each treatment with study drug (Visit 2 and 3 for group 1 to 4 and Visit 2 for group 5 to 6). Safety and tolerability will be assessed by vital signs (blood pressure and heart rate), physical examination, 12-lead electrocardiogram (ECG), collection of AEs and laboratory tests (haematology, clinical chemistry and urinalysis) at time points specified in Table 1 to Table 4. Subjects with suspected influenza will be tested to confirm presence and strain of the influenza virus.

Local tolerability will also be assessed by the subjects' recording of the presence or absence of local administration site reactions (pain and pruritus) and solicited AEs (runny nose, nasal congestion, sneezing, fever [temperature >38°C], chills, sweating, headache, tiredness, muscle aches, joint pain, cough, sore throat, nausea) in an electronic diary for 10 days after each dosing occasion, starting in the evening the day after treatment.

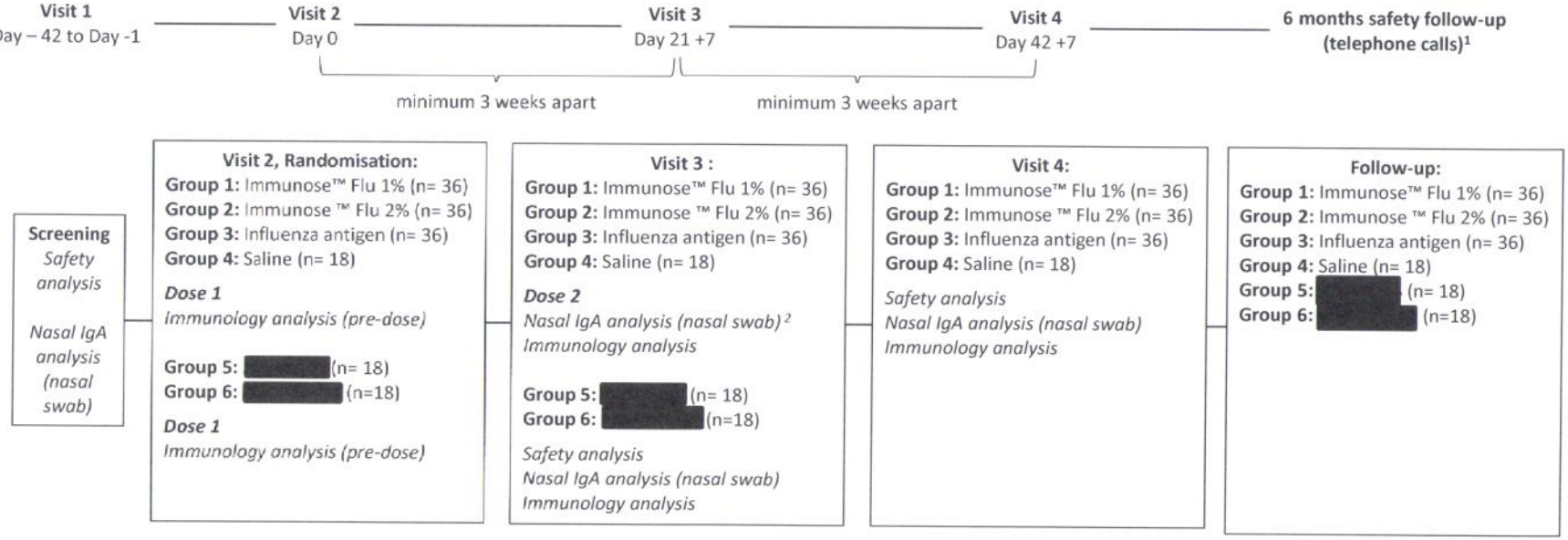
Immune responses against the vaccine antigens will be evaluated in both nasal and blood samples. Influenza-specific immune responses will be evaluated in nasal samples (IgA) and in serum (HI and VN) and PBMC (T-cells) from blood samples. Blood samples for peripheral blood mononuclear cell (PBMC) preparation and subsequent T-cell analysis will be taken from 10 subjects per treatment group at the same time points as the other immunology blood samples. Sampling for immunology analyses at visits and time points specified in Table 1 to Table 4.

The total study duration for the subjects in the study will be up to 36 weeks (group 1 to 4) and 33 weeks (group 5 and 6) including a 6 week screening period and a 180 days follow-up period after the last vaccination.

Further details on the investigational medicinal products are provided in Section 13.1. Procedures for maintaining the blind is described in Section 13.10. Randomisation procedures are outlined in Section 13.7.

Safety assessments are described in detail in Section 15 and efficacy assessments are described in Section 16.

Figure 1 Study flowchart and blood sampling schedule



[1] Telephone follow-up approximately every 8th week ±7 days following the last visit to the clinic for a total of 3 occasions during 180 days starting after the last vaccination.
[2] Nasal swab for group 1 to 4 at Visit 3 will be performed at least 60 minutes prior to the pre-dose administration site inspection for local tolerability assessments.

Table 1 Schedule of events group 1 to 4

	Visit 1 Screening	Visit 2	Visit 3[1]	Visit 4[1]	Safety follow-up (telephone calls)		
Day (+/- visit window)	Day -42 to Day -1	Day 0	Day 21 (+7)	Day 42 (+7)	Day 81±7	Day 141±7	Day 201 ±7
Week (+/- visit window)	Week-6 to week -1	Week 0	Week 3 (+1 week)	Week 6 (+ 1 week)	Week 11 (±1 week)	Week 20 (±1 week)	Week 28 (±1 week)
Informed consent	X						
Eligibility criteria[2]	X	X[2]	X[2]				
Demographics	X						
Medical history	X						
Physical examination	X			X			
Weight and height	X						
Electrocardiogram (ECG)	X			X			
Pregnancy test (urine dipstick)[3]	X[3]	X[3]	X[3]				
HIV, Hepatitis B and C blood sampling	X						
Urine drug screen	X						
Haematology and clinical chemistry blood sampling	X			X			
Urinalysis sampling	X			X			
Prior and concomitant medications	X	X	X	X			
Randomisation		X					
Diary instructions		X	X				
Diary check-up			X	X			
AEs[4, 5]		X[4]	X	X	X[5]	X[5]	X[5]
Vital signs[6]	X	X[6]	X[6]	X			
Nasal swab[7]	X		X[7]	X			
Immunology blood sampling[8]		X[8]	X[8]	X[8]			
Administration site inspection for local tolerability[9]		X[9]	X[9]				
Dose administration[10]		X[10]	X[10]				

[1] At least 3 weeks between Visit 2 and Visit 3 and between Visit 3 and Visit 4.
[2] Continued eligibility criteria are defined in Section 12.6.
[3] Pregnancy test should be performed in females at screening and pre-dose at Visit 2 and Visit 3.

[4] Pre-treatment events will be collected from signing of the ICF until first dose and AEs will be collected post study drug administration.
[5] SAEs and AEs of special interest (Section 14.1.5).
[6] Recording of vital signs (heart rate and blood pressure) should be performed prior to dosing and at 30 minutes and 120 minutes post study drug administration (before the subject leaves the clinic). Temperature should be measured prior to each dosing occasion.
[7] Nasal swab for IgA analysis should be performed at least 60 minutes prior to the pre-dose assessment of local tolerability at Visit 3. Instructions are provided in Section 16.1.
[8] Blood sampling for serum from all subjects and blood sampling for PBMC preparation and T-cell analysis as randomized (10 subjects per group).
[9] Assessment of local tolerability parameters should be performed prior to dosing and at 15 and 120 minutes post-study drug administration according to specific instructions (Section 15.1).
[10] Nasal study drug administration according to specific instructions (Section 13.5.1).

Table 2 Timing of assessments on treatment days group 1 to 4

Time point	Assessment	Visit 2	Visit 3	Comment
Pre-dose	Continued eligibility criteria	X[1]	X	
	Pregnancy test (urine dipstick)	X[1]	X	
	Vital signs including body temperature	X[1]	X	
	Concomitant medications	X[1]	X	
	Randomisation	X		
	Diary check-up		X	
	Pre-treatment events/AEs	X	X	
	Nasal swab for IgA analysis		X	At least 60 minutes before local tolerability assessments at Visit 3
	Immunology blood sampling	X	X	
	Local tolerability assessments	X[2]	X[2]	By independent evaluator[2]
0	Dose administration	X	X	
15 min +5min	Local tolerability assessments	X[2]	X[2]	By independent evaluator[2]
30 min ±5 min	Vital signs	X	X	
120 min ±5 min	Local tolerability assessments	X[2]	X[2]	By independent evaluator[2] Timing of local tolerability assessment is prioritised over timing of vital signs assessments
120 min ± 20 min[3]	Vital signs	X[3]	X[3]	
Post-dose	AEs	X	X	
	Diary instructions	X	X	

[1] Prior to randomisation.
[2] The same evaluator should perform the pre- and post-dose assessments.
[3] Taken before the subject leaves the clinic.

Table 3 Schedule of events group 5 and 6

	Visit 1 Screening	Visit 2	Visit 3[1]	Safety follow-up (telephone calls)		
Day (+/- visit window)	Day -42 to Day -1	Day 0	Day 21 (+7)	Day 60±7	Day 120±7	Day 180±7
Week (+/- visit window)	Week-6 to week -1	Week 0	Week 3 (+1 week)	Week 8 (±1 week)	Week 17 (±1 week)	Week 26 (±1 week)
Informed consent	X					
Eligibility criteria	X	X[2]				
Demographics	X					
Medical history	X					
Physical examination	X		X			
Weight and height	X					
Electrocardiogram (ECG)	X		X			
Pregnancy test (urine dipstick)[3]	X[3]	X[3]				
HIV, Hepatitis B and C blood sampling	X					
Urine drug screen	X					
Haematology and clinical chemistry blood sampling	X		X			
Urinalysis sampling	X		X			
Prior and concomitant medications	X	X	X			
Randomisation		X				
Diary instructions		X				
Diary check-up			X			
AEs[4, 5]		X[4]	X	X[5]	X[5]	X[5]
Vital signs[6]	X	X[6]	X[6]			
Nasal swab[7]	X		X			
Immunology blood sampling[8]		X[8]	X[8]			
Administration site inspection for local tolerability[9]		X[9]				
Dose administration[10]		X[10]				

[1] At least 3 weeks between Visit 2 and Visit 3.
[2] Continued eligibility criteria are defined in Section 12.6.
[3] Pregnancy test should be performed in females at screening and pre-dose at Visit 2.

[4] Pre-treatment events will be collected from signing of the ICF until first dose and AEs will be collected post study drug administration.
[5] SAEs and AEs of special interest (Section 14.1.5).
[6] Recording of vital signs (heart rate and blood pressure) should be performed prior to dosing and at 30 minutes and 120 minutes post study drug administration (before the subject leaves the clinic). Temperature should be measured prior to each dosing occasion.
[7] Nasal swab for IgA analysis according to specific instructions (Section 16.1).
[8] Blood sampling for serum from all subjects and blood sampling for PBMC preparation and T-cell analysis as randomized (10 subjects per group)
[9] Assessment of local tolerability parameters should be performed prior to dosing and at 15 and 120 minutes post-study drug administration according to specific instructions (Section 15.1).
[10] Nasal or intramuscular study drug administration according to the manufacturers' instructions.

Table 4 Timing of assessments on treatment day group 5 and 6

Time point	Assessment	Visit 2	Comment
Pre-dose	Continued eligibility criteria	X[1]	
	Pregnancy test (urine dipstick)	X[1]	
	Vital signs including body temperature	X[1]	
	Concomitant medications	X[1]	
	Pre-treatment events	X	
	Randomisation	X	
	Immunology blood sampling	X	
	Local tolerability assessments	X[2]	By independent evaluator[2]
0	Dose administration	X	
15 min +5min	Local tolerability assessments	X[2]	By independent evaluator[2]
30 min ±5 min	Vital signs	X	
120 min ±5 min	Local tolerability assessments	X[2]	By independent evaluator[2] Timing of local tolerability assessment is prioritised over timing of vital signs assessments
120 min ±20 min[3]	Vital signs	X[3]	
Post-dose	AEs	X	
	Diary hand-out and instructions	X	

[1] Prior to randomisation.
[2] The same evaluator should perform the pre- and post-dose assessments.
[3] Taken before the subject leaves the clinic.

12. STUDY POPULATION

12.1 SELECTION OF STUDY POPULATION

Subjects will be recruited using a register of healthy volunteers at CTC and from advertising in media. The total sample size and the sample size per treatment group were determined as outlined in Section 18.2.

Approximately 243 subjects are planned to be screened in order to identify 162 subjects eligible for randomisation.

12.2 INFORMED CONSENT

It is the responsibility of the Investigator, or any authorised designee, to give each potential subject adequate verbal and written information about the nature of the study, its purpose, expected duration, the benefits and risks involved in study participation before any study specific assessments are performed.

All subjects will be given the opportunity to ask questions and will be informed of their right to withdraw from the study without prejudice.

Each subject should be informed in writing that the data from the study will be stored and analysed, maintaining confidentiality in accordance with local data protection laws.

The subjects will also be informed about the possibility of audits by authorised representatives of the company and/or concerned competent authorities in which case a review of those parts of the medical and laboratory records relevant to the study may be required.

After this explanation and before entering the study, the ICF must be signed and personally dated by the subject and by the person who conducted the informed consent discussion. A copy of the subject information sheet, including the signed ICF, will be provided to the subject.

The date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

12.3 SUBJCETC SCREENING AND SUBJECT IDENTIFICATION LOGS

Investigators must keep a record of all subjects that were considered for enrolment even if they were not subsequently enrolled. This information is necessary to verify that the subject population was selected without bias. The reasons for non-eligibility are to be defined in terms of one or more of the eligibility criteria.

A screening number will be allocated to each subject in connection to the informed consent process at screening (Visit 1). The screening number is generated automatically by the eCRF. The screening number allow for identification of subjects irrespective of their possible eligibility for the main study. Randomised subjects will be assigned a randomisation number.

12.4 INCLUSION CRITERIA

For inclusion in this study, subjects must fulfil all of the following criteria:

1. Signed informed consent prior to any study related procedures.
2. Male or female 18-39 years of age (both inclusive) at the time of the first treatment with study drug.
3. Subjects who the Investigator believes will comply with the requirements of the protocol.
4. BMI: 18.0 and 30.0 kg/m^2 (inclusive).

5. Judged by the Investigator to have no serious illness based on medical history, physical examination, ECG, vital signs and blood and urine assessments at screening.
6. From the signing of the informed consent until 2 months after the last vaccination (Visit 3 for group 1 to 4 and Visit 2 for group 5 and 6) female subjects have to use contraceptive methods with a failure rate of < 1% to prevent pregnancy (combined [oestrogen and progestogen containing] hormonal contraception associated with inhibition of ovulation [oral, intravaginal, transdermal], progestogen- only hormonal contraception associated with inhibition of ovulation [oral, injectable, implantable], intrauterine device [IUD], intrauterine hormone-releasing system [IUS], bilateral tubal occlusion, sexual abstinence). Any male partner should be willing to use condom or should be vasectomized.

12.5 EXCLUSION CRITERIA

Subjects must not be included in this study if any of the following exclusion criteria is fulfilled:

1. Diagnosis of laboratory-confirmed influenza in the 2015/2016 season.
2. Use of any investigational drug product within 3 months before screening or planned use during the study period, including the safety follow-up.
3. Administration of an influenza vaccine during the 6 months before screening.
4. Previously received another vaccine within 28 days before administration of the study vaccine, or is scheduled to receive another vaccine during the study period, excluding the safety follow-up.
5. Any contra-indication to [redacted]
6. Any contra-indication to [redacted]
7. History of any anaphylactic reaction and/or serious allergic reaction following a vaccination, a proven hypersensitivity to any component of the study vaccine (e.g., to eggs or egg product as well as ovalbumin, chicken protein, chicken feathers, influenza viral protein, kanamycin, gentamycin and neomycin sulphate).
8. Diagnosis of asthma with poor disease control as assessed by the Investigator.
9. Potent immunosuppressive therapy including cytostatics, antibodies, drugs acting on immunophilins, interferons and other drugs used to prevent rejection of organ transplants, within 6 months before screening.
10. Use of any parenteral or oral corticosteroids within 30 days prior to screening. Inhaled steroids are allowed.
11. Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination.
12. Any progressive or severe neurologic disorder, seizure disorder or Guillain-Barré syndrome.
13. History of Guillain-Barré syndrome within 6 weeks of receipt of prior inactivated influenza virus vaccine.
14. Received blood, blood products and/or plasma derivatives or any administration of immunoglobulin preparation within the three months prior to Visit 2, or planned during the study.
15. Participation in blood donation within 3 months or plasma donation within 1 month prior to Visit 2.
16. History of substance or alcohol abuse within the past 2 years.
17. History or any illness/condition that, in the opinion of the Investigator, might interfere with the results of the study or pose additional risk to the subjects due to participation in the study.

18. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and HIV.
19. Pregnant or lactating female or intent to become pregnant during the clinic phase (up until and including Visit 4 for group 1 to 4 and Visit 3 for group 5 and 6) and for 2 months after the last vaccination.
20. History of Bell's palsy.
21. Ongoing regular use of intranasal sprays including corticosteroids and decongestants.
22. Ongoing cough, sinusitis, allergic rhinitis, nasal polyps or obstruction, including septum deviation significant enough to prevent bilateral administration of study vaccine.
23. Known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
24. Subjects that are prone to nosebleed.

12.6 CONTINUED ELIGIBILITY CRITERIA (TO BE CHECKED BEFORE FIRST AND SECOND DOSE)

At Visit 2, subjects **must not** be randomised if they comply with any of the following criteria:

1. Temperature ≥38.0°C and/or any acute illness within 3 days prior to study treatment.
2. Ongoing cough, sinusitis, allergic rhinitis, nasal polyps or obstruction, including septum deviation significant enough to prevent bilateral administration of study vaccine.
3. Blocked nose (subject must be able to breath in an out of each nostril when blocking the other nostril by pressing a finger against the wing of his/her nose).
4. Use of nasally administered prescription or nasally over-the counter (OTC) medications within 2 days prior to treatment.
5. Use of other investigational product since screening.
6. Participation in blood donation since screening (plasma donation within 1 month of Visit 2) or planned during the study.
7. Planned influenza vaccination during the study period including the safety follow-up period.
8. Laboratory-confirmed influenza since last visit.

Subjects who fulfil any of the continued eligibility criteria may be rescheduled for a second evaluation of continued eligibility at the discretion of the Investigator.

12.7 RESTRICTIONS

12.7.1 General restrictions

The following restrictions must be respected by the subjects during the participation in the study:

- Contraception requirements as detailed in Inclusion criterion #6.
- Subjects must not donate blood or plasma during the study until 3 months after Visit 4 (group 1 to 4) and Visit 3 (group 5 and 6).
- Subjects must not use any other investigational drug product during the study period including the safety follow-up period.

Restrictions related to concomitant therapy are described in Section 12.7.2.

12.7.2 Prior and concomitant therapy

From screening until the last visit to the clinic, subjects should abstain from intake of oral corticosteroids and potent immune modulating drugs as indicated in exclusion criteria #9 and #10.

Use of nasally administered prescription or nasal OTC medications within 2 days prior to each treatment are not allowed.

Influenza vaccination is not allowed during the study period including the safety follow-up period. Vaccination with other licenced vaccines are allowed after the last visit to the clinic.

12.8 WITHDRAWAL OF A SUBJECT, DISCONTINUATION OF TREATMENT AND STUDY TERMINATION

12.8.1 Subject withdrawal

Subjects have the right to withdraw consent at any time, for whatever reason, without prejudice. Subjects who withdraw will be asked about the reason(s) for withdrawal, and the presence of any AEs.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject for follow-up assessments.

Subjects may be discontinued from the study at any time at the discretion of the Investigator. Specific reasons for discontinuing a subject from further assessments are:

- Withdrawal of informed consent.
- Subject lost to follow-up (*i.e.* drop-outs).
- Death.
- Any violation of, or deviation from, study protocol procedures which, in the judgment of the responsible physician, could adversely affect the subjects or the integrity of the study.
- The Investigator considers that it is not in the interest of the subject to continue.
- Any AE, clinically significant laboratory abnormality, inter-current illness or significant worsening of inter-current illness which, in the opinion of the Investigator, is posing a risk for the subject.
- Occurrence of an exclusion criterion which is clinically relevant and affects the subject's safety, if discontinuation is considered necessary by the Investigator and/or Sponsor.
- Taking another investigational drug treatment during the subject's involvement in the study i.e., from screening to the last follow-up telephone call.

12.8.2 Study termination

Eurocine Vaccines AB, in collaboration with the Investigator and after detailed consultation with PCG, reserves the right to terminate the study prematurely for the event of an outbreak of pandemic influenza early during the study conduct, for scientific or administrative reasons or for any other valid and/or ethical reason. If the study is prematurely terminated, the Investigator should promptly inform the subjects and take necessary steps to finalise their engagement in the study, preferably according to the scheme for the final safety assessments to be conducted at Visit 4 (group 1 to 4) and Visit 3 (group 5 to 6), respectively. All relevant study material must then be collected and accountability completed.

If the study is prematurely terminated by the Sponsor, subjects who have received at least one dose of investigational medicinal product (IMP) should as far as possible be followed for safety according to the protocol, excluding sampling for immune parameters.

12.8.3 Procedure for withdrawal

The date and reason for withdrawal of a subject or the premature termination of the study must be documented in the eCRF.

After withdrawal of a subject, all AEs or complications should be followed for as long as the Investigator considers it necessary.

Subjects who have received at least one dose of study drug should as far as possible be followed for safety according to the protocol. Blood and nasal sampling for immunology parameters will not be performed.

Subjects who have received one dose of study drug and are diagnosed with laboratory-confirmed influenza prior to the second dose should not receive a second vaccination but should, as far as possible, be followed for safety according to the protocol. Blood and nasal sampling for immunology parameters will not be performed.

Subjects who withdraw before receiving any IMP should not participate in any further study related procedures.

12.8.4 Replacement of withdrawn subjects

Randomised subjects will not be replaced.

Subjects who develop suspected influenza during the study period should be tested using a diagnostic test at the discretion of the Investigator (Section 15.10). If influenza is confirmed no further treatment with study drug should be given. Safety should as far as possible be followed according to the protocol excluding further sampling for immune parameters. Subjects diagnosed with influenza before receiving any study drug should not participate in any further study related procedures.

13. STUDY TREATMENTS

13.1 TREATMENT GROUPS

Subjects in the study will be randomised to one of 6 treatment groups in a 2:2:2:1:1:1 ratio as outlined in Table 5. For details on each study drug, refer to Section 13.2. For details on treatment procedures, see Section 13.5.

13.2 IDENTITY OF THE INVESTIGATIONAL MEDICINAL PRODUCTS

13.2.1 TEST PRODUCTS

13.2.1.1 Immunose FLU (group 1 and 2)

Immunose FLU consists of influenza antigens (4 split inactivated virus suspensions; 15 µg HA/strain/0.3 mL) and Endocine in concentrations of 1% (group 1) or 2% (group 2). The influenza strains are the ones recommended by WHO for QIV for the northern hemisphere for season 2016/2017.

Endocine is composed of lipids (monoolein and oleic acid) that are formulated into liposomes.

Immunose FLU is a homogeneous, slightly turbid to turbid liquid and will be provided as 0.4 mL dispensed in 3 mL, flat bottomed, amber glass vials with white polypropylene screw caps.

13.2.1.2 Influenza antigen (group 3)

The influenza antigen consists of the same influenza antigens (4 split inactivated virus suspensions; 15 µg HA/strain/0.3 mL) as Immunose FLU.

The influenza antigen is a homogeneous, slightly turbid to turbid liquid and will be provided as 0.4 mL dispensed in 3 mL, flat bottomed, amber glass vials with white polypropylene screw caps.

13.2.1.3 Saline (NaCl) (group 4)

The saline solution will be provided as 0.4 mL in 3 mL, flat bottomed, amber glass vials with white polypropylene screw caps.

The solution is clear and colourless.

13.2.2 COMPARATORS

13.2.2.1 [redacted] (group 5)

[redacted] contain the influenza strains as recommended by the WHO for [redacted] the northern hemisphere for the 2016/2017 season.

[redacted].

13.2.2.2 [redacted] (group 6)

[redacted] influenza virus strains as recommended by the WHO for [redacted] the Northern hemisphere for the 2016/2017 season.

[redacted]

Table 5 Treatment groups

Group	Subjects (n)	Test product/comparator	Antigen dose	Antigen type	Number of strains	Adjuvant (Endocine)	Route	Doses (n)	Volume (µl)	Storage
1	36	Immunose FLU (1 %)	15 µg HA/strain	Inactivated split virus	4	1%	intranasal	2	300	Refrigerator, 2-8°C
2	36	Immunose FLU (2 %)	15 µg HA/strain	Inactivated split virus	4	2%	intranasal	2	300	Refrigerator, 2-8°C
3	36	Influenza antigen	15 µg HA/strain	Inactivated split virus	4	-	intranasal	2	300	Refrigerator, 2-8°C
4	18	Saline (NaCl)	-	-		-	intranasal	2	300	Refrigerator, 2-8°C
5	18	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	1	[redacted]	Refrigerator, 2-8°C Light sensitive
6	18	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	1	[redacted]	Refrigerator, 2-8°C Light sensitive

13.3 MANUFACTURING, PACKAGING AND LABELLING OF INVESTIGATIONAL MEDICINAL PRODUCTS

Immunose FLU and the influenza antigen for nasal administration will be manufactured, packed and labelled by [redacted].

Saline will be packed and labelled by [redacted].

[redacted] will be manufactured by [redacted] and packed and labelled by [redacted].

[redacted] will be manufactured by [redacted] and packed and labelled by [redacted].

The test products will be provided in labelled boxes containing IMP for 2 dose administrations. The comparators will be provided in labelled boxes with IMP for 1 dose administration.

All IMP will be shipped to the pharmacy, Akademiska hospital (Uppsala, Sweden) before distribution to each site.

The labels on the boxes and IMP containers will include all information required by applicable regulations.

13.4 STORAGE AND HANDLING OF INVESTIGATIONAL MEDICINAL PRODUCTS

All IMPs and comparators should be stored in a safe place with limited access at 2 to 8°C. Temperature logs should be kept for the refrigerator where the IMP is stored. The temperature should be noted on a daily basis (working days only unless automatic temperature readings are available). The IMPs must not be frozen.

[redacted] should be stored protected from light.

[redacted] must be protected from light [redacted]

13.5 DOSES AND TREATMENT REGIMENS

13.5.1 Doses and treatment regimens group 1 to 4 (test products)

The test products (Immunose FLU, influenza antigen and saline, respectively) will be administered in 2 doses delivered at least 3 weeks apart. Up to 4 weeks between administrations is allowed.

A short instruction for nasal administration of Immunose FLU, influenza antigen and saline is provided below and a complete instruction is provided separately.

Prior to administration, the content of each vial should be mixed carefully. Upon administration, the subject should sit in an upright position and lean the head backwards (Figure 2 and Figure 3). A calibrated pipette and pipet tips must be used.

A total of 150 µl of IMP should be administered in each nostril. The nose drops must be applied on the upside nasal cavity.

Figure 2 Head position of subject upon nasal administration of test products (group 1 to 4)

Figure 3 Administration of test products (group 1 to 4)

13.5.2 Doses and treatment regimens group 5 and 6 (comparators)

The comparators ( ) will be administered as a single dose according to the manufacturer's instructions.

will be administered

will be administered

13.6 INVESTIGATIONAL MEDICINAL PRODUCT ACCOUNTABILITY

It is the responsibility of the Investigator to establish a system for handling the IMPs, to ensure that:

- Deliveries of such products are correctly received and recorded by a designated person
- IMPs are handled and stored safely and properly
- IMPs are given only to study subject in accordance with the CSP
- All unused IMP and empty containers are stored until they have been checked by the monitor
- It is possible to reconcile records of all used and unused stocks as confirmed by Investigator's signature

The monitor(s) should collect any unused IMP from the sites and return the IMP to Eurocine Vaccines AB or representative when the dosing period is over.

13.7 METHOD OF ASSIGNING SUBJECTS TO TREATMENT GROUPS

13.7.1 Randomisation

At visit 2, subjects will be consecutively allocated to the 6 treatment groups in a 2:2:2:1:1:1 ratio. Group 1 to 3 will consists of 36 subjects and group 4 to 6 will consist of 18 subjects. The randomisation will be stratified by site and for each stratum an appropriate block size will be used to ensure the ratios between treatment groups.

Randomisation codes will be provided by PCG Clinical Services AB to [redacted].

13.8 ASSESSMENT OF TREATMENT COMPLIANCE

Following administration, the site personnel should evaluate whether the administration was successful or not (Y/N). Dripping, sneezing and swallowing in association with the administration must be commented on in the eCRF.

13.9 CONTINUATION OF MEDICATION AFTER THE STUDY

No subject will be offered continued treatment with any test product at the end of their study participation.

13.10 BLINDING

Treatments for group 1 to 4 will as far as possible be handled in a double blinded fashion. The influenza antigen without Endocine, and Immunose FLU with Endocine in different concentrations (1% and 2%, group 1 and 2, respectively) are slightly turbid to turbid liquids and is hence different from saline, which is clear and more transparent.

To maintain the blind, all test products (Immunose FLU with 1% and 2% Endocine, influenza antigen without Endocine and saline) will be provided in amber coloured bottles. The administration of the products will be carried out using coloured, yet transparent, pipet tips to partially mask the appearance of the test products but to allow for inspection of the volume prior to administration.

There is a risk that the person who administers the test products will be unblinded. Therefore, this person will not be involved in any study related evaluations. An independent evaluator will assess local tolerability reactions pre and post administration.

The comparators, [redacted] and [redacted] will be administered in an open label fashion [redacted]

13.10.1 Breaking the treatment code (unblinding)

Treatment code envelopes will be provided for each randomised subject. The code envelopes should be kept in a secure place with limited access. In case of emergency where it is crucial for the Investigator, or any other treating physician, to know which treatment has been given to the subject, the code envelope may be opened. If the code is broken, this must be documented on the treatment code envelope and in the subject's hospital records with date and name of the Investigator who decided to break the code.

In the event of an SAE, the Investigator may only break the treatment code if the appropriate future management of the subject necessitates knowledge of the current treatment. When an SAE may be a serious adverse drug reaction (SADR), the procedures for potential expedited reporting must be followed (Section 15.3.10).

14. STUDY ASSESSMENTS

The timing and frequency of the study visits are presented in Figure 1 and in the overall schedule of events for group 1 to 4 (Table 1) and group 5 and 6 (Table 3), respectively. A more detailed description for the timing of assessments is provided in Table 2 and Table 4 for group 1 to 4 and group 5 and 6, respectively.

14.1 STUDY VISITS

14.1.1 Visit 1, Screening

After written informed consent has been obtained, the following activities and assessments will be performed:

- Evaluation of eligibility criteria.
- Collection of demographic data.
- Recording of medical history.
- Physical examination.
- Recording of vital signs (heart rate and blood pressure).
- Recording of weight and height.
- 12-lead ECG.
- Pregnancy test, urine dipstick.
- Blood sample for viral screen (HIV, hepatitis B and hepatitis C).
- Urine drug screen (dipstick).
- Urine sampling for assessment of urinalysis parameters.
- Blood sampling for assessment of haematology and clinical chemistry parameters. Fasting is not required.
- Nasal swab for nasal IgA analysis (pre-treatment sample, to be stored and analysed with the other immune samples. Performed at screening to save time at visit 2 especially for group 1 to 4).
- Recording of prior and concomitant medication.

Eligible subjects will be scheduled for Visit 2.

14.1.2 Visit 2

Subjects will arrive at the study clinic on Day 0. A re-assessment of eligibility will be made, see Section 12.6. Eligible subjects will be randomised to one of 6 treatment groups as detailed in Section 11 and 13.1.

The activities and assessments to be performed at Visit 2 are outlined below. For details on the timing of each assessment, refer to Table 2 (group 1 to 4) and Table 4 (group 5 and 6).

The following activities and assessments will be performed **before randomisation:**

- Pregnancy test (urine dipstick).
- Recording of vital signs (heart rate, blood pressure and body temperature).
- Recording of concomitant medication.
- Collection of pre-dose events.

The following activities and assessments will be performed **after randomisation** but **pre-dose:**

- Blood sampling for immunology analyses.

- Inspection of nasal cavities and recording of local tolerability parameters (group 1 to 4 and group 6).
- Inspection of administration site (upper arm) and recording of local tolerability parameters (group 5).

The **study drug administration** will be performed as summarized in Section 13.5.1 and Section 13.5.2 and according to the manufacturers' instructions as applicable.

The following activities and assessments will be performed **post-dose:**

- Recording of local tolerability parameters at 15 and 120 minutes post study drug administration.
- Recording of vital signs at 30 minutes and approximately 120 minutes post study drug administration (before the subject leaves the clinic).
- AE collection.
- Diary instructions for daily AE recording during 10 days starting in the evening the day after treatment.

14.1.3 Visit 3

The activities and assessments to be performed at Visit 3 are outlined below. A re-assessment of eligibility will be made prior to dosing, see Section 12.6. For details on the timing of each assessment, refer to Table 1, Table 2 and Table 3.

14.1.3.1 Visit 3 group 1 to 4

The following activities and assessments will be performed **pre-dose:**

- Pregnancy test (females only, urine dipstick).
- Review of diary since the previous visit.
- AE collection.
- Recording of concomitant medication.
- Recording of vital signs (heart rate, blood pressure and body temperature).
- Nasal swab for nasal IgA sampling (at least 60 minutes before assessment of the pre-dose assessment of local tolerability).
- Blood sampling for immunology analyses.
- Inspection of nasal cavities and recording of local tolerability parameters.

Nasal **study drug administration** will be performed as summarized in Section 13.5.1.

The following activities and assessments will be performed **post-dose:**

- Recording of local tolerability parameters at 15 and 120 minutes post study drug administration.
- Recording of vital signs at 30 minutes and approximately 120 minutes post study drug administration.
- AE collection.
- Diary instructions for daily AE recording during 10 days starting in the evening the day after treatment.

14.1.3.2 Visit 3 group 5 and 6

The following activities and assessments will be performed:

- Review of diary since the previous visit.

- AE collection.
- Recording of concomitant medication.
- Recording of vital signs (heart rate and blood pressure).
- 12-lead ECG.
- Physical examination.
- Urine sampling of urinalysis parameters.
- Blood sampling for assessment of haematology and clinical chemistry parameters. Fasting is not required.
- Nasal swab for nasal IgA analysis.
- Blood sampling for immunology analyses.

14.1.4 Visit 4 (group 1 to 4 only)

The activities and assessments to be performed at Visit 4 are outlined below. For details on the timing of each assessment, refer to Table 2.

The following activities and assessments will be performed:

- Review of diary since the previous visit.
- AE collection.
- Recording of concomitant medications.
- Recording of vital signs (heart rate and blood pressure).
- 12-lead ECG.
- Physical examination.
- Urine sampling of urinalysis parameters.
- Blood sampling for assessment of haematology and clinical chemistry parameters. Fasting is not required.
- Nasal swab for nasal IgA analysis.
- Blood sampling for immunology analyses.

14.1.5 Safety follow-up (telephone calls)

AEs of special interest and SAEs will be collected during 6 months after the last vaccination through telephone calls approximately every 8th week ±7 days following the last visit to the clinic for a total of 3 occasions

The following will be collected:

- Potential immune-mediated diseases (pIMDs). pIMDs are a subset of AEs that include both clearly autoimmune diseases and also other inflammatory and/or neurologic disorders which may or may not have an autoimmune aetiology.
- SAEs

The questions to be asked are:

- Have you, since the last contact, visited any healthcare facility or hospital? If yes, please describe the reason for the visit, the assessment given and what treatment you received.
- Have you, since the last contact, experienced any worsening in a chronic disease that you are diagnosed with? If yes, please describe the symptoms.
- Are your pregnant? If yes, which week?

The information will be collected in the eCRF. The questions translated into Swedish are found in Appendix 4.

14.2 EVENT DESCRIPTIONS

14.2.1 Screening and informed consent

The procedures for screening and obtaining informed consent are described in Section 12.

14.2.2 Eligibility criteria

Eligibility criteria should be checked during screening as detailed in Section 12.4 and 12.5. On dosing days, eligibility will be re-assessed based on the continued eligibility criteria detailed in Section 12.6.

14.2.3 Demography

The following demographic data will be recorded: initials, gender, birth date and ethnic origin (Caucasian, Asian or Pacific Islander, African descent, Mixed/multi-racial, or other). Full information about the subject's social security number (personnummer) and name is to remain confidential in the records of the respective Investigator.

14.2.4 Weight and height

Weight and height will be measured without shoes at screening. BMI will be calculated from the height and weight recorded and will be rounded to one decimal.

14.2.5 Medical history

Medical history should be obtained by interview and include descriptions of all relevant diseases prior to screening as judged by the Investigator.

Pre-dose events occurring between the signing of ICF and first dose will also be registered in the medical history log of the CRF.

14.2.6 Prior and concomitant medications

Medication history (prior medications) is needed for 3 months prior to screening. Prescription medications, OTC medications, and herbal products should be asked for.

Any medication used from screening until the last clinical visit (Visit 4 for subjects in group 1 to 4 and Visit 3 for subjects in group 5 and 6) will be recorded in the eCRF.

The Investigator or designee should assess changes in concomitant medications throughout the study by asking the subject at each visit. Any changes reported by the subject should be recorded in the eCRF.

Medications will be coded according to the WHO Anatomic Therapeutic Chemical classification system (ATC) classification system.

14.2.7 HIV and Hepatitis B/C

Subjects will be tested for HIV (standard HIV antibody test) and hepatitis B (HBsAg and anti-HBc) and hepatitis C (anti-HCV) prior to inclusion in the study. Any positive result at screening will exclude the subject from participating in the study.

14.2.8 Pregnancy test

All females will do a pregnancy test (urine dipstick) at screening and prior to each dosing occasion.

14.2.9 Urine drug screen

Subjects will be screened for drugs of abuse at screening using a urine dipstick. The following substances will be included in the screen panel:

- Cocaine (COC)
- Amphetamine (AMP)
- Marijuana (THC)
- Methadone (MTD)
- Morphine (MOP)
- Benzodiazepines (BZO)
- Buprenorphine (BUP)

Additional drug screens may be performed during the study at the discretion of the Investigator.

15. SAFETY ASSESSMENTS

Safety will be assessed by recording pre-defined local administration site reactions, frequency and severity of AEs, routine safety laboratory parameters, vital signs, 12-lead ECG and physical examination. The timing of assessments is shown in Table 1 to Table 4.

15.1 LOCAL TOLERABILITY ASSESSMENTS

The administration area (nasal cavity for group 1 to 4 and group 6 and upper arm for group 5) will be inspected by an independent evaluator (i.e. a person different from the one doing the administration), pre-dose and at 15 and 120 minutes post-dose to record the presence or absence of swelling and redness (none, mild, moderate or severe). At the same time points, each subject will assess presence or absence of pain and pruritus in the administration area (none, mild, moderate or severe as defined in Section 15.3.7.1). The independent evaluator will thus be blinded for all groups receiving intranasal vaccination (i.e. all test products, saline and [redacted]).

Local tolerability reactions classified as moderate or severe will be reported as AEs.

For all inspections, the same type of lamps will be used at both sites. To facilitate the investigation, nostrils will be slightly widened using a nose speculum.

15.2 SUBJECT DIARY FOR ASSESSMENT OF LOCAL TOLERABILITY AND SOLICITED AES

The subjects will be provided with an electronic diary and will be asked to record the presence of any administration site reactions (pain or pruritus) for 10 days starting in the evening the day after each treatment.

In addition, the subjects will be asked to record the occurrence of solicited AEs (fever [temperature >38°C], chills, sweating, tiredness, headache, muscle aches (not associated with the administration area), joint pain (not associated with the administration area), runny nose, nasal congestion, sneezing, cough, sore throat and nausea for 10 days starting in the evening the day after each treatment.

All reactions will be classified as none, mild, moderate or severe by the subjects. The duration of each reaction (hours) will also be recorded. Reactions classified as moderate or severe will be reported as AEs.

The diary will also contain a question as to whether the subject has taken any antipyretic medication or any other type of medication to treat the recorded symptoms. Any medication taken will be recorded as a concomitant medication.

15.3 ADVERSE EVENTS

Any apparent side effects experienced by the subject will be assessed from the time the subject signs the informed consent and throughout the last visit to the clinic (Visit 4 for group 1 to 4 and Visit 3 for group 5 and 6).

The study personnel will document any pre-dose events, i.e. events reported after the signing of informed consent and before administration of the first dose in the medical history log of the eCRF.

The study personnel will document all AEs in the AE log of the eCRF, whether observed by the Investigator or spontaneously reported by the subject.

SAEs and AEs of special interest will be documented during the 6 months follow-up period starting after the last vaccination (Visit 3 or Visit 4).

The AE reporting period is described in Section 15.3.12.

15.3.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject who has received IMP. The occurrence does not necessarily need to have a causal relationship with the IMP. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the administration of IMP, whether or not causally related.

The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a subject recipient presenting for medical care, or upon review by a subject monitor who is scrutinising relevant source data.

Clarifications:

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as "acute appendicitis" and the resulting appendectomy noticed under Comments. Pre-study conditions, which led to elective surgery during the time of the study, are not to be reported as AEs.

If an abnormal laboratory value or vital sign is associated with corresponding clinical signs and symptoms, the sign/symptom should be reported as the AE and the associated laboratory result or vital sign should be considered additional information that is to be collected in the eCRF.

15.3.2 Adverse event questioning and recording

The Investigator is to record all directly observed AEs and all AEs spontaneously reported by the subject in the subject's personal records (source data) and in the eCRFs using concise medical diagnostic terminology.

The occurrence of an AE may come to the attention of personnel during a site visit or during interview of a subject presenting for medical care. The question asked will be "Have you had any health problems since your last evaluation?". AEs may also be identified upon review by a monitor who is scrutinising relevant source data.

All AEs must be graded for:

- Seriousness (Section 15.3.3)
- Intensity (Section 15.3.7.1)
- Causality (possible relationship) to IMP (15.3.7.2)

15.3.3 Definition of serious adverse event

An AE is considered serious if it:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect. The criterion congenital anomaly/birth defect in the offspring is valid for pregnancies with conception within 2 months after completion of the treatment series.
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the SAEs defined above)

An AE is also serious if it resulted in any other medically important condition that may not result in one of the above mentioned criteria, but may still be considered serious when, based upon appropriate medical judgement, it may jeopardise the subject and may require significant medical or surgical intervention to prevent one of the outcomes listed above.

15.3.4 Definition of a serious drug reaction

The term SADR is to be used whenever either the Investigator or Sponsor or designee assessed the SAE as possibly or probably related to the IMP.

15.3.5 Definition of a suspected unexpected serious adverse reaction

A suspected unexpected serious adverse reaction (SUSAR) is any SADR whose nature or intensity is not consistent with the current version of the IB or the respective SmPC for comparators.

15.3.6 Definition of adverse events of special interest

Adverse events of special interest include pIMDs occurring during the 6 months safety follow-up period (Section 14.1.5 and 15.3.11).

15.3.7 Rating scales

15.3.7.1 Intensity

The expression intensity of adverse events means the intensity of the event in the opinion of the subject. The grades of intensity are defined as follows:

Mild: Does not interfere with the subject's daily activities and requires no or minimal medication.

Moderate: Interferes with the subject's daily activities and may require medication.

Severe: Interrupts the subject's daily activities markedly and requires medical consultation.

The intensity of each AE is to be graded as above by the Investigator.

15.3.7.2 Causality

The relationship between the IMP and each AE has to be classified by the Investigator using one of the following terms:

Unlikely: The onset of the AE and administration of IMP are such that the medication is not likely to have any reasonable association with the AE.

Possible: It might be possible that the AE could have been caused by the IMP.

Probable: It is probable that the AE is caused by the IMP.

15.3.8 Assessment of outcome

The Investigator must assess the outcome of an AE using the definitions below:

Recovered: the subject has recovered completely, and no symptoms remain.

Recovering: the subject's condition is improving, but symptoms still remain.

Recovered with sequelae: the subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally, but has some motor impairment).

Not recovered: the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).

Death

15.3.9 Reporting procedures for serious adverse events

Starting from administration of the first dose of IMP, all SAEs must be reported by the Investigator to the Sponsor and to PCG within 24 hours after awareness of the SAE.

The SAE reporting will be performed electronically via the eCRF. The site staff will enter all available information regarding the SAE in the AE log for the specific subject. As soon as the event is saved as serious in Viedoc™, an e-mail alert will be sent to predefined recipients to highlight that an SAE has occurred.

A designated person at PCG will review the SAE report to ensure that the report is complete and correct. If any important information is missing or is unclear, queries will be raised. Investigators or other site personnel will inform PCG on any follow-up information on a previously reported SAE as soon as he or she becomes aware of it. Whenever an SAE is updated in Viedoc, a new e-mail alert will be sent.

The designated person at PCG will provide the medical monitor with an expectedness form for assessment of causality and expectedness. The medical monitor will provide his causality (unlikely, possibly or probably related to the blinded IMP) and expectedness (expected or unexpected) assessment. The reference documents for assessment of expectedness will be the IB for Immunose FLU, influenza antigen and saline, the SmPC for [redacted] and the prescribing information for [redacted].

Paper reporting

In case no internet access is available, the SAE should be reported using a paper copy of the SAE form, which will be available at the site. A scanned copy of the completed, signed and dated report should be faxed or e-mailed to:

PCG Clinical Services
Att. PCG SAE EUR003

Fax number: +46 (0)18 4444 823

E-mail: SAE@pharmaconsultinggroup.com

The site personnel should notify the medical monitor via phone or e-mail about the submission of the SAE report. As soon as there is internet access to Viedoc, the SAE should be reported electronically as well.

15.3.10 Reporting of suspected unexpected serious adverse events

The term SADR is used whenever either the Investigator or medical monitor deems the SAE as possibly or probably related to the IMP. Should an SADR be assessed as unexpected by the medical monitor, it is a SUSAR. Under such circumstances, an assigned, certified E2B reporter at PCG and the medical monitor will be unblinded. Based on treatment given, the medical monitor will reassess his causality treatment. In case the event is still regarded as a SUSAR, the E2B reporter at PCG will report the SUSAR to the CA, via the EudraVigilance database, and to the IEC in accordance with local regulations and PCGs SOPs within the following timelines:

- 7 calendar days if fatal or life-threatening (follow-up information within an additional 8 days)
- 15 calendar days if non-fatal and non-life-threatening (follow-up information as soon as possible)

The medical monitor is responsible for providing medical review to the SAE narrative in the Council for International organisations of medical sciences (CIOMS) form.

The Sponsor or designee also has the obligation to, once a year throughout the clinical study (or on request), submit a safety report to the CA and the IEC taking into account all new available safety information received during the reporting period.

The Sponsor is responsible for informing the Investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

15.3.11 Safety follow-up

SAEs and AEs of special interest will be collected during 6 months after the last vaccination through telephone calls approximately every 8th week ±7 days following the last visit to the clinic for a total of 3 occasions (see Section 14.1.5).

The following will be collected:

- pIMDs, i.e. a subset of AEs that include both clearly autoimmune diseases and also other inflammatory and/or neurologic disorders which may or may not have an autoimmune aetiology.
- SAEs

15.3.12 Reporting period for adverse events

Collection of pre-dose events starts after the subject has singed the ICF and continues until the first administration of IMP.

Collection of AEs starts upon administration of the first dose of IMP and continues until the subject's last visit to the clinic (Visit 4 for group 1 to 4 and Visit 3 for group 5 and 6).

Any apparent side effects experienced by the subject will be assessed from the time the subject signs the informed consent and throughout the last visit to the clinic.

In addition, SAEs and AEs of special interest will be documented during the 6 months follow-up period (Section 15.3.11).

15.3.13 Follow-up on adverse events (serious and non-serious)

All AEs collected during the reporting period until the subject's last visit to the clinic and which are assessed as possibly or probably related to the IMP must be followed until resolution or until assessed by the Investigator as stable.

All SAEs collected during the reporting period until the subject's last visit to the clinic must be followed up until the subject has recovered, stabilised or recovered with sequelae. The Investigator must report to the Sponsor all relevant new information. Relevant information includes discharge summaries, autopsy reports and medical consultation.

SAEs that occur during the 6 month safety follow-up period and that are assessed by the Investigator or medical monitor as possibly or probably related to the IMP must be followed up until the subject has recovered, stabilised or recovered with sequelae.

15.4 PREGNANCY

A pregnancy in itself will not be considered an SAE. If a subject becomes pregnant during the study treatment period (including 2 months after the last vaccination), the pregnancy should be reported to PCG and the Sponsor using the pregnancy report form. The subject and offspring must be monitored for AEs during the entire pregnancy. Any AE occurring to the offspring during the pregnancy or at birth must be reported.

In case of spontaneous abortion, stillbirth, congenital anomaly or birth defect, death or any other serious infant condition, the event should be reported as an SAE according to the procedures described in 15.3.9.

15.5 LABORATORY SAFETY ASSESSMENTS

Blood samples for analysis of clinical chemistry and haematology parameters will be collected by venepuncture at visits specified in Table 1 and Table 3. Samples will be analysed using routine methods at the local hospital.

Urine analysis will be performed at the clinic using dipsticks.

The following safety laboratory parameters will be measured:

Clinical chemistry (serum)

Urea nitrogen
Creatinine
Total protein
Total bilirubin
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Alkaline phosphatase
Gamma-glutamyl transferase (GGT)
Creatinine phosphokinase (CPK)
C-reactive protein (CRP)

Urine analysis:

pH
Glucose
Ketones
Nitrites
Blood
Leukocytes

Haematology:

White blood cell count (WBC) and differential (neutrophils [% abs], lymphocytes [% abs], monocytes [% abs], basophils [% abs])
Haemoglobin
Red blood cell count (RBC)
Platelet count

15.6 PROCEDURES FOR BIOLOGICAL SAMPLES

The anticipated total volume of blood that will be drawn from each subject during the study will not exceed 150 mL per subject, which is less than half the volume drawn during a regular blood donation.

15.6.1 Chain of custody for biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The study site will keep full traceability of collected biological samples from the subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory or process laboratory) will keep full traceability of the samples while in their storage and during use until used, shipped or disposed of.

The Sponsor will keep oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers as applicable.

15.7 PHYSICAL EXAMINATION

A physical examination will be performed at the time points specified in Table 1 and Table 3 and will cover the following body systems: ear, nose and throat, cardiovascular system, pulmonary system, skin, abdomen and neurological system.

It will be recorded in the eCRF if any abnormalities were observed in the physical examinations. Any abnormalities will be specified and documented as clinical significant or not clinically significant. Any post-dose clinically significant findings will be recorded as AEs.

15.8 VITAL SIGNS

Systolic and diastolic blood pressure and heart rate will be measured in supine position after 10 minutes rest at visits and time points specified in Table 1 and Table 3. The blood pressure will be measured using an arm cuff of appropriate size. Body temperature will be measured orally using a digital thermometer.

15.9 12-LEAD ECG

12-lead ECG will be recorded in supine position after 10 minutes rest at time points specified in Table 1 and Table 3. Any abnormalities will be specified and documented as clinically significant or not clinically significant. Any post-dose clinically significant ECG findings will also be reported as AEs.

15.10 INFLUENZA TEST

Subjects who develop suspected influenza during the study period should be tested using a diagnostic test at the discretion of the Investigator.

Influenza testing will be performed at Klinisk mikrobiologi (Uppsala, Sweden) according to standard procedures.

16. EFFICACY ASSESSMENTS

16.1 NASAL SWAB

Nasal swab will be performed at visits and time points specified in Table 1 to Table 4. Briefly, nasal swab samples will be obtained from both nares of subjects by sampling with a cotton-tipped wooden swab that has been premoistened with sterile phosphate buffered saline (PBS). The swab will be placed into the anterior nares and rolled across the mucosa for a few seconds with moderately constant pressure. The swab will then be placed in a small volume of PBS and vigorously shaken and wrung out. Samples will be frozen before shipment to Vismederi (Siena, Italy) for IgA analysis. Procedure specific details will be described in a separate document.

16.2 IMMUNOLOGY BLOOD SAMPLING

Blood sampling for analysis of influenza-specific immune responses will be performed according to standard methods at visits and time points specified in Table 1 to Table 4. Details of the sampling procedures will be described in a separate laboratory manual.

Blood samples allocated for antibody analysis will be centrifuged and the serum will be frozen. Serum samples will be shipped to Vismederi (Siena, Italy) for analysis of HI and VN titres and possibly also for SRH and NA-specific antibodies.

Blood samples for T-cell analysis will be shipped to the Rudbeck Laboratory (Uppsala, Sweden) for PBMC preparation. PBMCs will subsequently be shipped to Vismederi (Siena, Italy) for T-cell analysis.

17. DATA MANAGEMENT

Data management based on GCP refers to the activities defined to achieve safe routines to efficiently enter subject information into a database, avoiding errors.

The data management activities, such as procedures for data validation, will be described in a separate data management plan (DMP). The eCRF will be designed in accordance with the CSP.

17.1 THE WEB-BASED ECRF

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by PCG. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete,

or inaccurate. Clinical data will be entered directly from the source documents, which are to be defined at each site before inclusion of the first subject.

Authorised study site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorised study site personnel prior to the study being initiated and any data being entered into the system for any subject.

Captured data will be monitored electronically. Source data review and source data verification (SDV) will be performed at the site as described in the monitoring guidelines. Any inconsistencies will be presented as queries, either as automatically generated queries if raised by the logical data checks of the eCRF system, or by manually generated queries if raised by the off line data validation checks or the data review performed by e.g. the monitor. Queries shall be resolved in a timely manner by a trained member of the site staff.

17.2 ENTERING OF DATA INTO THE ECRF

All data must be entered in English. The eCRFs should always reflect the latest observations made during the subjects participating in the study. Therefore, the eCRFs should be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that the same individual who made the initial baseline determinations completes all corresponding safety evaluations.

The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable or unknown, the Investigator should indicate this in the eCRF. The Investigator will be required to electronically sign off the clinical data.

17.3 THE QUERY PROCESS

The monitor will review the eCRFs and evaluate them for completeness and consistency. The eCRFs will be compared with the respective source documents to ensure that there are no discrepancies between critical data in accordance with the monitor guidelines.

All entries, corrections, and alterations are to be made by the Investigator or designee. The monitor cannot enter data in the eCRFs. Once clinical data have been submitted to the central server via the eCRF, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged. Roles and rights of the site personnel responsible for entering clinical data into the eCRF will be determined in advance. If additional corrections are needed, the responsible monitor or project manager will raise a query in the electronic data capture application.

The appropriate investigational personnel will answer the queries in the eCRF. This will be audit trailed by the electronic data capture application meaning that the name of investigational personnel, time, and date is logged.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to each clinic for filing in the Investigator site file after finalization of the CSR.

17.4 SOURCE DOCUMENTS

Data entered into the subjects medical record at the clinic will be considered as the source data and will be transferred to the relevant sections of the eCRF. Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, that verifies the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. Source documents include laboratory notes, memoranda, material dispensing records, subject files, etc.

The eCRF is considered as source data when data is entered directly into the eCRF.

The Investigator is responsible for maintaining source documents. These will be made available for inspection by the monitor at each monitoring visit. All supportive documentation submitted with the eCRF, such as laboratory or hospital records, should be clearly identified with the study ID and subject number. Any personal information, including name, should be removed or rendered illegible to preserve individual confidentiality.

17.5 USER ID

The eCRF records will be automatically appended with the identification of the creator, by means of their unique UserID. Specified records will be electronically signed by the investigator to document his/her review of the data and acknowledgement that the data are accurate. This will be facilitated by means of the Investigator's unique UserID and password; date and time stamps will be added automatically at time of electronic signature. If an entry in an eCRF requires change, the correction should be made in accordance with the relevant software procedures.

17.6 AUDIT TRAIL

All changes made to data entered in the eCRF will be fully recorded in a protected audit trail, and a reason for the change will be required.

Once all data have been entered, verified, and validated, the database will be locked.

18. STATISTICS

18.1 STATISTICAL ANALYSIS PLAN

A Statistical analysis plan (SAP) will be prepared and finalised prior to the code-breaking of the subjects' treatment. This document will provide further details regarding the definitions of analysis variables and analysis methodology to address all study objectives.

A blinded data review will be conducted prior to code-breaking of the subject's treatment assignment. This review will assess the accuracy and completeness of the study database, subject evaluability, and appropriateness of the planned statistical methods.

18.2 SAMPLE SIZE DETERMINATIONS

A total of 162 subjects are planned to be randomised in a 2:1 ratio to treatment groups 1 to 3 or treatment groups 4 to 6, respectively (Table 5). The sample sizes are not based on statistical criteria with a power calculation but on what is assessed adequate based on previous experience in similar studies. Sample sizes are larger in the groups receiving antigen alone or antigen with Endocine since the evaluation of the effect of Endocine on safety/tolerability and immune response compared with no Endocine is a key objective.

18.3 DATABASE LOCK

After all subjects have completed the last visit to the clinic (visit 4 group 1 to 4, and visit 3 group 5 and 6), a clean file meeting will take place and an unblinded analysis of all data (baseline data, efficacy data and safety data) collected during the clinical phase will be conducted. The outcome of the analyses will be documented in a full ICH-E3 compliant CSR.

An additional clean file meeting will be conducted after completion of the last telephone follow-up call prior to final database lock. Data from the safety follow-up period will be summarised in an addendum to the CSR.

18.4 ANALYSIS SETS

The classification of subjects to each analysis set will be decided at the first clean file meeting prior to database lock. The classification will be documented in the clean file protocol and in the CSR.

18.4.1 Full Analysis set

The full analysis set (FAS) is used to describe the analysis set that is as complete as possible and as close as possible to the intention-to-treat (ITT). The FAS will consist of all randomised subjects who received at least one dose of study medication and have at least one post-randomisation efficacy assessment.

The FAS analysis will be the primary analysis for evaluation of immune response and immunology efficacy data and will be performed for all efficacy endpoints unless otherwise stated. Subjects will be analysed according to the treatment group to which they were randomised.

18.4.2 Per-protocol Analysis Set

The per-protocol analysis set (PPAS) is a subset of the FAS and consists of all subjects without any major protocol deviations, i.e. a set of subjects that participated in the study as intended.

Analysis based on the PPAS will be used to assess the robustness of the results from the FAS analyses. Subjects will be analysed according to treatment which they received.

Subjects diagnosed with influenza after start of treatment will not be included in the PPAS.

18.4.3 Safety Analysis Set

The safety analysis set will consist of all subjects who received at least one dose of study medication. The safety analysis set will be used for evaluation of safety data. Subjects will be analysed according to the treatment which they received.

18.5 STATISTICAL METHODS

18.5.1 Demographics and other baseline characteristics

Subject disposition will be presented for all enrolled subjects (including screening failures). The number and percentage of randomised subjects who completed the study and discontinued from the study will be provided, as well as reasons for early discontinuation. The reasons for screening failures will also be summarised.

Demographic information and baseline characteristics will be summarised by treatment group and in total for the following:

Demographics

- Age (years);
- Height (cm);
- Weight (kg);
- BMI (kg/m^2)
- Gender (male/female).

Medical history and concurrent diseases;

Prior and concomitant medications.

18.5.2 Safety analysis

Incidences will be descriptively compared between the treatment groups. For categorical variables the frequency and proportion will be presented. Percentages will be based on the number of subjects with evaluable data within each treatment group. Continuous variables, including change

from baseline, will be presented using appropriate descriptive statistics. Safety analyses will be based on the safety analysis set.

18.5.2.1 Adverse Events

All AEs will be coded using the medical dictionary for regulatory activities (MedDRA). Data will be summarised using preferred term (PT) and primary system organ class (SOC) and presented by treatment group and in total.

Nature, incidence, severity and the Investigator's causality assessments will be reported for each AE. AEs, SAEs, drug-related AEs, AEs leading to withdrawal and AEs of special interest will be listed and summarised by incidence and treatment group.

Pre-treatment events, collected from the signing of the ICF until the first dose, will be listed and summarised by incidence and treatment group.

18.5.2.2 Local tolerability assessments

The administration-site reactions swelling, redness, pain and pruritus in the nose or arm will be classified as none, mild, moderate or severe. The evaluations will be performed at study visits 2 and 3 for group 1 to 4 and visit 2 for group 5 and 6, both before and after dose. The results will be summarised graphically and in frequency tables.

18.5.2.3 Subject diary for assessment of local tolerability and solicited AEs

The administration-site reactions pain and pruritus will be recorded by the subject in a diary and classified by the subject as none, mild, moderate or severe. The evaluations will be summarised in frequency tables. Solicited AEs (fever [temperature >38°C], chills, sweating, tiredness, headache, muscle aches [not associated with the administration area], joint pain [not associated with the administration area], runny nose, nasal congestion, sneezing, cough, sore throat and nausea) will be recorded by the subject and listed and summarised graphically by incidence and treatment group.

18.5.2.4 Clinical laboratory parameters

Clinical laboratory parameters, including change from baseline, as well as number and percentage of subjects with any clinically significant findings will be summarised by treatment group.

18.5.2.5 Physical examinations

Number and percentage of subjects with any abnormal physical examination findings will be summarised by treatment group in contingency tables.

18.5.2.6 Vital signs

Vital signs, including change from baseline will be summarised by treatment group.

18.5.2.7 ECG

Number and percentage of subjects with any clinically significant ECG findings will be summarised by treatment group in contingency tables.

18.5.3 Efficacy analyses

All efficacy analyses will be performed on both the FAS and PPAS.

18.5.3.1 Secondary analyses

HI and VN titres in blood for all treatment groups will be analysed descriptively and also using analysis of covariance (ANCOVA) with terms for baseline value, treatment and site. Comparisons of

treatment groups will be performed at a 2-sided, 5% significance level. In addition to hypothesis testing, adjusted means and 95% confidence intervals will be presented for the respective treatment differences. The following comparisons will be made:

- Group 1 vs. Group 2, 3, 4, 5 and 6;
- Group 2 vs. Group 3, 4, 5 and 6;
- Group 1+2 vs. Group 3, 4, 5 and 6.

All comparative statistical analyses will be performed in an exploratory manner and conclusions should be interpreted with caution as the number of subjects per group is limited.

Additional descriptive analyses will be performed on the GMTs and the pre-/post-treatment GMRs will be calculated. The percentage of subjects with seroprotection (post-treatment HI titre ≥40) and seroconversion (either a pre-treatment HI titre <10 and a post-treatment titre ≥40 or a pre-treatment HI titre ≥10 and a fourfold increase in HI titre post-treatment) will also be presented for each treatment group.

18.5.3.2 EXPLORATORY ANALYSIS

The exploratory efficacy endpoint IgA in nasal secretions will be analysed in a similar manner to the HI and VN titre analysis.

18.5.3.3 POST-HOC EXPLORATORY ANALYSIS

The following may be selected/analysed if considered valuable based on the results of the secondary analyses:

- Influenza-specific T cell responses;
- SRH analysis;
- Functional analysis of IgA in nasal swab
- Measurement of neuraminidase (NA) specific antibodies.

Post-hoc exploratory analyses may only be performed on selected subjects, time points and virus strains but will always be performed both pre- and post-treatment. Details of any post-hoc exploratory analysis will be presented in the CSR. Note that exploratory endpoints may be reported in addenda after the primary CSR.

19. STUDY MANAGEMENT

19.1 CLINICAL MONITORING

Before the initiation of the study, PCG will:

- Determine the adequacy of the facilities
- Discuss with the Investigator and study personnel their responsibilities with regard to CSP adherence, local regulations, and the duties of PCG.

At an initiation meeting, PCG will comply with the ICH-GCP Guidelines (ICH-GCP 8.3.20) and document that the study procedures were reviewed with the Investigator and the Investigator's staff.

During the study, a monitor will pay visits to the investigational site in order to:

- provide information and support to the Investigator
- confirm that facilities remain acceptable
- confirm that the investigational team is adhering to the CSP
- confirm that data are being accurately recorded in the eCRFs

- ensure that accountability checks for the IMP are being performed
- check SAE recording and reporting procedures
- conduct SDV which will require direct access to all original records for each subject (*e.g.* medical records), in accordance with the monitoring guidelines.

The monitor will be available (by phone, fax and e-mail) between visits whenever the Investigator or other study personnel at the investigational site needs information, advice or help.

All documentation and correspondence pertaining to the study (raw data, letters etc.) should be kept in accordance with ICH-GCP.

19.2 AUDITS AND INSPECTIONS

The purpose of an audit or inspection is to systematically and independently examine all study-related activities to document that they were conducted, recorded, analysed and accurately reported according to the CSP and the background regulatory demands.

Audits or inspections may therefore be performed at the study sites during or after the study. Visits may thereby be paid by authorised representatives of Eurocine vaccines AB by PCG or by a CA. These visits may include source data verification and confidentiality documents are therefore created.

The Investigator should contact the monitor immediately if they are contacted by a CA about an inspection at their study site.

19.3 TRAINING OF STUDY PERSONNEL

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have a detailed knowledge of and training in the procedures that are to be executed by them.

The Investigator will maintain records of all individuals involved in the study (medical, nursing and other personnel) with their function and study related duties delegated.

There will be training in nasal swab, local tolerability assessment and dose administration at the initiation meeting or at another occasion before study start. Only trained personnel are allowed to perform these activities.

19.4 CHANGES TO THE STUDY PROTOCOL

Any proposed change to the approved final CSP will be documented in a written and numbered CSP amendment. Study procedures will not be changed without mutual agreement between the Investigator, Eurocine Vaccines AB and PCG.

All amendments including substantial changes to the protocol must be submitted to appropriate IEC and Competent Authority for approval, according to applicable national regulations.

The amendment or a new version of the CSP must be approved by the CA and the IEC as applicable before implementation. Approval must also be obtained for the written subject information and ICF, if applicable.

19.5 PROTOCOL DEVIATIONS

Deviations from the CSP, deemed necessary for an individual subject, will be reported in the eCRF giving the reason and date. If necessary, the Investigator (or designee) will contact the monitor to inform about the deviation.

19.6 EMERGENCY PROCEDURES

The principal investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes an SAE and is to be reported as such.

19.6.1 Emergency contacts

In case of a medical emergency, the Investigator must contact the medical monitor at Eurocine Vaccines AB. For contact details, refer to Section 8.

SAE reporting procedures are described in Section 15.3.9.

19.6.2 Procedures in case of an overdose

An overdose is a dose in excess of the dose specified for each treatment group and dosing occasion in this CSP.

Any overdose should be recorded as follows:

- An overdose with associated AEs will be recorded as the AE diagnosis/symptoms in the AE Log of the eCRF
- An overdose without associated symptoms will only be reported in the subject's medical records.

19.7 STUDY TIME TABLE AND DECLARATION OF END OF STUDY

The end of the clinical part of the study is defined as the last visit of the last subject participating in the study (Visit 4 for subjects in group 1 to 3 and Visit 3 for subjects in group 4 to 6).

The end of the safety follow-up part of the study is defined as the last follow-up telephone call to the last subject participating in the study.

The study is expected to start in Q3 2016 and to be completed by Q4 2016 (clinical part) and Q2 2017 (safety follow-up).

19.8 STUDY REPORTING AND PUBLICATION OF STUDY RESULTS

A CSR in compliance with ICH E3, describing the conduct of the study, the statistical analyses performed and the results obtained, will be prepared by PCG. The final CSR will be submitted to the CA and to the IEC within 12 months after completion of the study.

If the study duration exceeds one year, the Sponsor must submit an annual safety report to the CA and IEC.

The results from this study will be submitted for publication at the discretion of the Sponsor.

19.9 ARCHIVING

The Investigator shall keep records of the study for 10 years after CSR finalisation in accordance with ICH E6. This includes any original source documents related to the study, the subject identification list (providing the sole link between the named subject source records and anonymous eCRF data), the original signed ICFs and detailed records of disposition of IMP. Eurocine Vaccines AB or designee should be contacted before any study-related documentation is planned for destruction.

The Sponsor will archive the study master file in accordance with ICH E6 and applicable regulatory requirements.

19.10 DISCLOSURE AND CONFIDENTIALITY

All unpublished information concerning the test product and research carried out by the Sponsor, including patent applications, manufacturing processes, basic scientific data, etc., is considered

confidential and the sole property of the Sponsor. Disclosure to third parties must be limited to those undertaking legitimate peer review of the scientific and ethical aspects of the study and to those participating, including the recipients of drugs, so that customary medical care and informed consent can be achieved.

The subjects have the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete. Eurocine vaccines AB or designee, whose responsibilities require access to personal data agree to keep the identity of each subject confidential. This agreement is to be substantiated in a separate document (Sekretessavtal).

19.11 INSURANCE

Subjects will be covered under Eurocine Vaccines AB liability insurance policy through the Swedish Pharmaceutical Insurance (*Läkemedelsförsäkringen)*. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering services performed by CTC.

20. QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA)

This study will be conducted in compliance with the protocol, relevant SOPs, GCP and the applicable regulatory requirement(s).

The Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He or she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

21. REFERENCES

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