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

Protocol version: 4.1 Dated 28 April 2017

Sponsor code: FLU-v 003

EudraCT number: 2015-001932-38

Principal Investigator/centre

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Sponsor

PepTcell Limited (trading as SEEK)

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GCP Statement

This study will be performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements

1. Signatures and Agreement with Protocol

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

We, the undersigned, agree to conduct this study according to this Study Protocol. We agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the study takes place.

Sponsor's Representative	Gregory Stoloff CEO PepTcell Limited (trading as SEEK) 45 Beech Street London EC2Y 8AD UK	Date: _____	Signature: _____
Medical Monitor	Bryan Murray, MBBS Boyd Consultants Ltd 3a Station Cottages St Neots, Cambridgeshire PE19 1QF UK	Date: _____	Signature: _____

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

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Medical Monitor: Bryan Murray, MBBS

Date: _____ Signature: _____

Investigator Name: Paul Groeneveld

Site Address: Isala Hospitals
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Dokter van Deenweg 1
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The Netherlands
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Date: _____ Signature: _____

2. Study Synopsis

<table>
<tr><td colspan="2">Title of the study: A randomised, double-blind, placebo-controlled, single-centre phase IIb trial as part of the EU-funded UNISEC project to assess the immunogenicity and safety of different formulations and dosing regimens of FLU-v vaccine administered subcutaneously in healthy adults aged 18-60 years.</td></tr>
<tr><td>EudraCT number:
2015-001932-38</td><td>Protocol code:
Sponsor: FLU-v 003</td></tr>
<tr><td colspan="2">Sponsor or sponsor's representative in the European Union:
PepTcell Limited (trading as SEEK), 45 Beech Street, London EC2Y 8AD, UK</td></tr>
<tr><td colspan="2">Investigators
Paul Groeneveld</td></tr>
<tr><td colspan="2">Study Centre
Isala Hospitals
Isala Academy, department of Innovation and Science
Dokter van Deenweg 1
8052 BP Zwolle
The Netherlands
PO box 10400
8000 GK Zwolle</td></tr>
<tr><td>Planned study period: 1 Year</td><td>Phase of Development: Phase IIb</td></tr>
<tr><td colspan="2">Objectives: To investigate the immunogenicity and safety of different formulations and dosing regimens of FLU-v in healthy adults.</td></tr>
<tr><td colspan="2">Study design: A randomised, double-blind, controlled single-centre study,</td></tr>
<tr><td colspan="2">Planned number of subjects: 222</td></tr>
<tr><td colspan="2">Medical condition or disease under investigation: Influenza</td></tr>
<tr><td colspan="2">Inclusion criteria
- Healthy males or healthy non-pregnant females (as indicated by a negative blood pregnancy test done during the screening visit) between the ages of 18 and 60 years, inclusive;</td></tr>
</table>

- Women of childbearing potential (not surgically sterile or postmenopausal for greater than or equal to one year) and men must agree to practice appropriate contraception (a combination of barrier and hormonal methods for women and a condom for men) from screening and until at least 30 days (up to Study Day 51 for females) and 90 days (up to Study Day 111 for males), after the last vaccination.
- A subject is in good health, as determined by a comprehensive clinical assessment {vital signs (heart rate, blood pressure, oral temperature)}, blood chemistry test (electrolytes, renal/kidney function, liver function, C-reactive protein, complete blood count), medical history, general physical examination, self-reported illness} and the clinical judgment of the investigator;
- Able to understand and comply with planned study procedures;
- Provides signed informed consent form

Exclusion criteria

- Has a known allergy to any of the components of the vaccine.
- Has a history of severe reaction following immunization.
- Persons with immune deficiency/disorder, whether due to genetic defect, immunodeficiency disease, or immunosuppressive therapy.
- Women who have a positive pregnancy test during the screening visit or who are breastfeeding.
- Has a history of any of the following (reported by subjects):
    - Acute disseminated encephalomyelitis (ADEM);
    - Neoplastic disease – current or previous;
    - Asthma or severe allergic disease;

- Bleeding disorders
- Chronic Hepatitis B and/or C infection;
- Chronic liver disease;
- Diabetes mellitus;
- Guillain-Barré syndrome;
- HIV;
- Rheumatoid arthritis or other autoimmune diseases;
- Severe renal disease;
- Transplant recipients;
- Unstable or progressive neurological disorders.

- Receipt of medicines/treatments that may affect evaluation of immunogenicity such as:
  - Oral or parenteral steroids, high-dose inhaled steroids (greater than 800 micrograms/day of beclomethasone dipropionate or equivalent) or other immunosuppressive or cytotoxic drugs (azathioprine (Imuran), cyclosporine (Neoral, Sandimmune, SangCya); monoclonal antibodies such as basiliximab (Simulect), daclizumab (Zinbryta), infliximab (Remicade), rituximab (MabThera), alemtuzumab (Campath and Lemtrada), omalizumab (Xolair), abatacept (Orencia), adalimumab (Humira and Exemptia) and etanercept (Enbrel)basiliximab (Simulect), daclizumab (Zenapax), and muromonab (Orthoclone OKT3); corticosteroids such as prednisone (Deltasone, Orasone); tacrolimus (Prograf, Advagraf, Protopic); Glatiramer acetate (Copaxone); Mycopehnolate (Cellcept); Sirolimus (Rapamune); (within 6 months of vaccination in this study)
  - Immunoglobulin or other blood products (plasma, blood cells, coagulation factors, haemoglobin)(within 3 months of vaccination in this study);

- An experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month of vaccination in this study, or expects to receive an experimental agent (during the study period).
- Influenza antiviral medication (Amantadine (Symmetrel); Rimantadine (Flumadine); Zanamivir (Relenza), Oseltamivir (Tamiflu) (within 4 weeks of vaccination in this study).

- Has received any influenza vaccine within 6 months of vaccination in this study.
- Has influenza-like illness (a sudden onset of symptoms **and** at least one of the four systemic symptoms-*fever or feverishness, malaise, headache, myalgia* **and** at least one of the three respiratory symptoms-*cough, sore throat, shortness of breath*) or acute respiratory infection (a sudden onset of symptoms **and** at least one of the four respiratory symptoms-*cough, sore throat, shortness of breath, coryza* (Rhinitis) **and** a clinician's judgement that the illness is due to an infection) within 6 months prior to vaccination in this study. These symptoms must have stopped the subject from carrying out their normal daily activities such as attending work or school for a period of at least 3 days.
- Has an acute illness, including an oral temperature greater than 38 degrees Celsius, within 1 week of vaccination.
- Has a history of alcohol or drug abuse within the last 2 years deemed unsuitable for inclusion by the investigator.
- Any abnormal haematology values and/or serum chemistries judged by the Investigator as clinically significant.

Being considered ineligible is based on the judgement of the investigator and in the event of uncertainty about the participant's medical status regarding any of the exclusion criteria mentioned, the participant's primary care physician will be consulted. Consultation of the primary care physician will only take place after having received written approval from the participant, and will concern medical information about

exclusion criteria only.

Test product, dose and mode of administration:

FLU-v vaccine in emulsion: 500 µg in WFI (water for injection, 0.25ml) and ISA-51 adjuvant (0.25ml) on day zero.

FLU-v vaccine in suspension: 500 µg in 0.01M HCl (0.25ml) and 0.01M NaOH (0.25 ml) on day zero and day 21.

All doses administered by subcutaneous injection in the upper section of arm.

Reference product, dose and mode of administration:

Two reference products will be used. An emulsion (0.5ml) containing WFI (0.25ml) and ISA-51 (0.25ml) will be used as the reference product for FLU-v vaccine in emulsion. Saline (0.5ml) will be used as the reference product for FLU-v in suspension. Both products will be administered by subcutaneous injection in the upper section of arm.

Duration of treatment: 21 days

Criteria for evaluation:

Immunogenicity

Primary:

To evaluate the cellular immune responses at 42 and 180 days as the change in level of TH1 cytokine production from baseline (day 0) following FLU-v vaccination, given as a suspension or as emulsion (adjuvanted), compared to placebo.

Secondary:

- To compare the effects of FLU-v with placebo, given as a suspension or emulsion (adjuvanted) on immune responses as measured using TH2 cytokines
- To evaluate the antibody responses specific to FLU-v at 42 and 180 days from baseline following FLU-v vaccination.

Exploratory:

To assess the effect of previous influenza vaccinations on the immunogenicity of FLU-v.

Safety

To evaluate the incidence of solicited AEs in all groups until 21 days after the last dosing of study vaccine (FLU-v). (Primary)

To evaluate the incidence and nature of unsolicited AEs and SAEs in all subjects during the whole study period. (Primary)

Clinical Efficacy

To evaluate the efficacy of FLU-v vaccine in the reduction of the incidence of RT-PCR confirmed influenza A and/or B infection in all subjects during the influenza season 2016-2017. (Exploratory)

To evaluate the efficacy of FLU-v vaccine in the reduction of symptom scores using diary entries and a scoring system among RT-PCR confirmed influenza A and/or B infection cases during the influenza season 2016-2017. (Exploratory)

Statistical methods:

A detailed Statistical Analysis plan will be prepared before the data lock of the trial database. All measured variables and derived parameters will be listed individually by subject number and treatment group. The data will be summarized in appropriate tables of descriptive statistics by study treatment. Mixed models repeated measures analysis will be used to compare the effects of FLU-v with placebo, whether given as suspension (unadjuvanted) or as emulsion (adjuvanted) on the normalized cytokine values at days 42 and 180. The baseline (Day 0) value will be used as a covariate and/or to adjust the post-treatment values. The terms in the model will include age group, time, treatment, formulation, and the interaction between treatment and time in order to see whether FLU-v has similar effects when given as a suspension or in adjuvanted form. Estimates for the effects of both formulations relative to the corresponding placebo will also be derived. The incidence of adverse events will be summarised for the Safety population in tables for each treatment group but no formal analysis will be conducted. The severity of symptom scores will be compared between treatments using analysis of variance for those subjects who develop flu, if numbers allow.

In a post-hoc exploratory analysis, the effect of previous influenza vaccines on the immunogenicity of FLU-v will be assessed. For this purpose, subjects' data will be stratified as follows:

-subject never previously received an influenza vaccine.

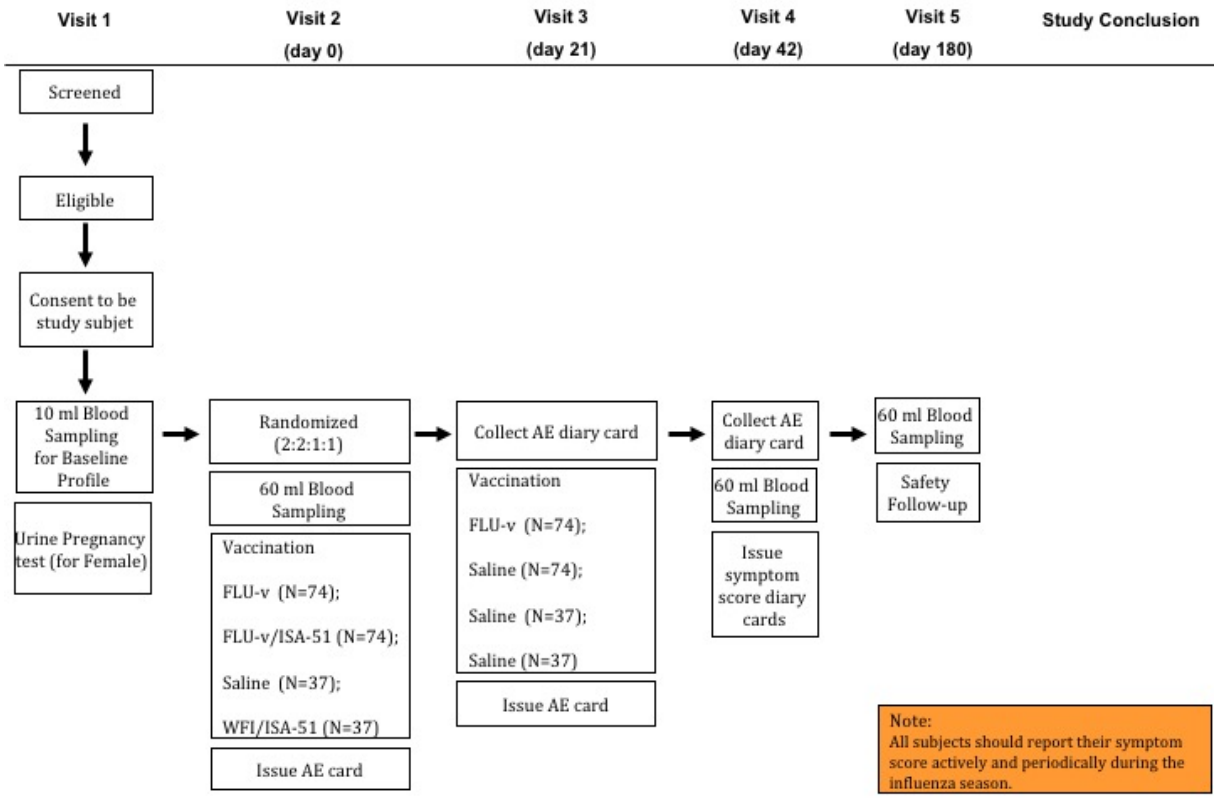
-subject received an influenza vaccine within the previous 24months.

-subject received an influenza vaccine over 24 months ago.

Determination of sample size:

Though a variety of immune parameters will be analyzed, the minimum sample size has been determined on the basis of the FLU-v-specific IFN-γ responses (assessed by ELISA) recorded in an earlier trial. IFN-γ is one of the most important markers of cell-mediated immunity for influenza protection. Taking the likely pattern of response and a loss to follow up into account, we will need to include 222 subjects in order to detect a 2.5-fold increase in the normalized value (change from baseline) for adjuvanted and unadjuvanted FLU-v relative to placebo.

Flow chart of study design



3. Table of Contents

4. List of Abbreviations and Definitions of Terms

AE	Adverse Event
CA	Competent Authority
CI	Confidence Interval
CMI	Cell Mediated Immunity
eCRF	electronic Case Report Form
CTL	Cytotoxic T Lymphocyte
CV	Curriculum Vitae
ECDC	European Centre for Disease Prevention and Control
EU	European Union
EudraCT	European Drug Regulatory Affairs Clinical Trials
FAS	Full analysis set
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator's Brochure
ICF	Informed consent form
IMP	Investigational Medicinal Product
ITT	Intention to treat
MMRM	Mixed models repeated measures
NP	Nucleoprotein
PBMC	Peripheral Blood Mononuclear Cells

PP	Per protocol
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SC	Subcutaneous
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCC	Trial Coordination Centre
Th	T helper

5. Investigators and Study Administrative Structure

5.1 Sponsor Representatives

Gregory Stoloff;

Dr. Olga Pleguezuelos;

Luca Landucci;

Emma Zuccaro

5.2 Sponsor Medical Monitor

Bryan Murray, MBBS
Boyd Consultants Ltd
3a Station Cottages
St Neots, Cambridgeshire
PE19 1QF
UK

5.3 UNISEC Project Coordinator

Prof. Dr. Eelko Hak (University of Groningen)

5.4 Investigators

Dr Paul Groeneveld
Isala Hospitals
Isala Academy, department of Innovation and Science
Dokter van Deenweg 1
8052 BP Zwolle
The Netherlands
PO box 10400
8000 GK Zwolle

5.5 Qualified Physicians Who Are Responsible for (all) Trial-site Related Medical (or Dental) Decisions

The same as the Investigators.

5.6 Clinical Laboratories and Other Medical and/or Technical Departments and/or Institutions Involved in the Trial

Institution	Role and responsibility
Sjef van de Leur Isala Hospitals The Netherlands	Haematology and blood chemistry tests
Fredrik Oftung Norwegian Institute of Public Health Norway	Antibody responses and exploratory CMI assays.
Steve Norley Robert Koch Institute Germany	Multiparametric FACS analysis and IFN-γ ELISA (Primary CMI endpoints)
Prof. dr H.G.M Niester PhD University Medical Centre Groningen University of Groningen Department of Microbiology Division of Clinical Virology The Netherlands	RT-PCR assay on influenza A and/or B viruses

6. Summary

Rationale: Current seasonal influenza vaccines mainly induce immune responses against viral membrane glycoproteins. These proteins, however, undergo continuous mutations by a process called antigenic drift. To prevent immune escape, annual vaccination with the latest predicted viral strains is adopted. Such vaccination strategy not only poses inconvenience and cost-inefficiency, but also results in poor protective effectiveness when the vaccinated strains are mismatched with the actual circulating strains. The latter point is especially of concern during a pandemic outbreak, when a large geographical area is affected and the general population is naïve to the newly re-assorted viral strain due to antigenic shift.

Objective: To evaluate the safety and immunogenicity of the influenza vaccine (FLU-v, as a suspension or adjuvanted as emulsion) targeting conserved immunogenic regions of influenza A and B viruses in healthy adults, in particular to show that the TH1 cytokine response at 42 and 180 days after the first injection is greater in the adjuvanted FLU-v and unadjuvanted FLU-v than in the placebo.

Study design: A total of 222 study participants will be recruited. The study follows a factorial design where the two factors are treatment (FLU-v / placebo) and formulation (unadjuvanted / suspension, adjuvanted / emulsion). Subjects will be randomised in two strata (age 18 to 40, age 41 to 60) to one of the following treatment regimens:

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

Each administration will be given subcutaneously. Solicited and unsolicited adverse events (AEs) will be collected by AE questionnaire/diary card. Adverse events (AEs) and serious adverse events (SAEs) will be collected for the entire study period. The

treatments will be administered starting in third quarter of 2016 in order to provide protection for the subsequent influenza season starting in December 2016. Blood samples will be taken from all subjects on day 0 (before FLU-v vaccination), 42 (21 days after the second dosing) and 180 (159 days after the second dosing) for the evaluation of FLU-v-specific cellular and humoral immune responses. Clinical symptom scores to ascertain severity and the incidence of RT-PCR-confirmed influenza A and/or B infection will be recorded during the subsequent influenza season (December 2016 to March 2017) to decide clinical efficacy of the tested vaccines.

Study population: Healthy volunteers aged 18-60 years.

Intervention: FLU-v investigational influenza vaccine lyophilised product containing 500 micrograms of total peptides reconstituted in either 0.01M HCl (0.25ml) and 0.01M NaOH (0.25ml) to achieve a volume of 0.5ml, or emulsified in WFI (water for injection, 0.25ml) and ISA-51 (0.25ml) to achieve a volume of 0.5ml.

Primary study parameters/endpoints: For immunogenicity: To compare the change from baseline (Day 0) between treatments in cellular immune responses, specifically TH1 cytokines, in all groups 42 and 180 days following FLU-v vaccination. For safety: (1) To evaluate the solicited AEs in all subjects until 21 days after the last dosing of the study vaccine (FLU-v); (2) To evaluate the unsolicited AEs and SAEs in all subjects for the entire study period after the first dosing of FLU-v.

Secondary study parameters/endpoints: To evaluate the humoral immune responses specific to FLU-v and TH2 cytokines from baseline in all groups 42 and 180 days following FLU-v vaccination.

Exploratory study parameters/endpoints: For immunogenicity: To evaluate the change from baseline in cellular immune responses based on additional CMI assays such as ELISPOT (Enzyme-Linked ImmunoSpot) in all groups at 42 and 180 days following FLU-v vaccination, in a subset of subjects chosen After un-blinding the study a subset of subjects will be selected (high FLU-v responders, FLU-v non-responders and placebo

similarly represented) to assess broadness of protection against different influenza strains. The analytical lab will remain blinded.

Clinical efficacy: (1) To evaluate the efficacy of FLU-v vaccine in reducing the incidence of RT-PCR confirmed influenza A and/or B infections in all subjects during the influenza season 2016-2017. (2) To evaluate the efficacy of FLU-v vaccine in the reduction of symptom score among RT-PCR confirmed influenza A and/or B infection cases during the influenza season 2016-2017. The relationship between efficacy and cellular and humoral response will be explored if possible.

The effect of previous influenza vaccination on the immunogenicity of FLU-v will be assessed in a post-hoc exploratory analysis after stratification of the data based on exposure to the influenza vaccine in the previous 24 months or over.

Exploratory endpoints may be reported separate from the main clinical study report.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The intended application of the IMP is as a prophylactic vaccine to prevent influenza virus infection by inducing an enhanced influenza-specific immune response. The FLU-v vaccine is designed to be delivered either as a naturally particulate suspension (i.e. no adjuvant) or emulsified in adjuvant. Particulate and emulsified protein preparations are preferentially taken up by phagocytic cells (e.g. dendritic cells) responsible for inducing primary immune responses.

Treatment with FLU-v of up to four doses of 263 µg/occasion or up to two doses of 553 µg/occasion, with or without adjuvant, was well tolerated in animals with no signs of systemic toxicity. In pre-clinical studies, subcutaneous administration of ISA 51 resulted in no systemic toxicity. At the injection sites and occasionally in adjacent tissue the injected material remained surrounded by a mild chronic inflammatory response. As the IMP is provided in one presentation (500 µg of the combined peptide) in lyophilized form for suspension in a sealed single-use vial, overdose is highly unlikely.

In a single centre, randomised, double blind phase I study of the safety, tolerability and immunogenicity of FLU-v no safety or tolerability concerns were identified at the doses administered (250 µg and 500 µg FLU-v) to the subjects in this clinical study and no safety or tolerability concerns were identified following administration of the adjuvant

to the subjects (1). FLU-v vaccine candidate was demonstrated to be immunogenic in humans, as measured by ex vivo γ-interferon production (1).

Clinical experience with Montanide ISA 51 dates back to the 1990s and most trials were related to cancer and Acquired Immune Deficiency Syndrome (AIDS). Currently, cancer trials in melanoma, colorectal, prostate, cervical, brain cancer and leukemia are ongoing. Frequency of vaccination is often between 2 and 4 weeks, and the number of injections can reach up to 40. Route of immunization is most often subcutaneous and volume of injection can reach up to 3mL. Most common local reactions are local pain, tenderness, erythema and granuloma at the injection site. Less frequently, mild to moderate transient indurations and swelling are described. General reactions are mainly 'flu-like symptoms such as chills, fever and headaches. Lethargy and nausea are also observed. The intensity is usually mild or moderate. No biological changes are generally observed. As the Placebo vaccine is provided in a sealed single-use vial, overdose is highly unlikely (Influenza virus (FLU-v) vaccine Investigator's Brochure, Edition 2.0, 07 September 2015).

7. Introduction

7.1 Background

Influenza is one of the major respiratory person-to-person transmittable viral infections in humans. The disease itself comes in two distinct forms: seasonal epidemics that occur every year in the winter period, and (global) pandemics that occur with unpredictable frequency and which can take place in any season. Over the past decade seasonal influenza has resulted in an estimated 80,000 to 500,000 deaths per year, depending on the severity of the flu outbreak. Deaths occur especially in vulnerable populations, such as the elderly and chronically ill subjects (2). Direct influenza-associated health care costs for Europe are estimated at more than €50 million per million of population per year (i.e. for the whole of Europe €2.5 billion/yr.), and costs are likely to increase further due to an ageing of the population (3).

Pandemic influenza arises when new influenza strains enter the human population by transmission from farm animal species. These viruses are antigenically very different from seasonal influenza strains and are therefore not or very poorly recognized by pre-existing immunity. For this reason, they can spread very easily in the entire population.

7.2 Rationale

Several strategies have been developed in recent years to broaden the response to vaccines, so that cross-clade recognition and neutralization of H5N1 viruses could be achieved. Yet, the emergence of the 'Mexican flu' or 'swine flu' caused by an H1N1 virus strain in 2009 demonstrated the unpredictable nature of pandemic influenza viruses. The Mexican Flu pandemic underlined the fact that a truly universal influenza vaccine will be required to tackle not only different clades generated by antigenic drift (with slightly changed HA), but also different virus strains generated by antigenic shift (with completely different HA subtypes). Consequently, universal influenza vaccines have to target viral proteins, or domains of viral proteins, which are highly, conserved across a number of (or even all known) influenza A and B virus strains.

FLU-v vaccine developed by SEEK is a sterile equimolar mixture of four synthetic polypeptides that cover highly conserved regions found in the NP, M1 and M2 proteins of influenza A and B viruses. Please refer to the Investigator's Brochure of FLU-v for detailed information. FLU-v is expected to achieve cross-protection against influenza

disease by inducing cell-mediated immunity (cytotoxic T lymphocytes (CTLs) and/or T helper (Th) cells) and non-neutralizing antibodies. Epitopes recognized by T and B cells are often highly conserved among influenza subtypes indicating that these cells have 'natural' cross-reactive potential (4). In addition, recent data demonstrates that human CTLs elicited by seasonal influenza recognize and lyse cells infected by H5N1 or pdmH1N1 virus (5). Together with data indicating age-related relative protection against newly emerging virus strains, these results imply that CTLs (though not providing sterilizing immunity) have at least a disease-mitigating potential in humans.

The aim of this randomised, double-blind, parallel group, placebo-controlled, single-centre phase IIb study will be to evaluate the immunogenicity and safety of FLU-v vaccine in healthy adults (aged 18-60). The immunogenicity of FLU-v will be evaluated by CMI and humoral responses. The safety will be evaluated based on reported AEs and SAEs during the study period. In addition, an exploratory endpoint of clinical efficacy will be evaluated, which will be determined by reductions in occurrence of influenza and the clinical symptom severity in RT-PCR confirmed cases of influenza A and/or B infection during the 2016-2017 influenza season.

8. Objectives

Vaccination with FLU-v generates a population of T cells that can recognise conserved influenza antigens on the surface of infected cells. On recognition, T cells are activated and secrete Th1 cytokines such as INF-γ, TNF-α, and IL2 and release granzyme and perforin. In addition, FLU-v specific non-neutralising antibodies may also bind to these antigens on the surface of infected cells activating complement and cytotoxic responses lead by Natural Killer (NK) cells. The T cell and antibody actions will result in the destruction of infected cells.

The objectives of this study are to determine the safety of the vaccine and assess which one of the two treatment groups, one dose adjuvanted vs two dose unadjuvanted, induces the strongest immune response which is measured by quantifying the increase in Th1 and non-neutralising antibody responses after vaccination compared to baseline and corresponding placebo vaccination. The relationship between clinical efficacy and the immune response will also be explored.

8.1 Primary objectives

(1) Cellular Immunogenicity

- To compare the cellular immune responses as measured by the change from baseline (Day 0) in TH1 cytokines between FLU-v administered as a suspension and as emulsion (IFA-51 adjuvanted) at 42 and 180 days following FLU-v vaccination.

(2) Safety

- To evaluate the solicited AEs in all subjects until 21 days after the last dosing of the study vaccine (FLU-v).
- To evaluate the unsolicited AEs and SAEs in all subjects during the whole study period.

8.2 Secondary Objective

- To evaluate the FLU-v specific antibody responses in all subjects from baseline at day 0 to day 42 and day 180 following FLU-v vaccination.
- To compare the TH2 cytokine response between treatments from baseline to day 42 and day 180 following FLU-v vaccination.

8.3 Exploratory objective

(1) Cellular Immunogenicity

- To evaluate the change from baseline in cellular immune responses based on additional CMI assays at 42 and 180 days following FLU-v vaccination, in a subset of subjects.
- To evaluate the effect of previous influenza vaccines on the cellular immune responses to FLU-v.

After un-blinding the study a subset of subjects will be selected (high FLU-v responders, FLU-v non-responders and placebo similarly represented) to assess broadness of protection against different influenza strains. The analytical lab will remain blinded.

(2) Clinical Efficacy

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

Exploratory endpoints may be reported separately from the main clinical study report.

9. Study Design

After providing consent, subjects will enter a screening period of approximately 7 days to verify eligibility for the trial. All screening evaluations will be reviewed and once confirmed as eligible, subjects will be considered enrolled and will progress to the randomisation visit. Randomised subjects will then enter a 21-day treatment period, followed by 2 follow-ups on day 42 and 180, for a maximum of one year (from screening to study conclusion) study participation. Subjects will attend clinic visits as shown in the Flow Chart and detailed in section 11.3.

9.1 Study Groups and Treatments

Subjects will be randomised in a (2:2:1:1) fashion, double-blinded (see note below) and placebo–controlled. Randomisation will be stratified by age group (18 to 40, 41 to 60) to ensure that the age distribution in all treatment groups is broadly similar. (This is because older subjects may be more prone to infection than younger subjects though they are not expected to respond differently to the treatment regimens). Each subject's participation will be for a maximum of one year (from screening to study conclusion) enrolling 222 healthy adults aged 18-60 years. The treatment groups are as follows:

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

9.2 Description and Justification of Control

The placebo for arm 1 is 0.5ml saline. Its chemical composition (NaCl in water) is the same as when mixing HCl and NaOH in equimolar amounts. The placebo for arm 2 is 0.5 mL emulsion of WFI (0.25 mL) and the adjuvant [Montanide ISA-51-VG; Seppic](0.25ml) where Montanide ISA 51 is defined as a mixture of a highly purified mineral oil (Drakeol 6VR) and a surfactant (Mannide monooleate). When mixed with an aqueous phase at a

50/50 ratio, it renders a water in oil emulsion and is used as vaccine adjuvant to enhance the immune response.
In pre-clinical studies, subcutaneous administration of ISA 51 resulted in no systemic toxicity. At the injection sites and occasionally in adjacent tissue the injected material remained surrounded by a mild chronic inflammatory response.
No safety or tolerability concerns were identified following administration of the adjuvant to the subjects in a Phase 1 study (EUDRACT No: 2009-013910-28).

9.3 Description and Justification of Dosage and Administration

Different doses were tested in previous human studies and the dose selected for this study, 500ug, is the one that performed the best in the previous studies from both a perspective of safety/tolerability as well as for efficacy.

In a previous study (EUDRACT/IND NUMBER: 2009-014716-35) FLU-v was administered subcutaneously as a sterile lyophilized mixture at a nominal total peptide dose of 500 μg/vial, resuspended in 0.5 mL sterile WFI and the suspension emulsified with 0.5mL of adjuvant [Montanide ISA-51-VG; Seppic] to give a 1 mL volume for subcutaneous injection.

The dose selected (500ug) is considered both safe and well-tolerated and with the potential for eliciting immunogenicity based on the results of pre-clinical studies, as follows:
A number of repeat dose toxicity studies, conducted in mice and rats investigated the systemic toxicity potential and immunogenicity of FLU-v when administered alone or in combination with the adjuvant ISA 51. All the toxicity studies were carried out using the tetravalent formulation of FLU-v used in the current study. All the studies demonstrated that following subcutaneous vaccination with FLU-v alone or with the adjuvant, there was no effect on bodyweight gain, food consumption, organ weight or ophthalmoscopy (where ophthalmic examination was carried out). The test substance was clinically well-tolerated with no signs of systemic toxicity and thorough histopathological evaluation did not reveal any signs of systemic toxicity.

In repeat dose toxicity studies in the mouse using FLU-v alone and adjuvanted FLU-v, cell-mediated responses were evident with enhanced responses when FLU-v was administered with adjuvant. Increases in total white blood cell counts were evident in most of the studies but were considered related to an immune response to FLU-v and, as such, did not represent an adverse effect.

Subcutaneous injection of FLU-v alone or with adjuvant was also well-tolerated in a local tolerance study in rabbits, with no systemic sign of reaction to treatment.

Generally, injection site responses and microscopic and macroscopic changes in animals receiving FLU-v were considered likely to be due to the effects of the adjuvant, ISA 51.
In addition, treatment with FLU-v of up to four doses of 263 µg/occasion or up to two doses of 553 µg/occasion, with or without adjuvant, was well-tolerated in animals with no signs of systemic toxicity. The IMP is provided in one presentation (500 µg of the combined peptide) in lyophilized form for suspension in a sealed single-use vial. At this dose, based on the pre-clinical studies, the dose is likely to be well-tolerated and safe in humans.

This protocol describes that the dose of FLU-v continues to be the same as in previous trials, 500ug, however the total volume has decreased from 1ml to 0.5ml in order to minimise injection site specific adverse effects. Additionally, rather than reconstitute FLU-v directly in saline as in previous trials, it will now be reconstituted in HCl first followed by pH neutralisation with NaOH. This method is a more time-effective way of reconstituting FLU-v to be administered in suspension unadjuvanted. The final result after mixing equal volumes of 0.01M HCl and 0.01M NaOH is NaCl in water which is the same composition as saline.

9.4 Vaccination Schedule

Group	Admin 1 (day 0)	Admin 2 (day 21)
1	FLU-v in pH neutral HCl/NaOH [suspension]	FLU-v in pH neutral HCl/NaOH [suspension]

2	FLU-v adjuvanted with ISA-51 [emulsion]	Saline
3	Saline	Saline
4	WFI and ISA-51 [emulsion]	Saline

9.5 Duration of the Study

The study will require 5 visits (including the screening visit) for each subject. The total study time is a maximum 1 year from first subject first visit to study conclusion.

9.6 Method of Assigning Subjects to Treatments

(1) Assignment of Subject Numbers

The Trial Coordination Center (TCC) of University Medical Center Groningen will be in charge of the randomisation list. Each subject screened in Zwolle will be allocated a 3 digit screening number representing the sequential order in which they are screened e.g. 001, 002 etc. On randomisation, subjects will be allocated the next available 3-digit randomisation number in the appropriate age group in the randomisation list. Randomisation numbers generated by the ALEA system will be sent out by TCC by courier directly to the unblinded pharmacist at Isala Clinic in Zwolle. No other person, except the responsible person at TCC (who generated the list) and the unblinded pharmacist, will have the access of the randomisation list.

(2) Randomisation Method

The randomisation system is hosted and set up by the Trial Coordination Center (Clinical Research Organization) in the UMCG, however the implementation of the randomisation itself will be performed on-site in Zwolle. The subjects will only have study-visits in Zwolle.

In accordance with the protocol, subjects will attend a screening visit (visit 1) in Zwolle which will be completed [-7]to[-1] days before the subject is randomised. Each screened subject will then receive a sequential identification number as described above.

Subjects will retain this unique number, whether or not they are ultimately randomised to receive treatment. If the subject meets the inclusion/exclusion criteria, blood samples are taken and the subject is requested to return for a Day 0 visit (visit 2). At the day 0 visit the blood sample results are reviewed and if the subject continues to meet the inclusion/exclusion criteria, the subject can be randomised. When a subject is to be randomised, the next randomisation number in sequence within the appropriate age group stratum will be allocated to the subject, and the treatment description then made up in accordance with the Pharmacy Instructions.

Randomisation will be performed using a web-based system (ALEA) by the Trial Coordination Centre at the University Medical Centre Groningen and which is fully backed up on a daily basis. Randomisation will be stratified according to age (age 18 to 40, and age 41 to 60) with the 2:2:1:1 treatment ratio being maintained in appropriately sized blocks within each stratum to ensure similar age distribution between the groups.

9.7 Blinding

To maintain blinding, the preparation of IMP and Control will be performed by a qualified person other than the person administering the injection. The study will be double-blind although it is not possible to completely mask the presence of adjuvant in the injections at Day 0 since the solution and the emulsion are physically distinguishable. Due to the local injection site reactions likely from Montanide ISA-51 adjuvant, masking of syringe barrels is deemed superfluous. However, the blinded study personnel will remain blinded to the presence or absence of FLU-v vaccine. The label on the syringe will need to carry the necessary trial randomisation information. This is explained in detail in the pharmacy manual.

The study pharmacist/designee responsible for the preparation of dosing materials is unblinded to the treatment each subject will receive. The unblinded study pharmacist/designee will prepare dosing solutions and will use the study randomisation code to assign each subject to the appropriate treatment group. The unblinded study pharmacist/designee will not reveal the treatment code to study personnel or perform

any other study related duties. The unblinded study pharmacist/designee will prepare syringes containing the study medication assigned by the randomisation scheme labelled with the subject's randomisation number. An identical label will be attached to the top of the case report form. This material will be taken to the blinded study personnel administering the injections.

The Pharmacy file, including the randomisation list, vaccine supplies and all associated documentation, will be stored in a locked cabinet within the Pharmacy where only unblinded Pharmacy personnel have granted access.

9.8 Diary Cards

(1) Symptom Diary Card/Diary Score

A symptom diary card will be issued to all subjects which they are instructed to record the card daily during the influenza season (December 2016 to March 2017). Scores on a scale of 0 to 3 (see below for definitions) will be requested daily for the following symptoms: fever (≥38^{o}C), malaise, headache, myalgia (muscle and joint pain), cough, sore throat, shortness of breath, runny nose, stuffy nose, sneezing and earache. The trial centre must be contacted immediately if subjects feel unwell for 24h, with a sudden onset of at least one respiratory (cough, sore throat, shortness of breath, runny nose, stuffy nose, sneezing and earache) and one systemic symptom (fever, malaise, headache and myalgia (muscle and joint pain). Nasal and tonsil swabs (2 swabs in total) should be taken from the reported subjects within 3 days from the trial centre being contacted or within 4 days of the onset of symptoms, whatever time is shorter. The completed diary cards should be posted/faxed/emailed back to the trial site by subjects every two weeks during the duration of the influenza season.

The same list of symptoms in the diary card will be used in those subjects with confirmed influenza (by RT-PCR) to assess severity of disease. Each symptom is assessed on a scale of 0-3: no symptoms=0, just noticeable=1, bothersome but can still

so activities=2, bothersome and cannot do daily activities=3. The sum of all the scores constitutes the daily symptom score.

(2) AE Diary Card

To follow solicited AEs, 2 AE diary cards will be issued to all study subjects. Solicited AEs include fever, chills, joint pain, muscle pain, headache, backache, abdominal pain, chest pain, pain in arm, itching on body, swelling/tender lymph nodes, rhinorrhea, nasal stuffiness, difficulty breathing, sore throat, cough, fatigue, loss of appetite, vomiting, diarrhea and injection site reaction. The cards will need to be completed by the subjects and returned on day 21 and 42.

9.9 Clinical Efficacy Follow-up

- Active and periodic reports of the symptom diary card/diary score from all subjects during the influenza season;
- Clinical sampling and laboratory confirmation by RT-PCR.

9.10 Immunogenicity Follow-up

Cellular and humoral immunogenicity of FLU-v vaccine as explained in section 6 and section 8 will be evaluated in all subjects. 60 ml of blood will be sampled on the scheduled visits at day 0, 42, and 180. 50 ml and 10 ml of the collected blood will be used for the assessment of cellular and humoral immune response, respectively.

9.11 Safety Follow-up

Adverse events reported after FLU-v vaccination will be collected and evaluated for all subjects.

(1) Solicited AEs (subjects will return on day 21 (second vaccination) and 42 with AE diary card/questionnaire).

(2) Unsolicited AEs and SAEs (collection throughout the whole study period).

9.12 Data Collection and Management

Data management will be conducted by TCC, which is ISO 9001:2008 certified for "supporting researchers in the design, supervision, analysis and reporting of biomedical research, including project management, protocol design and review, EC/CA submission, data entry, data management, monitoring, biometrics, medical writing and software development", hence qualified to meet the essential requirements of ICH-GCP guidelines for GCP according to the European directives. An interactive website will be the major communication instrument ensuring integration of the program communication and the trial study.

(1) Electronic case report forms (eCRFs)

- Will be optimized for scalability, in accordance with standards for electronic data entry and flexible data export (GCP);
- Will be developed as web-based report forms integrated into the Web Content Management System. Windows Internet Explorer is required to support online data entry.

(2) Data

- Will be stored in the databases for consistency checks and further processing;
- Will be backed up on a daily basis;
- Will be subject to secure access control management to allow secure entry, access, analysis and export of data by users regardless of their locations;
- Will be subject to plausibility and consistency check during the entry process to enforce high data integrity, authentication and data protection according to the relevant user access levels and server and communication security mechanisms will be used;
- All nominal subject data will be anonymised to ensure personal data protection. If several reports taken at different times have to be correlated, pseudo-anonymization instead of complete anonymization will be used.

We will ensure that the data protection authority of the trial participating nation accepts the security and privacy procedures.

9.13 From Enrolment to Study Conclusion

(1) Scheduled visits at day 0 and 21 for vaccination.

(2) Scheduled visits at day 0, 42 and 180 for cellular and humoral immunogenicity follow-up.

(3) Scheduled visit at day 21 and 42 for solicited AE follow-up.

(4) Unsolicited AEs and SAEs will be followed throughout the study period for all subjects.

9.14 During the Influenza Period (December 2016 to March 2017)

A symptom diary card will be issued to all subjects. For details please refer to point (1) Symptom Diary Card/Diary Score under section 9.8 Diary Cards.

In the event of missing data for any timepoint in the influenza symptom diary card, the study doctor will ask the subject about any symptoms during the missing day(s). However, no protocol deviation will be recorded for missing influenza symptom scores.

10. Study Population

10.1 Number of Subjects

A total of 222 healthy adults aged 18-60 years will be randomised.

10.2 Eligibility Criteria

Subject eligibility should be reviewed and documented by an appropriate qualified member of the Investigator's study team before subjects are included in the study.

(1) Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Healthy males or healthy non-pregnant females (as indicated by a negative blood pregnancy test during the screening visit) between the ages of 18 and 60 years, inclusive;
- Women of childbearing potential (not surgically sterile or postmenopausal for greater than or equal to one year) and men must agree to practice appropriate contraception (a combination of barrier and hormonal methods for women and a condom for men) from screening and throughout the study treatment and for at least 30 days (up to Study Day 51 for females) and 90 days (up to Study Day 111 for males). After the last dose of the IMP);
- Is in good health, as determined by a comprehensive clinical assessment {vital signs (heart rate, blood pressure, oral temperature), blood chemistry test (electrolytes, renal/kidney function, liver function, C-reactive protein, complete blood count), medical history, general physical examination, self-reported illness} and the clinical judgment of the investigator;
- Able to understand and comply with planned study procedures;
- Provides signed informed consent form.

(2) Exclusion Criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Has a known allergy to any of the components of the vaccine.
- Has a history of severe reaction following immunization.

- Persons with immune deficiency/disorder, whether due to genetic defect, immunodeficiency disease, or immunosuppressive therapy.
- Females (child bearing potential) having a positive blood pregnancy test during the screening visit or women who are breastfeeding.
- Has a history of any of the following (reported by subjects):
  - Acute disseminated encephalomyelitis (ADEM);
  - Neoplastic disease – current or previous;
  - Asthma or severe allergic disease;
  - Bleeding disorders
  - Chronic Hepatitis B and/or C infection;
  - Chronic liver disease;
  - Diabetes mellitus;
  - Guillain-Barré syndrome;
  - HIV;
  - Rheumatoid arthritis or other autoimmune diseases;
  - Severe renal disease;
  - Transplant recipients;
  - Unstable or progressive neurological disorders.
- Receipt of medicines/treatments that may affect evaluation of immunogenicity such as:
    - Oral or parenteral steroids, high-dose inhaled steroids (greater than 800 micrograms/day of beclomethasone dipropionate or equivalent) or other immunosuppressive or cytotoxic drugs (azathioprine (Imuran), cyclosporine (Neoral, Sandimmune, SangCya); monoclonal antibodies such basiliximab (Simulect), daclizumab (Zinbryta), infliximab (Remicade), rituximab (MabThera), alemtuzumab (Campath and Lemtrada), omalizumab (Xolair), abatacept (Orencia), adalimumab (Humira and Exemptia) en etanercept (Enbrel); corticosteroids such as prednisone (Deltasone, Orasone); tacrolimus (Prograf, Advagraf, Protopic); Glatiramer acetate (Copaxone); Mycopehnolate (Cellcept); Sirolimus (Rapamune) (within 3 months prior to vaccination);

- Immunoglobulin or other blood products (plasma, blood cells, coagulation factors, haemoglobin)(within 3 months prior to vaccination in this study);

o An experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month prior to vaccination in this study, or expects to receive an experimental agent (during the study period).

- Influenza antiviral medication (Amantadine (Symmetrel); Rimantadine (Flumadine); Zanamivir (Relenza), Oseltamivir (Tamiflu) (within the 4 weeks prior to vaccination in this study).

- Has received any influenza vaccine within 6 months prior to vaccination in this study.

- Has influenza-like illness (a sudden onset of symptoms, **and** at least one of the four systemic symptoms-*fever or feverishness, malaise, headache, myalgia* **and** at least one of the three respiratory symptoms-*cough, sore throat, shortness of breath*) or acute respiratory infection (a sudden onset of symptoms **and** at least one of the four respiratory symptoms-*cough, sore throat, shortness of breath, coryza* **and** a clinician's judgement that the illness is due to an infection) within 6 months prior to vaccination in this study. These symptoms must have stopped the subject from carrying out their normal daily activities such as attending work or school for a period of at least 3 days.

- Has an acute illness, including an oral temperature greater than 38 degrees C, within 1 week prior vaccination.

- Has a history of alcohol or drug abuse within the last 2 years deemed unsuitable for inclusion by the investigator

- Any abnormal haematology values and/or serum chemistries judged by the Investigator as clinically significant.

- Ineligible subject based on the judgement of the investigator.

- In the event of uncertainty about the participant's medical status regarding any of the exclusion criteria mentioned, the participant's primary care physician will be consulted. Consultation of the primary care physician will only take place after having received written approval from the participant, and will concern medical information about exclusion criteria only.

11. Study Procedure

11.1 Enrollment

Individuals volunteering to participate into the study will be invited for a study screening visit. During this visit,

- Subject Information Sheet 'SIS' and Informed Consent Form 'ICF' will be explained and given to the subjects.
- Subject eligibility will be assessed and a signed/dated informed consent will be obtained.
- Baseline information including demographics and self-reported medical and medication use history, influenza vaccination, alcohol, drug and cigarette consumption history/habits will be collected
- A blood sample (15 ml) will be taken to examine a standard blood picture, pregnancy test (females) and antibodies against circulating influenza viruses.
  Only subjects showing no abnormalities/non pregnancy (females) with the entry blood tests will be accepted for the study and later randomised to the study treatments.

11.2 Outline of Study Procedures (see next page)

Time		Screening	Day 0	Day 21	Day 42	Day 180	Unscheduled	
Contact	**Number of subjects**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit**	**Phone call**
I. Study enrollment								
Check inclusion criteria	300	√	√					
Check exclusion criteria	222	√	√					
General physical examination	222	√						
Medical and medication history	222	√						
History of influenza vaccination (within 6 months)	222	√						
Alcohol and drug usage history/habits	222	√						
Smoking history/habits	222	√						
Demographic data	222	√						
Informed consent	222	√						
Blood sampling (15 ml for baseline blood profile, for all participants)	222	√						
Blood pregnancy test (only for female) part of the 15ml baseline sample	Depends[1]	√						
II. Study procedure								
Randomisation	222		√					

Pre-vaccination body temperature (judged by clinical investigator)	222		**√**	**√**				
Blood sampling (60 ml)	222		**√**		**√**	**√**		
Vaccination	222		**√**	**√**				
30-minute post-vaccination observation	222		**√**	**√**				
Issue symptom diary card/diary score	222			**√**				
Issue AE diary card/questionnaire	222		**√**	**√**				
III. Study follow-up								
Examination of AE diary card/questionnaire	222			**√**	**√**			
Reporting of severe/high-grade solicited AEs by the subjects								
Physical examination of severe/high-grade AEs	depends[1]						**√**	
Evaluation of severe AEs (soon after reporting by the subject until resolution of the event)	depends[1]							**√**
Reporting of unsolicited AEs and SAEs	222	**Reported throughout the entire study period**						
Reporting of diary card/symptom score	222	**Self-recording daily during the influenza period.** **Self-reporting when reached the symptom criteria for swab sampling.**						

ILI (nasal and tonsil swabs) sampling	depends[1]						√	
ILI follow-up (till resolution of the event)	depends[1]							√
Recording of (urgent) hospitalization/care visit of all cause	depends[1]							√

Note:

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

11.3 Detailed Description of Study Procedures

During influenza season:

All subjects will be instructed and taught to complete the symptom diary card on a daily basis and to call the trial site(s) immediately when reaching the pre-defined criteria (the presence of one respiratory and one systemic symptom). Nasal and tonsillar swab samples (2 in total) should be collected from the reported subjects within 3 days from the centre being contacted or within 4 days of the onset of symptoms, whichever time is shorter. The completed diary cards will then be posted/emailed/faxed back to the trial site(s) every two weeks during the entire influenza season. If the cards are not returned, the trial site personnel will call those participants to remind them. In the event of missing data for any timepoint in the influenza symptom diary card, the study doctor will ask the subject about any symptoms during the missing day(s). However, no protocol deviation will be recorded for missing influenza symptom scores.

Screening, visit 1

- Check inclusion and exclusion criteria.
- Obtain written informed consent.
- Collect subject baseline information including medical and medication history, influenza vaccination history, alcohol, drug usage and smoking history/habits and demographic data (age, gender and ethnicity).
- General physical examination.
- 15 ml of blood will be collected for baseline blood profile (haematology, serum chemistry and antibodies against circulating influenza viruses) and pregnancy test (females)

Day 0, visit 2 (Within 1 week of visit 1)

- Review results from haematology values and blood chemistries.
- Examine pre-vaccination body temperature, judged by investigators.
- Check protocol violations from subjects. Obtain and record on-going medications.
- Randomise subjects as per protocol.

- 60 ml Blood sampling prior to vaccination. (50 ml for CMI measurements and 10 ml for the antibody response analysis)
- Vaccinate subjects. (One dose administered subcutaneously)
- The subjects will be observed for at least 30 minutes.
- Issue AE diary card/questionnaire. Instruct subjects how to use the diary.
- Instruct subjects to contact investigators immediately if they develop signs of SAEs.
- Record any AEs and SAEs.
- Report any SAEs to the sponsor

Day 21, visit 3

- Check protocol violations from subjects. Obtain and record on-going medications.
- Collect and verify completed AE diary card. The information will be transcribed to eCRF in English. The trial site should contact the participants before the visit and remind them to bring the AE diary card. No replacement of the card will be made.
- Check pre-vaccination body temperature.
- Vaccinate subjects. (One dose administered subcutaneously)
- The subject will be observed for at least 30 minutes.
- Issue AE diary card/questionnaire.
- Issue symptom diary card/diary score to complete during the influenza season 2016-2017. Instruct subjects when to call the study site to report a possible influenza-like illness (ILI) and to arrange nasal and tonsil swabs sampling.
- Record any AEs and SAEs.
- Report any SAEs to the sponsor

Day 42, visit 4

- Check protocol violations from subjects. Obtain and record on-going medications.
- Collect and verify completed AE diary card. The information will be transcribed to eCRF in English. The trial site should contact the participants before the visit and remind them to bring the AE diary card. No replacement of the card will be made.

- 60 ml of blood will be collected from all subjects. (50 ml for CMI measurements and 10 ml for antibody response analysis)
- Record any AEs and SAEs.
- Report any SAEs to the sponsor

Day 180, visit 5

- Check protocol violations from subjects.
- 60 ml of blood will be collected from all subjects. (50 ml for CMI measurements and 10 ml for antibody response analysis)
- Record any AEs and SAEs.
- Report any SAEs to the sponsor

From Visit 5 to the end of study (1st April 2017)

- Collect AE/SAEs from subjects until the end of the study
- Collect patient symptom diaries from the subjects every two weeks
- Arrange clinic appointments for any subjects that feel unwell for 24 hours with a sudden onset of a least one respiratory (*cough, sore throat, shortness of breath, runny nose, stuffy nose, sneezing or earache*) and one systemic symptom (*fever, malaise, headache and myalgia (muscle and joint pain*)). Nasal and tonsil swabs will be taken.

11.4 Sample Handling and Analysis

Biological samples including blood, nasal and tonsil swabs that are drawn for the trial study will be stored and accessed according to national guidelines. The storage duration is 10 years for the data and samples. Samples will be destroyed and data will be permanently removed from the computer network. When transferring biological samples from one country to another, trial participants will confirm and make sure that they comply with the Oviedo convention and the Recommendation Rec (2006) of the Committee of Ministers to member states on research on biological materials of human

origin (Adopted by the Committee of Ministers on 15th March 2006 at the 958th meeting of the Ministers' Deputies).

(1) Treatment and Storage of Biological Samples

PBMC isolated form subject's blood will be stored at -80°Celsius until shipped to the central laboratory where they will be stored in liquid nitrogen.
Serum samples will be stored at -20°Celcius before and after shipment.
Tonsil and nasal swab samples will be stored at -80 °Celsius.

(2) Shipment of Biological Samples

PBMCs, serum samples and nasal and tonsil swabs must be placed at in a container containing 2.5 -5 kg dry ice per 24 hours expected transport time (plus 24 hours in case of delays) complying with International Air Transport Association (IATA) requirements if shipment by air or complying with ADR or local regulation if transport by road. The completed Biological Specimen Listing Form should always accompany the shipment. The box must be clearly labelled with a Dry Ice Shipping Label.

(3) Laboratory Assays

At visit 1 (screening visit), 15 ml blood will be collected from all subjects for laboratory tests (haematology, serum chemistry, pregnancy test (females) and antibodies against circulating influenza viruses). Values for the haematology and serum chemistry parameters are to be within the normal ranges and pregnancy test negative, screening laboratory values outside the normal limits will be accepted into the study only after the Investigator or his/her designee has determined that the abnormal values are "not clinically significant". Haematology and serum chemistry parameters for analysis are outlined below:

Haematology	Serum Chemistry
Haemoglobin/Haematocrit/Total	Total bilirubin/ Uric acid/ Creatinine/ Total

and differential leukocyte count/ Red blood cell count/ Platelet count/ MCH/ MCHC/ MCV	protein/ Albumin/ AST/ ALT/ Alkaline phosphatase/ GGT/ Glucose/ Urea/ Sodium/ Potassium/ LDH/ Calcium/ Phosphate/ Cholesterol/ Triglycerides/ C reactive Protein

Serum samples from the collected blood will be stored at -20 °Celsius. At the end of the trial, the serum samples will be applied to serology tests to detect antibodies against the influenza virus strains that circulated whilst efficacy was monitored. This will help us to identify subjects who had pre-existing immunity to the circulating strains prior to the trial start. Endpoint analyses will be sub-analyzed with or without these subjects to account for subjects with pre-existent antibody responses to the strains of influenza circulating during the 2016-2017 season.

At visit 2, 4 and 5 (day 0, 42 and 180), 60 ml blood will be collected from all subjects for cellular immunogenicity measurement and FLU-v specific antibody responses. 50 ml of the collected blood will be used to isolate PBMCs and the isolated cells should be stored for later analysis. 10 ml of the collected blood will be used to measure antibody responses specific to FLU-v. Primary CMI measurements will be performed at the Robert Koch Institute (Germany). The Norwegian Institute of Public Health (Norway) will conduct additional exploratory CMI measurements. At RKI, IFN-γ ELISA and multiparametric FACS analysis will be performed according to the standardized procedures developed by the UNISEC consortium with adequate controls.

All swab samples will be stored at -80° Celsius and later posted to University Medical Centre Groningen Laboratory of Clinical Virology on dry ice. The samples will be tested for both influenza A and B viruses by generic RT-PCR assay targeting matrix gene. Multiplex and strain-specific primers will be used for typing and subtyping respectively.

<table>
<tr><th>Method</th><th colspan="3">Marker</th><th>Laboratory</th></tr>
<tr><td rowspan="4">Multi-parametric FACS analysis (Primary CMI)</td><td>Staining panel</td><td>Marker</td><td>Purpose</td><td rowspan="4">Robert Koch Institute</td></tr>
<tr><td rowspan="3">A</td><td>Viability dye</td><td>Exclude dead cells</td></tr>
<tr><td>CD3</td><td rowspan="2">Define lineage</td></tr>
<tr><td>CD4</td></tr>
</table>

			CD8	
			IFN-γ	Cytokine response
			IL-2	
			IL-4	
			TNF-α	
			CD107a	Cytotoxicity Marker
IFN-γ ELISA (Co-primary CMI)	IFN-γ			
Additional CMI assays (exploratory CMI)	Cytokine markers such as IFN-γ and granzyme B by ELISpot			Norwegian Institute of Public Health
Antibody assays	Flu-v-specific antibody responses			Norwegian Institute of Public Health
RT-PCR	Influenza A or B			University Medical Centre Groningen Laboratory of Clinical Virology

(4) Laboratory Readouts

Sampling time			Assay
Sample	Day	Visit number	
Nasal and tonsil swabs sampling	within 3 days of subject symptom report		RT-PCR
Blood sampling for 222 subject	0	2	Multi-parametric FACS analysis (222 samples), IFN-γ ELISA (222 samples), additional CMI assays (a subset of samples[1]) and antibody responses (222 samples)
	42	4	
	180	5	

[1] All blood samples taken for CMI measurement will be analyzed by multi-parametric FACS analysis and IFN-γ ELISA, which are the primary measurements for the CMI endpoint. Additional CMI assays (exploratory measurements for the CMI endpoint) will only be carried once the results from FACS analysis are complete and validated. The exploratory CMI assays will be performed on a subset of samples randomly selected from those available for both day 0 (reference) and day 42 and/or day 180.

11.5 Withdrawal of Individual Subjects

A window of ±3 days is allowed for the study visits.

Subjects will be advised that they are free to withdraw from the study at any time for any reason. In addition, the Investigator may withdraw a subject from the study in order to protect the subject's health. Investigators will withdraw subjects according to the following criteria:

- Withdrawal from the study is, in the Investigator's judgment, in the subject's best interest
- The development of any medical condition excluded in section 10.2
- Intolerable/unacceptable adverse experiences
- Major violation of study protocol procedures
- Subject unwilling to proceed and/or consent is withdrawn

In the event that a subject is withdrawn from the study due to a serious adverse event (SAE), the Investigator will evaluate the urgency of the event. If the situation warrants, the Investigator will take appropriate diagnostic and therapeutic measures. If the situation is not an immediate emergency, the Investigator at the clinical study facility will attempt to contact the Medical Monitor for consultation. No medical help, diagnosis, or advice will be withheld from the subject due to an inability to contact the Medical Monitor. The subject will be encouraged to remain available for follow-up monitoring by the Medical Monitor.

The Sponsor will be notified as soon as possible of any subject withdrawals.

Subjects who are withdrawn or discontinued after the start of study dosing will not be replaced. The investigator will ask the subject to complete the Study Termination Visit. The reasons for withdrawal will be recorded on the CRF and included in the final report, along with any AEs and any necessary medical treatment.

12. Safety Report

The Investigator and site staff are responsible for detection, recording and reporting of events that meet the criteria and definition of an AE or SAE (described below).

12.1 Definition of an Adverse Event

Any untoward medical occurrence occurring after the subject has given informed consent, whether or not considered related to the investigational product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) occurring after the subject has given informed consent.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency and/or intensity of the condition
- A new condition detected or diagnosed after informed consent, even though it may have been present prior to this
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication.

Examples of an AE do NOT include:

- A medical or surgical procedure (*e.g.* endoscopy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected before informed consent was obtained, that do not worsen.

12.2 Definition of a Serious Adverse Event

An SAE is any untoward medical occurrence that:

- 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, which hypothetically might have caused death, if it were more severe
- Requires hospitalization or prolongation of an existing hospitalization. In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-subject setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious. *Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE*
- Results in disability/incapacity. The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (*e.g.* sprained ankle), which may interfere or prevent everyday life functions but do not constitute a substantial disruption;
- Is a congenital anomaly/birth defect
- Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered

serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse

- All pregnancies will be reported and followed up. The Investigator will notify the Medical Monitor of any women partners who become pregnant during the study within 24 hours, who will then notify the Sponsor within 1 business day. Any resulting offspring will be monitored for up to 6 months *post-partum,* unless otherwise medically indicated.

12.3 Clinical Laboratory Abnormalities, Other Abnormal Assessments

Abnormal laboratory findings (*e.g.* clinical chemistry and haematology) or other abnormal assessments (*e.g.* ECGs, vital signs) that are judged by the Investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE (as defined in Sections 12.1 and 12.2, respectively). In addition, any clinical symptom or diagnosis or abnormal laboratory test thought to represent an AIDS defining illness will also be classed as a medically significant event and reported as an SAE.

The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

12.4 Time Period, Frequency, and Method of Determining AEs and SAEs

As a consistent method of soliciting AEs, the subject should be asked a non-leading question such as "How do you feel?"

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (*i.e*. before informed consent is obtained) should be recorded as Medical/Surgical History. Any medical occurrence, which is reported *after* informed

consent is obtained but *prior* to starting active or randomised treatment, will be documented as an AE.

Any signs and symptoms present at the time of informed consent will be documented as baseline signs and symptoms.

All AEs occurring after administration of the dose of study medication and on or before the final visit must be reported as AEs. All AEs must be recorded, irrespective of whether they are considered drug related.

At each visit/assessment in the period defined above, AEs will be evaluated by the Investigator and recorded.

Any AEs already documented at a previous assessment and designated as ongoing, should be reviewed at subsequent visits as necessary. If these have resolved, this should be documented.

12.5 Unblinding

It is unlikely that knowledge of a subject's treatment assignment will influence the nature of any immediate treatment necessary should a serious adverse event, such as anaphylaxis, occur. Where unblinding is deemed appropriate, the Principal Investigator should first contact SEEK's trial medical expert (please refer to 5.2 "Sponsor's Medical Expert for the Trial") before the blind is broken and the pharmacist or designee should not be contacted until unblinding has been agreed.

In the event of unblinding, a note to file should be prepared and signed by the Principal Investigator to include the date and reason for unblinding and personnel involved. The original file note should be given to the Unblinded Pharmacist for inclusion in the Pharmacy file and copies included in the subject's notes and site study file.

To comply with ICH E2A guidance and EU Clinical Trial Directive, any SAE associated with the use of the investigational product, which is unexpected and attributable/suspected is to be unblind prior to regulatory reporting. SEEK's medical expert is responsible for unblinding the treatment assignment in accordance with specified time frames for expedited reporting of SAEs.

12.6 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (*e.g.* hospital progress notes, laboratory, and diagnostics reports) relative to the event. The Investigator will then record all relevant information regarding the AE/SAE on the eCRF. It is not acceptable for the Investigator to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the appropriate AE/SAE eCRF pages. However, there may be instances when the Sponsor requests copies of medical records for certain cases. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to the Sponsor.

The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms. All SAEs that occur anytime after informed consent is given until study conclusion or early termination visit are considered reportable to the sponsor or sponsor's designee. Serious adverse events occurring after a subject is discontinued from the study will not be reported unless the Investigator feels that the study drug or a protocol procedure may have caused the event.

12.7 Evaluating AEs and SAEs

(1) Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgment. The intensity of each AE and SAE recorded in the eCRF should be assigned to one of the following categories:

- Mild: an event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities
- Moderate: an event that causes sufficient discomfort as to interfere with normal everyday activities

- Severe: an event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes, as described in Section 12.2 "Definition of a SAE".

(2) Assessment of causality

The Investigator is obliged to assess the relationship between investigational product and the occurrence of each AE or SAE. The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The Investigator will also consult the IB in the determination of his/her assessment.

The Investigator will provide the assessment of causality as per the instructions on the AE or SAE form. Causality will be assessed by the Investigator according to the following definitions:

- Unrelated: where an event is not considered related to the study drug
- Unlikely: although the relationship to study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations more likely
- Possibly related: the temporal relationship and the absence of a more likely explanation suggest the event could be related to the study drug
- Probably related: the known effects of the study drug or its therapeutic class, or based on challenge testing, suggest the study drug is the most likely cause
- Definitely related: this category applies to those AEs that are clearly a consequence of administration of the drug. It is likely that such events will be widely documented and generally accepted as having association with the study medication.

The steps taken as a result of an AE/SAE will be recorded, including any treatment administered because of the event, any adjustments of the dose of study drug or withdrawal of the subject.

12.8 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject and provide further information to the Sponsor on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE eCRF page(s) and SAE form will be updated. The Investigator will ensure that the follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

The Sponsor may request that the Investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The Investigator is obligated to assist. If a subject dies during participation in the study or during a recognized follow-up period, the Sponsor will be provided with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the Investigator. The updated SAE form should be resent to the Sponsor.

All SAEs must be reported to the SEEK Safety Team within 24 hours (or sooner if possible) of the Investigator (or designee) becoming aware of the situation.

12.9 Prompt Reporting of the SAEs to the Sponsor

All SAEs will be reported promptly to the SEEK Safety Team Medical Monitor:
Bryan Murray, MBBS
Boyd Consultants Ltd
3a Station Cottages
St Neots, Cambridgeshire
PE19 1QF
UK

The SEEK Safety Team will coordinate SAE reporting for all sites participating in this study. A form will be used for the collection of SAE data. The SAE form must be completed in English.

The SAE form should be completed as soon as possible from the time of the initial report, including the Investigator's assessment of causality. If the Investigator does not have all information to hand regarding an SAE, he/she should not wait to receive additional information before notifying the Medical Monitor of the event and completing the form. The form can be updated when additional information is received.

Investigators must notify the Medical Monitor and pharmacovigilence of all SAEs (whether considered treatment-related or not and whether expected or not) by email to:

bryan.murray@boydconsultants.comUpon receipt of an SAE form, the Medical Monitor will review the form for completeness and accuracy and for confirmation of the serious criteria, expectedness and causality. The Medical Monitor will notify the Sponsor in writing of all SAEs within 1 business day of being notified by the Investigator.

A business day is defined as 1 calendar day, excluding weekends and national holidays. The local clinical monitor will be notified by fax/email at the same time.

The Safety Team will generate queries as needed for all SAEs. If the Sponsor requires additional follow up information they will contact the Safety Team by e-mail or fax. The Safety Team will contact the Investigator for missing information and for the clarification of discrepancies on the initial report.

The Sponsor, in conjunction with the Safety Team, will decide on the need for expedited reporting to regulatory authorities, Investigators and the Independent Ethics Committees (IECs). The Sponsor is responsible for forwarding reportable SAEs to the appropriate regulatory authorities. Reports expedited to regulatory authorities will be forwarded to the Project Manager for distribution to all Investigators. The Safety Team will ensure that the Investigators notify their IEC as appropriate.

For medical queries, Investigators should contact the Medical Monitor of the SEEK Safety Team:

Bryan Murray, MBBS

Boyd Consultants Ltd
3a Station Cottages
St Neots, Cambridgeshire
PE19 1QF
UK

12.10 Regulatory Reporting Requirements for SAEs

The Investigator will promptly report all SAEs to the Sponsor in accordance with the procedures detailed in Section 12.9, "Prompt Reporting of SAEs to the Sponsor." Prompt notification of SAEs by the Investigator to the appropriate project contact for SAE receipt is essential, so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

The Sponsor (or responsible person, according to local requirements) will comply with the EU directive 2001/20/EU and the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities.

12.11 Post-Study AEs and SAEs

Any unresolved AE and SAEs will be followed for up to 30 days after the subject has completed the study. Following this time, any suspected unexpected serious adverse reactions (SUSARs) will be recorded. If the Investigator learns of any SAE, including a

death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the Investigator will promptly notify the Sponsor.

12.12 SAEs Related to Study Participation

An SAE considered related to study participation (*e.g.* procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly to the Sponsor (see Section 12.9, "Prompt Reporting of SAEs to the Sponsor").

12.13 Annual Safety Report

The duration of the trial, from the visit 1 (screening) to study conclusion, is approximately a maximum of 1 year for each subject. The Clinical Study Report (or End of Study Report) contains a review of safety data gathered. Please refer to 17.4 “End of Study Report” for details. A separate safety report will be provided to the ethical committee and regulatory authority in the Netherlands.

13. Investigational Medicinal Product

13.1 Name and Description

(1) FLU-v

FLU-v is an equimolar mixture of 4 synthetic polypeptides. These sequences represent predicted immunoreactive conserved regions identified *in silico* through multiple sequence (ClustalW) and immunogenicity analysis of all Influenza A and B protein sequences available at the National Centre for Biotechnology Information (NCBI) taxonomy database[5]. Each polypeptide represents a region of the consensus sequence where every consecutive amino acid is present in ≥70% of the sequence population tested and where >5 potential human T cell epitopes are found.

Chemical synthesis is a simpler and more easily scalable manufacturing alternative to biological systems (e.g. eggs or cell culture). Also, the final product is safer as the risks of contamination with infectious agents (e.g. Campylobacter, Salmonella, etc.), egg allergy in the population and increased incidence of Guillain–Barré syndrome are eliminated (6,7). Polypeptides are synthetically manufactured by American Peptide Company (USA) and 500ug of an equimolar mix will be vialed by Symbiosis (Scotland, UK) under sterile conditions in accordance with current Good Manufacturing Practice.

(2) 0.01M HCl and 0.01M NaOH suspension

FLU-v will be reconstituted in 0.01M HCl and later neutralised with the same volume of 0.01M NaOH. Both solutions are manufactured by Tiofarma (The Netherlands) under sterile conditions in accordance with current Good Manufacturing Practice.

(3) ISA-51

Montanide ISA-51 is a mixture of a highly purified mineral oil (Drakeol 6VR) and a surfactant (Mannide monooleate). When mixed with an aqueous phase in a 50/50 ratio, it renders a water in oil emulsion. This water in oil emulsion is used as vaccine adjuvant. It is manufactured by Seppic (Air Liquide, France) under sterile conditions in accordance with current Good Manufacturing Practice.

13.2 Summary of Findings from Non-Clinical Studies

(1) FLU-v

A series of pharmacology, safety and toxicology studies have been carried out in two animal species, the mouse and the rat. Pharmacology studies have demonstrated that FLU-v is able to generate both a cell mediated and humoral response in vaccinated animals and that the cell mediated response is enhanced when FLU-v is injected together with an adjuvant. A safety pharmacology study demonstrated that FLU-v had no biologically relevant effects on cardiovascular or respiratory parameters when administered subcutaneously alone or in combination with the adjuvant, in rats. Toxicology studies have demonstrated evidence of injection site responses and microscopic and macroscopic changes in animals receiving FLU-v and it is thought that these were likely to be due to the effects of the adjuvant used in the studies, ISA-51. In general, FLU-v was clinically well tolerated with no signs of systemic toxicity. FLU-v was also well tolerated in a local tolerance study in rabbits, with no local or systemic sign of reaction to treatment.

A safety pharmacology study in rats, under Good Laboratory Practice (GLP) principles, was carried out to measure potential side-effects of FLU-v on cardiovascular and respiratory parameters. FLU-v was administered subcutaneously alone or in combination with the adjuvant, ISA 51, to rats and was found to have no biologically relevant effects on cardiovascular or respiratory parameters.

Study	Species	N	Dose	Adj	Methods	Results
TR008	C57BL/6 mice	8/ group	2 doses of 2.5, 5 & 10nmol (27.6, 55.3 or 110.6ug) FLU-v **(tetrav)** or 5nmol NRP-v on Days 1 & 15	None	Measurement of IFN-γ	No dose response. Immune response higher with larger dosing volume.
TR009	C57BL/6 mice	8/ group	2 doses of 2.5, 5 & 10nmol (27.6, 55.3 or 110.6 ug) FLU-v **(tetrav)**	None ISA 51	Measurement of IFN-γ. Weight loss following intranasal flu challenge	Greatest IFN-γ response in lowest dose group without adjuvant. Lowest weight loss in lowest dose group with ISA 51.

TR010	C57BL/6-mice	10/ group	1 dose of 2.5nmol (27.6 ug) FLU-v **(tetrav)**, NRP-v, commercial or live flu virus	ISA 51 None for live virus	Weight loss following intranasal flu challenge	FLU-v gave equivalent protection to commercial vaccine. Sub-lethal dose of live virus gave best protection.
TR015	C57BL/6-Tg HLA-A*201 mice	8/ group	1 dose of 10nmol (110.6 ug) FLU-v **(tetrav)** or NRP-v	None ISA 51 ISA 720	Measurement of IFN-γ and IL-4 response.	Increased IFN-γ & human MHC-restricted Th1 immune response in FLU-v. Higher in both adjuvanted groups. No IL-4 response in any group.

Key messages from FLU-v non-clinical studies:

- FLU-v generates both cell mediated and Ab responses in mice and rats and cell-mediated responses are enhanced when FLU-v is given with adjuvant.
- Injection site responses and microscopic and macroscopic changes in animals receiving FLU-v likely to be due to the effects of the adjuvant, ISA 51.
- FLU-v was well tolerated with no signs of systemic toxicity.

(2) HCl/NaOH solution

Non-clinical studies on HCl/NaOH solution are irrelevant to the study endpoints (immunogenicity and safety).

(3) ISA-51

A number of potential adjuvants for use with the FLU-v vaccine were tested for their ability to enhance the immune response. In a number of experiments, the adjuvants ISA 51, Montanide® ISA 720 (ISA 720) and Quill saponin (QS 21) all boosted the cellular immune response to FLU-v, as measured by IFN- γ, although they had no effect on the antibody response.

ISA 51 in a final vaccine dose, prepared as instructed, is an emulsion of oil in water in which the micelles have an average diameter of approximately 4 μm. As particles between 2 and 5 μm are preferentially taken up by phagocytic cells and most antigen

presenting cells (e.g. dendritic cells) have phagocytic capacity, this peptide adjuvant combination increases the delivery of antigen to cells capable of triggering an immune response. In addition, these micelles contain, in themselves, both hydrophilic and hydrophobic environments and thus can accommodate, and efficiently carry, peptides of very different hydrophilic potentials. Therefore ISA 51 was chosen to test the ability of FLU-v as an adjuvant to enhance the immunogenicity of the FLU-v vaccine in clinical studies in humans.

13.3 Summary of Findings from Clinical Studies

(1) FLU-v

FLU-v is the only synthetic influenza vaccine that has been clinically shown to elicit a T cell response that (a) correlates with reduced viral shedding by 3 logs (r=-0.821; p=0.01) and morbidity scores by half (r=-0.786; p=0.02) and (b) recognizes widely divergent influenza strains[5]. For full information please refer to the IB.

(2) HCl/NaOH solution

Clinical studies on HCl/NaOH solution are irrelevant to the study endpoints (immunogenicity and safety).

(3) ISA-51

Please refer to the ISA-51 IB.

13.4 Summary of Known and Potential Risks and Benefits

(1) FLU-v

In study EUDRACT No. 2009-014716-35, all subjects reported AEs. 32 subjects reported mild AEs (16 FLU-v: 16 Placebo), 10 reported moderate AEs (6 FLU-v: 4 Placebo) and 1 subject had a severe adverse event (AE)(presyncope- Placebo). There were no serious adverse events (SAEs). Injection site AEs related to the investigational medicinal product (IMP) were frequently reported. Pain, swelling and erythema were most commonly reported by the FLU-v group within 24 hours post-vaccination. AEs related to the injection site corresponded with those reported for Montanide ISA-51-VG (Seppic), used as an adjuvant in the vaccine and Placebo preparations. 1 FLU-v subject had an

injection site lump removed 6 months following the study. 4 FLU-v (25%) and 3 Placebo (18.8%) subjects reported rhinorrhea which was considered IMP related. In general, changes in complete physical examination, clinical chemistry, haematology and urinalysis, vital signs, ECG, oral temperature or concomitant medications were not clinically significant and were resolved by the end of the study. 2 Placebo subjects had abnormal spirometry results at the Day 28 follow-up which were followed up post-study.

(2) HCl/NaOH solution

There are no potential risks and benefits expected from HCl/NaOH solution.

(3) ISA-51

Refer to 13.4 (1) FLU-v

13.5 Drug Packaging and Labelling

The study drug product will be supplied by SEEK. The study drug product will be manufactured and packaged in accordance with current Good Manufacturing Practice (GMP). Study drug supplies will be stored securely under the appropriate conditions at study sites according to the applicable local laws. Study drug product will be supplied to the study site after receipt of required documents in accordance with all applicable regulatory requirements and SEEK procedures. Labels will be in accordance with all applicable regulatory procedures.

13.6 Product Accountability

The trial site pharmacies will be responsible for recording the receipt of all vaccine supplies and for ensuring the supervision of the storage and allocation of these supplies. When a shipment is received, an assigned qualified person verifies the quantities received and the accompanying documentation and returns the acknowledgment of receipt to the Sponsor.

Drug administration will be recorded in the source documents, in the eCRFs and in the Drug Administration Record form. The latter includes the subject identification, quantity (volume) and date of administration. The containers from which the vaccine was administered to the subjects will be retained for dose confirmation.

At the end of the study, delivery records of study vaccine will be reconciled with used / unused stocks and appropriate forms will be completed, to verify that all used, unused or partially used supplies have been returned and that no study supplies remain in the Investigator's possession.

All unused vaccine supplies, partially used and empty containers will be returned to the Sponsor.

13.7 Study Drug Handling and Storage

Only subjects enrolled in the study may receive the study drug, in accordance with all applicable regulatory requirements.

Only authorized site staff may supply or administer the study drug. The study drug must be stored in a secure area with access limited to the Investigator and authorised staff only.

Authorised study site staff (e.g. a pharmacist) will verify and dispense each subject's study treatment supply and equipment. Subjects will receive their study dose under clinical supervision and observation.

13.8 Accountability of Study Supplies

All materials supplied are for use only in this clinical study and should not be used for any other purpose.

The Investigator is responsible for the investigational product accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, the Investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. The Investigator or designated site staff will document the amount of investigational product received from SEEK and the amount administered to subjects.

A Drug Dispensing Log must be kept current and will contain the following information:

- The identification of the subject to whom the drug was dispensed
- The date(s) of the drug dispensed to the subject.

The inventory must be available for inspection by the study monitor at any point during the study. Drug supplies, excluding empty containers, will either be collected at the end of the study by the study monitor or returned by the Investigator or designee to SEEK. When requested in writing by the Sponsor, unused drug supplies may be destroyed by the Investigator provided such disposition does not expose humans to risks from the drug. Records will be maintained by the Investigator of any such alternate disposition of the test drug. These records must show the identification and quantity of each unit disposed of, the method of destruction (taking into account the requirements of local law), and the person who disposed of the test substance. Such records must be submitted to the Sponsor.

13.9 Retention of Samples

It will be the responsibility of SEEK to ensure adequate samples of all study drug are retained in accordance with the regulatory guidelines.

14. Statistical Considerations

14.1 Endpoints

(1) Primary

- Cellular immunogenicity:

Cellular immune responses based on change from baseline (Day 0) in TH1 cytokines (IFN-γ, TNF-α, IL-2) on days 42 and 180.

- Co-Primary Safety

To evaluate the occurrence and intensity of all AEs (i.e. solicited and unsolicited, local and systemic) and their relationship to vaccination based on the AE diary card/questionnaire returned on day 21 and 42

To evaluate the occurrence of SAEs and their relationship to vaccination based on reports collected during the entire study period.

(2) Secondary

- Change from baseline (Day 0) in cellular immune TH2 cytokine responses (IL-4) on day 42 and 180
- Humoral immune responses (specific to FLU-v) on days 0, 42 and 180

(3) Exploratory

- Cellular immunogenicity:

Cellular immune responses will be evaluated by additional CMI assays on day 0, 42 and 180 in all groups, in a subset of subjects.

Effect of previous influenza vaccines on the immunogenicity of FLU-v will be assessed in a post-hoc analysis after stratifying the subjects' data based on whether they have ever been exposed to other influenza vaccines and if so, if it was in the previous 24 months or longer.

- Clinical efficacy

The efficacy of FLU-v vaccine in the reduction of the incidence of RT-PCR-confirmed influenza A and/or B infections and the reduction of symptom score among the confirmed influenza infection cases.

The relationship between clinical efficacy and immune responses will be explored.

Exploratory endpoints may be reported separately from the main clinical study report.

14.2 Estimated Sample Size

Vaccination with FLU-v generates a population of T cells that can recognise conserved influenza antigens on the surface of infected cells. On recognition, T cells are activated and secrete Th1 cytokines such as INF-γ, TNF-α, and IL2 and release granzyme and perforin. In addition, FLU-v specific non-neutralising antibodies may also bind to these antigens on the surface of infected cells activating complement and cytotoxic responses lead by Natural Killer (NK) cells. The T cell and antibody actions will result in the destruction of infected cells.

The objectives of this study are to determine the safety of the vaccine and assess which one of the two treatment groups, one dose adjuvanted vs two dose non-adjuvanted, induces the strongest immune response which is measured by quantifying the increase in Th1 and non-neutralising antibody responses after vaccination compared to baseline and corresponding placebo vaccination. The relationship between clinical efficacy and the immune response will also be explored.

Although a variety of immune parameters will be analysed, the minimum sample size has been determined on the basis of influenza-specific IFN-γ responses, one of the most important cell mediated immunity for influenza protection. The calculations were conducted based on results from the earlier Phase I trial, FLU-v-001 and full details are in a separate sample size document (13th March 2015).

The primary analysis objective of the study is to assess whether the active treatment (vaccine), in particular the adjuvanted version (administered as emulsion), produces a greater TH1 cytokine response than when given as a suspension (i.e. no adjuvant). It is

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

The proposed plan is to do a weighted allocation of patients to treatments, with twice as many patients allocated to the active treated group than to the placebo. Since there are two factors namely treatment and formulation, the study will follow a factorial design, so there are effectively 4 groups of patients. Three scenarios were used to derive the sample size and to investigate the power, of which the first (see below) was used as the model to drive power calculations. All calculations assumed that the final data will be log-transformed since the data is likely to be very skewed with some high values. Furthermore, analysing logged data allows estimates to be produced of fold-increases in cytokine responses, which are perhaps more readily interpretable. The assumptions are set out in Table 1.

Table 1 Scenario used for power calculations (Numbers in cells contain log_e response)

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

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

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

The most important test is that of the interaction between the effects of treatment and adding the adjuvant therapy. If this is non-significant then it is possible to conduct an overall test of the treatment effect. In particular, for Model 1, where there is an interaction, it is important to be able to detect this. Table 2 shows the total number of patients required for the study, in order to achieve 80% power for the various tests.

Table 2: Total sample size for study

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

The sample sizes in Table 2 do not allow for loss to follow up, assay problems or any other major protocol deviations. If a withdrawal rate of 15% is assumed, the figures need to be divided by 0.85. So, for example, 188 patients would need to be recruited to achieve 160 evaluable patients.

The final choice of sample size needs to be made considering the relative importance of the various tests and the likelihood of the proposed treatment differences occurring. As

a first step the interaction test is the most important: if there is no interaction the numbers required on each treatment to test for an overall treatment effect are relatively small. To test for interaction, equal allocation is more efficient than unequal allocation.

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

14.3 General considerations

1). General

A Statistical Analysis Plan (SAP) will be developed according to the CONSORT guidelines for the conduct and report of trials. This will provide full details of the analyses to be conducted, as well as methods of handling missing data. It will also provide table shells for the summary tables in the report. All subjects enrolled in the trial will be accounted for using a flowchart or table: this will provide numbers of subjects recruited, randomized and followed up as well as details of withdrawals. All subject data will be listed in data listings according to treatment group. Tables will be produced containing descriptive statistics, by treatment group, of the efficacy and safety variables, demographic variables, extent of exposure and other relevant outcomes. For continuous variables these will comprise mean (arithmetic or geometric as appropriate), median, standard deviation, minimum and maximum as well as the number of observations. For categorical variables counts and percentages will be shown. Graphs will be used where appropriate to complement the use of summary tables.

Any deviation from the original statistical plan will be described and justified in the study report.

2). Analysis populations

Three analysis populations will be defined as follows:

- Intention to treat (ITT): comprising all subjects randomized to receive treatment, irrespective of whether they receive any injections. The Full Analysis Set (FAS) will be derived from this population to include any subject with at least one set of post-vaccination data (i.e. at either Day 42 or Day 180)
- Per Protocol (PP): comprising those subjects who receive both injections, as per the randomisation schedule who provide a blood sample for ELISA determination of IFN-γ on Day 0, Day 42 and Day 180, and who do not have any major protocol violations regarding to eligibility criteria or study procedures
- Safety: comprising all subjects who received at least one injection

The primary efficacy analysis (TH1 cytokine response) will be conducted on the FAS, with the PP population providing supporting evidence of efficacy. All secondary analyses will be conducted on the ITT population; an additional analysis of the clinical efficacy of the vaccine based on flu symptom scores may also be conducted on the PP population if numbers allow.

Safety analyses will be conducted using the safety population.

All analysis populations will be determined at the end of the study when the database is complete before breaking the randomisation code in order to avoid any bias.

14.4 Methods of analysis

1) Primary outcome

The change from baseline in TH1 cytokines will be compared between the treatment groups using mixed models repeated measures analysis (MMRM), with the baseline (Day 0) level as a covariate. This takes into account the correlation between repeated observations made on the same individual. The model will include subject effects as random and fixed effect terms for age group (corresponding to the randomisation strata), time (day 42, 180), treatment (FLU-v or placebo), formulation (solution or emulsion) and the interaction between treatment and formulation. The terms comprising treatment formulation and their interaction together allow for the

evaluation of the overall difference between the four treatments. The significance of the interaction effect tests whether the effect of FLU-v relative to placebo differs according to whether it is administered as a solution or as an emulsion (i.e. adjuvanted). Additional terms may also include the interaction between time and each of the treatment/formulation terms to see whether the response over time differs. Since the data are likely to be skewed with some very high values, the data will be $\log_e$ transformed before analysis.

The analysis will provide estimates of the difference between groups at each time (Day 42 and 180) on the $\log_e$ scale which can be back-transformed to give ratios of geometric means. These therefore provided estimates of the fold-increases in response. The absolute means will give estimates of the ratio relative to baseline. All estimates of treatment differences will be accompanied by 95% confidence intervals. Summary tables and graphs will also be produced.

2) Secondary outcomes

The change from baseline in humoral response and TH2 cytokine (IL-4) will be handled in a similar manner to that described in 1).

3) Safety

For each of the following the incidence rate (number and percentage of subjects) will be presented for each vaccine group with associated 95% confidence intervals where appropriate:

(i) At least one AE (solicited and unsolicited);

(ii) At least one systemic AE (solicited and unsolicited);

(iii) With each individual solicited AE (local and systemic) and

(iv) Any AE (by preferred term, MedDRA- see below))

All adverse events will be coded according to the coding dictionary (MedDRA version 17.1 or higher) for System Organ Class (SOC) and Preferred Term (PT).

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

- SAEs

For each vaccine group the percentage of subjects with at least one SAE will be reported with 95% CI. SAEs and withdrawals due to AEs will be described in detail.

4) Clinical efficacy

For each vaccine group, the incidence rate of subjects with RT-PCR confirmed influenza A and/or B infection will be presented with 95% CI.

The severity of the influenza symptoms (based on the diary card with 1-3 score) among the laboratory-confirmed influenza cases will be summarised in tables of descriptive statistics but no formal statistical analysis will be performed as it is likely that the numbers of such subjects will be small (approximately 10-20% of subjects).

An exploratory analysis looking at the relationship between clinical efficacy (i.e infection rates and reductions in symptom scores) and the cellular and humoral responses will be performed. This will include graphical summaries of the data.

14.5 Handling of missing values

The MMRM method of analysis planned for the cellular and humoral responses is fairly robust to missing data under certain assumptions related to whether data are missing at random. The pattern of missing data in the four treatment arms will be assessed by plotting data according to withdrawal time to see whether the pattern of time differs accordingly. Sensitivity analysis will be conducted, for example using multiple imputation, and from the PP analysis to see whether the conclusions are similar. Note that missing data may arise through problems with assays: these are expected to be random occurrences and so the MMRM analysis should provide good estimates of

treatment effects even if some values are unusable. Note that every effort should be made to ensure that subjects are followed up and attend clinic visits in order to minimize the amount of missing data.

Sensitivity analyses may be conducted excluding any values which appear to be outliers to investigate their influence on results. If there is scientific evidence or explanation for one or more outliers, this will be presented and the results of the analyses will be discussed in the light of such explanations. However, the primary analysis will be that based on all data in the FAS. Note that by log transforming the data prior to analysis, the number of outliers may be reduced.

All summary tables will show the number of cases with missing data.

14. Multiplicity

Since there are several primary outcome variables, namely the four TH1 cytokines, there is a risk of inflation of the Type 1 error (i.e. obtaining false positive results). No formal adjustment of significance levels will be performed. However, the discussion of the results and the conclusion being drawn will be made in the light of the actual significance levels from the tests. In addition, the results of the analyses of each cytokine will be shown in a Forest plot to aid interpretation and to see whether results are consistent for all cytokines measured. A 2-fold increase in TH1 cytokine production would represent an important treatment effect, so this will also be taken into account when discussing the study results.

15. Ethics

15.1 Ethical Review Committees

The trial study protocol will be submitted to and approved by the Central Commission Research Involving Human Subjects in the Netherlands (Centrale Commissie Mensgebonden Onderzoek, CCMO).

The protocol will also be submitted to the Minister of Health, Welfare and Sport for the marginal review. If requested by medical ethical review committees and the Dutch health authority access may be given to medical records related to the program as stated by national law.

The following international conventions and declarations will be respected:

- Helsinki Declaration in its latest version;
- Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris on 12 January 1998;
- Beneficiaries should take into account to the opinions of the European Group of Advisers on the Ethical Implications of Biotechnology (1991 -1997) and the opinions of the European Group on Ethics in Science and New technologies (as from 1998);
- The Council of Europe additional Protocol to the European Convention on Human Rights and Biomedicine on Biomedical Research (CETS No.:195).

All relevant national and international provisions on data protection, subject's right and informed consent will be met, with the most up-to-date regulations. An ethics evaluation team is established for periodic discussion about relevant ethical issues, monitoring of ethical standards and a common statement on all ethical issues.

15.2 Ethical Conduct of the Study

The study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

All existing ethical and safety provisions of the Netherlands and of the EU will be strictly adhered. Relevant EU legislations include:

- The Charter of Fundamental Rights of the EU;
- Repeal of Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use;
- Commission Directive 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of such products;
- Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data and the revision of this directive once come into force;
- Council Directive 83/570/EEC of 26 October 1983 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC on the approximation laid down by law, regulation, or administrative action relating to proprietary medicinal products;
- Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions;

15.3 Description of Ethical Considerations Relating to the Trial

The trial is based entirely on voluntary participation.

- For each study visit, the subject will receive a travel compensation of approximately €15.00 and will only be given when the subject presents at each of the scheduled visits and when the corresponding travelling receipts are presented.
- No other compensation will be given.
- The vaccines will be provided by the sponsor, SEEK. All vaccines have been produced to GMP standard.

Procedural sampling i.e. nasal and tonsil swabs, blood sampling, will be performed according to standard clinical practice.
Over the course of the study, a total of 3 additional blood samples will be taken from all subjects.
All study procedures will be performed by experienced health care personnel and to standard clinical practice.
No serious risks are anticipated for the subjects.

15.4 Information and Consent

Prior to screening for the study each subject will be informed in detail about the study vaccine to be administered, and the nature of the clinical investigation with its risks and discomforts to be expected. The basic elements of informed consent as specified by the FDA (21 CFR 50.25) and ICH-GCP will be followed. The subjects will also be instructed that they are free to withdraw their consent and discontinue their participation in the study at any time without prejudice. Written consent will be obtained from each subject to be involved in the clinical trial by using the CCMO-approved Informed consent form (ICF) prior to the conduct of any study-related activity. A copy of the ICF will be submitted together with this protocol and must be approved by the CCMO prior to study commencement. Each subject will be given a copy of the written ICF, and each subject's chart will include the signed ICF for study participation. The original subject signed and dated ICFs will be maintained by the site for 15 years. Regulatory authorities may check the existence of the signed ICF in this central study folder if not having done so during the study.

Information regarding the inclusion lab tests, pregnancy test (for females), nasal and tonsil swabs and details of the medical register data/records usage will be provided in the SIS and ICF. Each Investigator involved will be responsible for the adequate storage of informed consents according to good clinical practice and national regulations.

Prior to the commencement, and where applicable, copies of ethical approvals/opinions/notifications by the CCMO and the Minister of Health, Welfare and Sport will be submitted to the European Commission.

15.5 Compensation for Injury

Appropriate liability insurance to cover against injury and damages arising from participation in the study will be provided by the Sponsor.

16. Provisional Timelines for the Study Phases

The duration of the trial, from visit 1 (first subject first visit) to study conclusion will be a maximum of 1 year.

The recruitment is estimated to take 1-4 months.

Prof. Eelko Hak is the working package leader on the clinical trial program (WP6) within the UNISEC Consortium and scientific coordinator of the trials conducted within this working package. Other members of his team include Marcy Heng Liu, postdoc in vaccinology, Eva van Doorn, PhD research fellow, and Denise Mailly, senior data manager at TCC.

The main tasks of this steering committee are to supervise the protocol for scientific content as well as the data management, statistics and report of the trial.

17. Administrative Aspects, Monitoring and Publication

17.1 Monitoring and Quality Assurance

(1) Study Monitoring

GCP stipulates that all clinical studies are adequately monitored. SEEK is responsible for ensuring the proper conduct of the study with regards to protocol adherence and the validity of the data recorded on the eCRFs. Subject confidentiality will be maintained throughout.

In accordance with applicable regulations, GCP, and SEEK procedures, SEEK study monitors or monitors appointed by SEEK (*e.g.* monitors employed by a Contract Research Organization) will contact the site prior to the subject enrolment to review the protocol and data collection procedures with site staff. In addition, the monitor will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrolment rate.

During these contacts, the monitor will:

- Check the progress of the study
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution.

This will be done to verify that the:

- Data are authentic, accurate, and complete
- Safety and rights of subjects are being protected
- Study is conducted in accordance with the currently approved protocol (and any amendments), ICH-GCP guidelines and all applicable regulatory requirements.

The Investigator will agree to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

At study closure, monitors will also conduct the following activities in conjunction with the Investigator or site staff as appropriate:

- Return of all study data to SEEK
- Ensure timely resolution of outstanding data queries
- Accountability, reconciliation and arrangements for unused investigational product
- Review of site study records for completeness.

(2) Quality Assurance

To ensure compliance with ICH-GCP and all applicable regulatory requirements, SEEK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the Investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

(3) Records Retention

SEEK will retain all original eCRFs, while the Investigator will retain a copy. Following closure of the study, the Investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (*e.g.* for an audit or inspection) and where feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems and staff. When permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (*e.g.* microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The Investigator must assure that all reproductions are legible and are a

true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the Investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

SEEK will inform the Investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or SEEK standards/procedures; otherwise, the retention period will default to 15 years.

The material to be stored shall include the following:

- Signed and dated copy of the final study protocol and any amendments
- Signed and dated Confidentiality Agreements
- Signed and dated letter of IEC approval, letter of constitution of the IEC and copies of any other correspondence relevant to the study with the IEC or regulatory authorities
- The IEC-approved subject information (SIS) and ICF
- Current curriculum vitae (signed and dated) of the Investigator and co-workers with major responsibilities in the trial
- Sample eCRF
- Signed subject ICF
- Subject screening, identification and enrolment logs
- Complete authorised drug accountability and reconciliation record
- Authorised signature and delegation list of investigator site staff
- Laboratory certificates and reference ranges (signed and dated)
- The End of Study Report

- Clinical raw data including the Medical Source Data Forms, all clinical laboratory report forms, subject eCRFs, drug accountability forms, and dispensing records, *etc*.

17.2 Amendments

All substantial amendments will be notified to the national regulatory and local medical ethical review committees in the country conducting the trial. Written approval of any substantial amendments will be obtained from such authorities before implementation. A 'substantial amendment' is defined as an amendment to the terms of the medical ethical review committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial;
- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of any intervention used in the trial.

Non-substantial amendments will not be notified to the authorities but will be recorded in the Trial Master File by the sponsor.

17.3 Annual Progress Report

The current study will last 1 year approximately (from first subject first visit to study conclusion). A summary of the progress of the trial in line with ICH Topic E2F will be submitted by SEEK to the relevant ethical review committees.

17.4 End of Study Report

SEEK will notify the relevant regulatory authorities and ethical review committees of the end of the study within the time frame requested. An End of Study Report will be

submitted to the relevant regulatory authorities within one year of the end of the trial. In case the study is ended prematurely, SEEK will notify the relevant regulatory authorities within the time frame requested, including the reasons for the premature termination.

Exploratory endpoints may be reported separately from the main clinical study report.

17.5 Public Disclosure and Publication Policy

In the grant proposal for the FP7-UNISEC project, the following statements are stated: (1) "The research results will be made suitable for (further) publications in international scientific journals. The partners in UNISEC will actively pursue publications, as these are read across Europe by the target groups of the dissemination of foreground." (2) "A "Plan for the use and dissemination of foreground intellectual property" will be prepared and maintained throughout the project duration and finalized at the end of the project. Prior to entering the European Commission contract, appropriate actions regarding knowledge management will be undertaken concerning the identification of -, access to - and exclusion of background intellectual property (IP), resulting in separate agreements. The coordinator will be responsible for the coordination of these actions and the development and maintenance of the "Plan for the use and dissemination of knowledge" and for ensuring compliance with articles II.29-30 of Part C of Annex II of the FP7 Contract. The policy of open access to scientific findings and publications resulting from publicly funded research, and the need to take into account the challenge of intellectual property (see Commission Recommendation C(2012) 4890), will be considered by the beneficiaries according to clause 39(on open access) of the FP7 model grant agreement."

18. References

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2. WHO. Influenza Fact Sheet 2009.

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4. Tan PT, Khan AM, August JT. Highly conserved influenza A sequences as T cell epitopes-based vaccine targets to address the viral variability. Hum Vaccine. 2011;7(4):402-9.

5. Boon AC, de Mutsert G, van Baarle D, Smith DJ, Lapedes AS, Fouchier RA et al. Recognition of homo- and heterosubtypic variants of influenza A viruses by human CD8+ T lymphocytes. J Immunol. 2004;172(4):2453-60.

6. Chua JV, Chen WH. Bench-to-bedside review: vaccine protection strategies during pandemic flu outbreaks. Crit Care. 2010;14(2):218.

7. Evans D, Cauchemez S, Hayden FG. "Prepandemic" immunization for novel influenza viruses, "swine flu" vaccine, Guillain-Barré syndrome, and the detection of rare severe adverse events. J Infect Dis. 2009; Aug 1;200(3):321-8.