Phase IIb Study of the Efficacy of FLU-v, a Broad Spectrum Influenza Vaccine in an H1N1 Influenza Healthy Human Challenge Model

Clinical Study Protocol

NCT Number: NCT03180801

EudraCT Number: 2016-002134-74

Last date of approval: 19th May 2017

CLINICAL STUDY PROTOCOL

Sponsor's study number: FLU-v-004

Phase IIb Study of the Efficacy of FLU-v, a Broad Spectrum Influenza Vaccine in an H1N1 Influenza Healthy Human Challenge Model

Version:	Final v3.0_18APR2017
Sponsor:	Gregory Stoloff, PepTcell Limited (t/a SEEK), Central Point, 45 Beech Street, London, EC27 8AD
Senior Clinical & Scientific leader	Dr Matthew J. Memoli Director, LID Clinical Studies Unit Laboratory of Infectious Diseases NIAID, NIH MSC 3203 33 North Drive Bethesda, MD 20892.
Principal Investigator:	Dr Balpreet Matharu Principal Investigator hVIVO Services Limited (hVIVO), Queen Mary BioEnterprises Innovation Centre, 42 New Road, London E1 2AX, UK. Dr Jeremy Dennison Principal Investigator Hammersmith Medicines Research Ltd Cumberland Avenue London, NW10 7EW
Study carried out at the facilities of:	hVIVO Services Limited, Queen Mary BioEnterprises Innovation Centre, 42 New Road, London. E1 2AX. UK. hVIVO Services Limited, Manchester Science Parks, Kilburn House, Lloyd Street North Manchester, M15 6SE. UK Hammersmith Medicines Research Ltd Cumberland Avenue London, NW10 7EW
Research Ethics Committee (REC) Ref:	16/NE/0227
EudraCT Number:	2016-002134-74

DETAILS OF AMENDMENTS			
Number	**Date**	**Type**	**Brief details**
1.1	25JUL2016	Amendment to the Protocol in response to the MHRA review dated 06JUL2016	• Clarification on requirement of competent authority approval prior to commencement of activities for Part B. • Addition of pregnant and breastfeeding women to the definition of vulnerable population. • Addition of pregnancy as criteria for subject discontinuation.
1.2	12AUG2016	Non-Substantial Amendment 02	• Version control and date updated throughout the document. • Changes to the group orders to harmonise with study schematic figure on page 48, at pages 32, 48, 56. • Addition of Principal Investigator approval section for Dr Dennison as PI at the HMR Pharmacy Site. • Section 1: Addition of PI details for Dr Dennison. • Section 7.3: Exclusion Criteria: **1)** Amendment to the smoking criteria **2)** Amendment to BMI criteria **3)** Amendment to the reference to vulnerable populations **4)** Amendment to criteria to prevent duplication • Section 9: Clarification on the conditions for end of Quarantine phase. • Table 9-2: Removal of LDH and Uric Acid from the Biochemistry panel as reflected in the Time & Events (T&E) schedule and throughout the protocol, as it does not add significant safety information in a healthy & young population. • Table 9-2: Removal of Biochemistry from Day 5 assessments as it does not add significant safety information. • Table 9-2: Clarification on the conduct of the safety blood analysis on the Follow-Ups (Day 35 and 63) will be conducted at PIs discretion. • Keynotes for T&E Schedule: 1) Clarifying the conduct of the FLU-PRO to be in line with the Protocol text. 2) #7: Clarifies that a viral challenge serology test may be repeated if required, if subjects fall out of the 90-day window. Additionally, clarifying that the screening

DETAILS OF AMENDMENTS			
			visit includes collection of PBMC and PAXgene from subjects invited to receive IMP before their vaccination visit. • Section 10 & 13: Clarification that HMR SOPs will be followed when appropriate. Section 13.2.3: Clarification that no protocol deviation will be recorded due to missing diary card data. Furthermore, the diary card data will be reviewed by a physician in retrospect and complete the information as accurately as possible by asking the subject about symptoms for the missing data sets. • Grammatical and formatting changes have been made throughout the document.
1.3	26Aug2016	Non-Substantial Amendment 03	• Section 5.3, page 32 - updated the order of group numbering • Section 9, page 47, updated the order of group numbering and removed the Figure 1 • Section 10, page 56, updated the order of group numbering • Exclusion criteria 1 - clarified exclusion criteria 1
2.0	26SEP2016	Substantial Amendment 01	• Section 7.2; Inclusion Criteria 9: Clarification to participant asthma history prior to entry into study. • Section 7.2; Inclusion Criteria 11: Clarification on the time-point for acquiring a subject's medical history from their GP. • Section 18.7.1; Informed consent procedure: Amended to include Investigator or delegate to conduct participant consenting. • Section 18.7.3; Information for General Practitioners: Amendment to the requirement for participant's medical history from their GP.
2.1	09DEC2016	Non-Substantial Amendment 04	• Protocol has been clarified throughout to allow variable entry to the Quarantine unit on Day -2 or Day -1. The changes are further reflected in the study design, Time & Events schedule and individual assessments where appropriate. • Sections 7.2 & 18.7.1: Clarification on the responsibilities of the Investigator or delegate in the consenting process for participants. • Keynotes to the Time & Events schedule & Section 14.1.3: Clarification on the collection of pre-vaccination PAXGene and PBMC samples in the Time & Events schedule.

DETAILS OF AMENDMENTS			
			• Section 18.7.3: Clarification on the confirmation of volunteers' medical history from their medical practitioner.
2.2	31JAN2017	Non-Substantial Amendment 05	• Correction of Section 7.2; Inclusion Criteria 9: Participant asthma history prior to entry into study to match the wording included in the MHRA and Research Ethics Committee -approved version 2.0 of the study protocol, which was incorrectly reverted to the original wording in protocol version 2.1.
3.0	18APR2017	Substantial Amendment 02	• Pages 1, 6 and 12 updated to reflect change in PI. • Section 6.3.1 and throughout updated to reflect exploratory endpoints may be reported separately from CSR. • Section 9.3.1 correction for consistency that subjects will be admitted to the Quarantine unit with a minimum 20 days not 21 days post second vaccination. • Section 9.3.1 and throughout a clarification that serology test and result must be available within 90 days of Day 0 inoculation • Section 13.1.2 inspection of the eyes removed from mandatory list in directed physical examination. • Section 13.2.2 language inserted to state how missing Flu-PRO data will be handled.

SPONSOR'S AUTHORISATION

This clinical trial protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational medicinal product (IMP), and with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki 1996[1] and the principles of the International Conference on the Harmonisation of Good Clinical Practice (ICH GCP)[2] as applicable to this clinical trial.

The Sponsor will provide an Investigator's Brochure (IB) and/or an Investigational Medicinal Product Dossier (IMPD), giving information about the chemistry, manufacture and controls, and the pharmacological and toxicological properties of the IMP, and summarising any clinical experience of the compound. Additional information regarding the IMP will be provided to the Principal Investigator (PI) when it becomes available. The Sponsor will also provide appropriate documentation relating to the clinical trial supplies, including certificates of analyses and confirmation of current Good Manufacturing Practice (GMP)[3] compliance in their manufacture.

A designated professional representative of the Sponsor will conduct visits to the investigational site(s) at appropriate intervals throughout the study, in order to verify adherence to the protocol and the accurate and complete recording of data in the source documents and drug inventory forms.

SIGNATURE OF THE SPONSOR'S REPRESENTATIVE

SIGN Gregory Stoloff **DATE** 19 APR 2017

Gregory Stoloff, PepTcell Limited (t/a SEEK)

PRINCIPAL INVESTIGATOR'S AGREEMENT

I have read the protocol, and agree to conduct the trial in accordance with the approved protocol, the Declaration of Helsinki 1996[1], the principles of ICH GCP[2], the current regulatory requirements as detailed in the Medicines for Human Use (Clinical Trial) Regulations (Statutory instrument [SI] 2004/1031)[4] and all subsequent amendments, the UK Data Protection Act 1998[5], any other applicable laws and guidance, and any Sponsor requirements.

I agree to conduct the procedures described in this protocol according to these guidelines and to appropriately direct and assist the staff under my control.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

SIGN _____ **DATE** 19 APR 2017

Dr Balpreet Matharu
Senior Investigator, hVIVO

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

SIGN J.M.T.J. D **DATE** 19 APR 2017

Dr Jeremy Dennison
Senior Research Physician, HMR

Note: The term 'Investigator' includes appropriately qualified persons to whom the PI has formally delegated his/her Investigator roles and responsibilities.

Contents

LIST OF TABLES

LIST OF APPENDICES

1 STUDY PERSONNEL CONTACT LIST

CONTACT	DETAILS
SPONSOR	PepTcell Limited (t/a SEEK)
SPONSOR'S REPRESENTATIVE	Gregory Stoloff, PepTcell Limited (t/a SEEK), Central Point, 45 Beech Street, London, EC27 8AD Phone/Mobile: +44 (0)207 153 6575 Email: gregory.stoloff@seekacure.com
SPONSOR'S MEDICAL EXPERT	Dr. Bryan Murray, Boyd Consultants Limited, 3a, Station Cottages, St Neots, Cambrigdeshire, PE19 1QF Phone: +44 (0)7833 204296 Email: bryan.murray@seekacure.com
SENIOR CLINICAL & SCIENTIFIC LEADER	Dr Matthew J. Memoli Director, LID Clinical Studies Unit Laboratory of Infectious Diseases NIAID, NIH MSC 3203 33 North Drive Bethesda, MD 20892. Phone: +1 301-443-5971 Email: memolim@niaid.nih.gov
PRINCIPAL INVESTIGATOR (PI) AND TRIAL SITE	Dr Balpreet Matharu hVIVO Services Limited, Queen Mary BioEnterprises Innovation Centre, 42 New Road, London E1 2AX. Phone: +44 (0) 20 7756 1396 Email: b.matharu@hvivo.com Dr Jeremy Dennison Principal Investigator Hammersmith Medicines Research Ltd Cumberland Avenue London, NW10 7EW Email: jdennison@hmrlondon.com

IMP CONTACT	Olga Pleguezuelos / Gregory Stoloff, SEEK, Central Point, 45 Beech Street, London, EC27 8AD Phone/Mobile: +44 (0) 207 153 6575 Email: gregory.stoloff@seekacure.com Email: olga.pleguezuelos@seekacure.com
PHARMACY AND ADMINISTRATION	hVIVO External Pharmacy Vendor, Hammersmith Medicines Research Ltd, Cumberland Avenue, London, NW10 7EW
OTHER PARTIES	The Investigator Site File (ISF)/Trial Master File (TMF) contains details of third party contractors, sub-investigators, laboratories, data management suppliers, statisticians and all other service providers.

2 PROTOCOL SYNOPSIS

SYNOPSIS	
TITLE	Phase IIb Study of the Efficacy of FLU-v, a Broad Spectrum Influenza Vaccine in an H1N1 Influenza Healthy Human Challenge Model
SPONSOR	PepTcell Limited (t/a SEEK)
SPONSOR PROTOCOL NO.	FLU-v-004
PHASE	Phase IIb
INDICATION	Prophylaxis of Influenza
EUDRACT NUMBER	2016-002134-74
STUDY DESIGN	A randomized, double-blind, placebo-controlled efficacy and safety trial of FLU-v administered as 2 vaccinations or 1 vaccination vs placebo prior to intranasal challenge with the Influenza A 2009 H1N1 human virus. In this study, the main efficacy comparisons are for each of the FLU-v groups to placebo. The study will also compare the two FLU-v dosing arms but will only have power to detect a significant difference in Mild to Moderate Influenza Disease (MMID) rates between the FLU-v groups and placebo and not against each other. Assuming significant efficacy of at least one dose of FLU-v against placebo is demonstrated in the current protocol (Part A), when the primary efficacy endpoint and key safety results are available, the protocol may be amended to include a second part (Part B) which will test the optimal FLU-v dose from Part A (in terms of efficacy and safety) versus current standard of care seasonal influenza vaccine. Details of Part B will be determined and documented in a future protocol amendment following the availability of the unblinded key efficacy and safety results from Part A. Approval will be sought via a substantial amendment from the competent authority before part B can commence.
SAMPLE SIZE	Up to 123 participants inoculated with the challenge virus
RANDOMISATION AND BLINDING	Randomisation will occur on day -43. Part A FLU-v 1 or 2 doses and placebo treatment arms will be double-blinded and randomised 1:1:1.

SYNOPSIS	
	Randomisation numbers will follow a 3 digit format e.g., 101-1NN. A copy of the randomisation code list will be sent to the unblinded pharmacist preparing the vaccines, so that vaccine or placebo doses can be prepared for each subject as appropriate. A designated unblinded statistician will be responsible for the computer generated randomisation schedule. Sealed copies of the randomisation code will be stored in a secure place. Following database lock, on receipt of authorisation from the Sponsor, a copy of the randomisation code list will be sent to the Study Statistician to conduct study unblinding. Part B Assuming significant efficacy of at least one dose of FLU-v against placebo is demonstrated in Part A, Part B will be conducted and test the optimal FLU-v dose from Part A (in terms of efficacy and safety) versus a current standard of care seasonal influenza vaccine. Details of Part B randomisation and blinding will be determined and documented in a future protocol amendment following the availability of the unblinded key efficacy and safety results from Part A. Approval will be sought via a substantial amendment from the competent authority before part B can commence.
CHALLENGE VIRUS	Live, recombinantly derived A/Ca/04/2009-like flu virus
CHALLENGE VIRUS ROUTE	Intranasal (both nostrils)
IMP SUPPLIER	PepTcell Limited (t/a SEEK)
IMP	FLU-v
IMP DOSE(S)	500 micrograms suspended in 0.5ml volume of water and adjuvant
PHARMACEUTICAL FORM	Lyophilised powder to be suspended for injection
IMP ROUTE	Subcutaneous Injection
CONTROL COMPOUND	Placebo (adjuvant Montanide ISA-51)
STUDY POPULATION	Healthy males and females aged 18 to 55 years of age
REPLACEMENT POLICY	Subjects will not be replaced post-influenza challenge on Day 0
PRIMARY OBJECTIVE	To determine the effect of FLU-v on reducing the incidence of Mild to Moderate Influenza Disease (MMID) defined as detectable viral shedding plus at least one symptom of influenza

SYNOPSIS	
PRIMARY ENDPOINT	Incidence of MMID
SECONDARY OBJECTIVES	To determine the overall effect of FLU-v on measurements of disease severity
SECONDARY ENDPOINTS	• Viral shedding duration • Viral shedding quantitation • Symptom duration • Total number of symptoms experienced • Symptom severity score as measured by FLU-PRO Symptom Questionnaire • Incidence of vaccine related adverse events (diary card for 21 days after each vaccine)
EXPLORATORY OBJECTIVES	To explore the immunological responses to FLU-v

EXPLORATORY ENDPOINTS	• Immunogenicity of FLU-v as measured by
	  ○ B-cell and T-cell responses to FLU-v both after vaccination as well as influenza challenge
	  ○ Immunophenotyping
	• Broadness of protection as measured by culturing PBMCs pre-influenza virus inoculation in study with PBMCs (same subject) infected with influenza and assess killing of infected cells by PBMCs from vaccinated subjects and use sera containing non-neutralising antibodies and test killing of human influenza infected cells by complement activation or ADCC assays

SAFETY OBJECTIVES	To determine the safety and tolerability of FLU-v after vaccination in healthy subjects subsequently challenged with Influenza A 2009 H1N1 human challenge virus
SAFETY ENDPOINTS	• Incidence of treatment-emergent AEs (TEAE), severity, seriousness and causality • Absolute values and change from baseline in routine clinical and laboratory parameters • Vaccine related physical examination findings • Concomitant medications
STUDY DESIGN	• Study-specific screening (SSS) within 90 days prior to challenge • Vaccination on Day - 43 • Vaccination on Day - 22

SYNOPSIS	
	• Entry to quarantine on Day -2 or Day -1 • Challenge Virus inoculation on Day 0 • Subjects will be resident in the Quarantine Unit for a total of approximately 10 days (from Day -2 or Day -1 to Day 7) • End of quarantine phase is achieved on Day 7 or until two negative diagnostic tests for influenza on two separate but consecutive days and are otherwise clinically stable. • Follow-up visits: Day 35 (+/- 3) and Day 63 (+/- 5) days.
PROCEDURES AND ASSESSMENTS	During the study the following assessments and procedures will be performed: • Written informed consent • Eligibility criteria • Height, body weight, body mass index (BMI), • Medical history • Demographics • Prior medications • Randomisation Dosing with FLU-v-004 or placebo • Challenge Virus inoculation • Complete physical examination • Directed physical examination • Vital signs (blood pressure [BP], respiratory rate [RR], heart rate [HR], peripheral arterial oxygen saturation [SpO_2]) • Tympanic temperature • 12-lead ECG • Spirometry • FLU-PRO Symptom Questionnaire • Bloods: o Challenge Virus serology o Haematology o Biochemistry o Cardiac enzymes o Hepatitis A, B and C and human immunodeficiency virus (HIV) serology o Serum follicle stimulating hormone (FSH) (post-menopausal females) o Beta-human chorionic gonadotropin (β-hCG) pregnancy test (females). o Whole and serum blood collection for immune response analysis • Nasopharyngeal swab o Respiratory virus screen o Viral load ($TCID_{50}$ and/or RT-qPCR).

SYNOPSIS	

	• Breath alcohol test
	• Urine:
	  ○ Urinalysis (dipstick)
	  ○ Pregnancy test
	  ○ Drugs of abuse.
	• Adverse events
	• Concomitant medications

END OF STUDY	The last subject's last scheduled visit (LSLV).
EXPECTED DURATION OF SUBJECT PARTICIPATION	Approximately **153** days or **22** weeks from screening to LSLV
OVERALL DURATION OF CLINICAL PHASE	Approximately 18 months
STATISTICS	The hypothesis is that vaccination with 1 dose or two doses of FLU-v + adjuvant will significantly reduce MMID when compared to individuals vaccinated with placebo. The primary objective of this study is to examine whether there is a difference in the MMID post inoculation rates between either of the 2 FLU-v arms and placebo. A sample size of 41 subjects per arm (total 123) will provide 80% power to detect a difference in MMID rates of 0.70 versus 0.40 (ie difference of 0.30) using a one sided 0.05 significance level. In this study, the main comparisons are for each of the FLU-v dose groups to placebo. The study will also compare the two FLU-v dosing arms but the study will not have adequate power to detect a significant difference in MMID rates at the predicted treatment rates. This study does not control for multiple comparisons since we are trying to maintain a small sample size but have high power to detect the predefined primary objective of efficacy versus placebo when FLU-v is truly beneficial. All comparisons will be made at the one sided 0.05 significance level. FLU-v Statistical Analysis Plan MMID rates in the 2 FLU-v groups and placebo group will be compared using a Fishers exact test. 95% confidence intervals around the difference in rates between the FLU-v groups will be presented. Adverse event rates between groups will also be compared using Fishers exact test. The secondary endpoints will be examined by calculating the median and interquartile range

SYNOPSIS	
	and then performing a two-sided Wilcoxon Rank Sum Test at the 0.05 significance level.
STOPPING CRITERIA	The study will be terminated if: • One or more subjects experience a vaccine related SAE or two or more subjects experience severe or clinically significant AEs within same organ class and confirmed by repeat sample if considered to be at least possibly related to the FLU-v vaccine. • One or more subjects experience any severe clinically significant illness from influenza challenge.

3 GLOSSARY OF ABBREVIATIONS

Abbreviation	Term
ABPI	Association of British Pharmaceutical Industries
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AP	Analytical Plan
API	Active Pharmaceutical Ingredients
APTT	Activated partial thromboplastin time
APTTR	Activated partial thromboplastin time ratio
AR	Adverse reaction
ASR	Annual Safety Report
AST	Aspartate aminotransferase
ATS	American Thoracic Society
AUC	Area under (the) curve
BD	Twice daily
BDRM	Blinded Data Review Meeting
β-hCG	Beta human chorionic gonadotropin
BMI	Body mass index
bpm	Beats per minute
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use
CK	Creatine kinase
COPD	Chronic obstructive pulmonary disease
CPE	Complete Physical Examination
CRF	Case report form
CRP	C-reactive protein
CSR	Clinical study report
CTA	Clinical Trial Agreement
CTL	Cytotoxic T lymphocytes
DMID	Division of Microbiology and Infectious Diseases
DMP	Data Management Plan
DPE	Directed Physical Examination
DSUR	Development Safety Update Report
DTA	Data Transfer Agreement
ECG	Electrocardiogram
eCRF	Electronic case report form
ECSC	European Coal and Steel Community
EMA	European Medicines Agency
ERS	European Respiratory Society
ESI	Event of Special Interest

ESR	Erythrocyte sedimentation rate
EU	European Union
FDA	Food & Drug Administration
FEV_1	Forced expiratory volume in 1 second
FI	Febrile illness
Fmoc	Fluorenylmethoxycarbonyl
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
HA	Haemagglutinin
HAI	Haemagglutinin inhibition
HBsAg	Hepatitis B surface antigen
HepA IgM	Hepatitis A antibody Immunoglobulin M
HCAb	Hepatitis C antibodies
HIV	Human Immunodeficiency Virus
HR	Heart rate
HRT	Hormone replacement therapy
HVC	Human Viral Challenge
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICF	Informed consent form
Ig	Immunoglobulin
IL	Interleukin
ILI	Influenza-like illness
IMP	Investigational medicinal product
IMPD	Investigational Medicinal Product Dossier
INR	International normalised ratio
IP	Induced protein
ISF	Investigator Site File
ITT	Intention-to-treat
ITT-I	Intention-to-treat- Infected
IUD	Intra-uterine device
IUS	Intra-uterine system
kg	Kilogram
KC	Keratinocyte-derived cytokine
LDH	Lactate dehydrogenase
LRT	Lower respiratory tract
LSLV	Last subject last (scheduled) visit

MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCP	Monocyte chemotactic protein
MCS	Microscopy, sensitivity and culture
MCV	Mean corpuscular volume
MDA	Melanoma differentiation-associated
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MIP	Macrophage inflammatory protein
mm Hg	Millimetres of mercury
MMID	Mild to Moderate Influenza Disease
MTD	Maximum tolerated dose
NA	Neuraminidase
NPS	Nasopharyngeal Swab
OTC	Over-the-counter
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHQ	Patient Health Questionnaire
PI	Principal Investigator
PP	Per protocol
PT	Prothrombin time
QA	Quality assurance
QC	Quality control
QIV	Quadrivalent Influenza Vaccine
QP	Qualified Person
RANTES	Regulated on Activation, Normal T Cell Expressed and Secreted
RBC	Red blood cell
REC	Research Ethics Committee
RIG	Retinoic acid inducible gene
RNA	Ribonucleic acid
RR	Respiratory rate
RVAT	Rapid virus antigen test
RT-qPCR	Reverse Transcription quantitative PCR
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAR	Serious adverse drug reaction
SI	Statutory Instrument
SME	Sponsor's Medical Expert
SOC	System, Organ, Class
SOP	Standard operating procedure
SpO_2	Peripheral arterial oxygen saturation
SSS	Study-specific screening

SUSAR	Suspected unexpected serious adverse reaction
SVS	Systemic viral symptoms
$TCID_{50}$	Tissue culture infective dose (50%)
TEAE	Treatment emergent adverse event
TLR	Toll-like receptor
TMF	Trial Master File
TNF	Tumour necrosis factor
TSH	Thyroid stimulating hormone
UK	United Kingdom
URT	Upper respiratory tract
US/USA	United States of America
USM	Urgent safety measures
VCU	Viral Challenge Unit
VIS	Volunteer information sheet
WBC	White blood cell
WHO	World Health Organization

4 DEFINITIONS

4.1 General

TERM	hVIVO DEFINITION
Completion (of a subject's participation in the study)	A subject is considered to have completed the study after his/her attendance at the last planned study visit on Day 63.
Enrolment (of a subject into the study)	A subject will be considered to be 'enrolled' into the study once he/she has signed the consent form.
Events of Special Interest (ESI)	Significant adverse events that are judged to be of special interest in a clinical trial because of their clinical importance, known class effects, or based on preclinical signals (e.g., nasal irritation).
Human Viral Challenge (HVC) study	A study to determine how a virus and the human body interact. Subjects are isolated in a hVIVO Quarantine Unit and infected (challenged) with a respiratory virus.
hVIVO	hVIVO acting as Site (including Manchester Screening, Whitechapel Screening and/or Whitechapel Quarantine)
Infectious titre	The titre of virus inoculum producing viral infection in a subject.
Investigator	The term 'Investigator' includes appropriately qualified persons to whom the PI has formally delegated his/her Investigator roles and responsibilities.
Quarantine group	A group of subjects who are admitted to and are resident in the Quarantine Unit for a particular quarantine period.
Quarantine period	The period of time when clinical trial subjects are isolated in the Quarantine Unit during a HVC study.
Quarantine Unit	A hVIVO isolation facility for HVC studies.
Randomisation (of a subject into the study)	A subject will be considered to be 'randomised' into the study once he/she has been allocated a randomisation number and dosed with study drug.
Randomisation number	The number allocated to a subject at randomisation.
Screening	A process of active consideration of potential subjects for enrolment in a trial.
Sponsor	Clinical Trial Sponsor
Subject number	The unique number assigned to a volunteer on the hVIVO volunteer database, which is used to identify the volunteer prior to randomisation.
FLU-PRO survey	A document in which the subject records his/her assessment of symptoms related to the study.
Titre	The term 'titre' applies to the quantity or concentration of virus inoculum or antibody.
Treatment group	A group of individuals who either receive the study drug, or the placebo or combinations of the aforementioned

TERM	hVIVO DEFINITION
Viral Challenge (or Challenge)	The inoculation of a subject with virus inoculum. By definition, the day of Viral Challenge is Day 0.

4.2 Study definitions of infection and illness

Illness/infection type	Definition
Viral shedding	A positive molecular test for influenza
MMID (Mild to moderate influenza induced disease)	Detectable viral shedding plus at least one of the following symptoms or signs or tests determined by the investigator to be related to influenza: • Arthralgia • Chills • Conjunctivitis • Coryza • Diarrhoea • Dry Cough • Dyspnea/Shortness of Breath • Fatigue/Tiredness • Fever (>38.0°C) • Headache • Myalgia • Nausea • Oxygen Saturation Decrease by ≥3% from baseline • Productive Cough • Rhinorrhea • Sore Throat • Sweats

5 BACKGROUND AND INTRODUCTION

5.1 Information about Influenza

The high morbidity and mortality associated with both pandemic and seasonal influenza and the threat of new pandemic strains emerging continues to keep influenza at the forefront of infectious disease and public health research. Mean annual estimates of influenza deaths due to seasonal influenza alone attributes up to 49,000 deaths in the US and 250,000 to 500,000 deaths in industrialized countries to influenza [44-46]. Pandemics can have an even more devastating effect. Public health agencies must continue to be prepared by attempting to reduce the public health impact of this important virus.

Currently, influenza vaccination is the cornerstone of prophylaxis and the most effective method available now to reduce the impact of influenza on the world's population each year. Current vaccines target the major surface protein, hemagglutinin (HA), and are standardized as stimulating anti-HA antibodies as the primary correlate of protection. Measurements of these serum antibodies to this surface protein have become the gold standard for evaluating vaccines. The Federal Drug Administration (FDA) and the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) both define "protective titre" as a haemagglutination inhibition (HAI) titre of $\geq$ 40[44].

In recent years epidemiologic evaluations however, have questioned if such antibody titres are the sole correlate of protection. Since the 2009 pandemic more questions have arisen regarding the performance of these types of vaccines, and epidemiologic data from as recently as the 2013 influenza season suggest that current seasonal vaccines targeting the HA are greatly underperforming especially in those that really need protection such as the elderly, young, and infirm[46].

Novel correlates of protection are being explored and the role of other aspects of the immune system, such as cellular immunity, has come to the forefront. Influenza specific cytotoxic T lymphocytes (CTL) have been shown to be involved in killing and removing cells that are infected with influenza viruses [49-54]. These cells may play an important role in the clearance of influenza virus both before and after clinical symptoms appear. Influenza has been shown to stimulate CTL responses not only specific to the surface proteins which may be subtype specific, but also to the internal proteins PB1, PB2, PA, NP, and M1[55-58], which may be more broad spectrum, in action since these proteins are not as variable as viral surface proteins.

Influenza virus is a highly variable virus. Most of its variability comes from the proteins on its surface, NA and HA. Current vaccines use these highly immunogenic proteins to induce production of neutralising antibodies, however because these proteins change from strain to strain, the antibody response is only efficient against the strain present in the vaccine and if the circulating strain is different to that in the deployed vaccine, it will not be protective.

5.2 Information about FLU- v

FLU-v, a novel peptide vaccine, aims to provide a broad-spectrum response and for that it uses as antigens viral proteins that are conserved throughout the different strains. These proteins are not located on the surface of the influenza virus like NA and HA are, but internally. These antigens are not capable of producing neutralising antibodies but can induce cytotoxic T cell responses and non-neutralising antibodies. When cells are infected with influenza, these internal viral antigens are presented on the surface of infected cells. Vaccination with FLU-v generates a population of T cells and non-neutralising antibodies that can recognise these 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. Non-neutralising antibodies will activate complement and/or induce cytotoxic responses led by Natural Killer cells (NK cells). These T cells and antibody actions will result in the destruction of infected cells.

5.2.1 Product description

FLU-v is a lyophilised product composed of four short peptidic active pharmaceutical ingredients (APIs) (FLU-5, FLU-7, FLU-8N and FLU-10). The APIs are manufactured by fluorenylmethoxycarbonyl (Fmoc) chemistry before reconstituting them in 50% acetic acid and mixed to prepare an equimolar solution of the four peptides. Glass vials are then filled with the solution under sterile conditions. Water and acetic acid are removed from the preparation by lyophilisation. The end product is a white to off white lyophilised solid that will need reconstitution prior administration by injection.

5.2.2 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.

Key messages from FLU-v non-clinical studies:

- FLU-v generates both cell mediated and antibody 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.

In animal models vaccination with FLU-v has been shown to reduce viral titre and severity of illness (please refer to section 5.2.2 in the IB v4.0 7th June 2016).

(2) 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 four μm. As particles between two 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.

5.2.2.1 Pharmacokinetics and metabolism

A number of ex vivo pharmacology studies have been conducted in transgenic and non-transgenic mice demonstrating the generation of an effective immune response following subcutaneous administration of FLU-v. Most of the studies that were conducted demonstrated the ability of FLU-v to generate a cell mediated Th1 type response to the influenza virus. In contrast, FLU-v did not generate a Th2 type response in any of the studies conducted. FLU-v vaccination was shown to generate both human and murine MHC class I specific immunity to influenza virus in a number of studies. Humoral immunity was investigated by measuring the levels of total IgG and IgG2a responses to each FLU-v peptides. A weak response to each peptide was detected and this was not dose dependent in all but one of the studies, nor was it boosted by adjuvant. One study demonstrated weak IgG and IgG2a responses that were inversely dose dependent. When FLU-v was administered subcutaneously alone or in combination with the adjuvant, ISA 51, to rats, it was found to have no biologically relevant effects on cardiovascular or respiratory parameters. ISA 51 was chosen to test the ability of

an adjuvant to enhance the immunogenicity of the FLU-v vaccine in clinical studies in humans because this peptide adjuvant combination increases the delivery of antigen to cells capable of triggering an immune response.

Standard pharmacokinetic studies were not performed as it was not possible to measure an intact test substance in the systemic circulation since the FLU-v peptides are processed rapidly by the immune system. Therefore, the cellular immune response was seen as a surrogate measure of exposure and an indication of the biological response to treatment with the peptides.

5.2.2.2 Toxicology

In repeat dose toxicity studies conducted in mice and rats to investigate the systemic toxicity potential and immunogenicity of FLU-v, all the studies demonstrated that following subcutaneous vaccination with FLU-v alone or with the adjuvant, ISA 51, at doses of up to 553 µg, there was no effect on the following parameters: bodyweight gain, food consumption, organ weight or ophthalmology. In one repeat dose toxicity study in the mouse, where FLU-v was administered alone, there was evidence of a cell mediated response (as measured by increase in IFN-γ) but no Ab response to FLU-v at doses of up to 553 µg. In other repeat dose toxicity studies, FLU-v, in combination with adjuvant, showed an increase in cell mediated response with limited evidence of a weak Ab response which was observed in a small number of animals immunised with 11.1 µg or more of peptide. However, due to the small number of responders, the weak response and the absence of a dose relationship or peak effect it was not possible to conclude that FLU-v with adjuvant elicited a humoral response in mice when administered subcutaneously on Days 1 and 15. All studies demonstrated evidence of an injection site response in animals receiving FLU-v either with or without adjuvant and adjuvant alone, although the response was more marked in those groups receiving both FLU-v and adjuvant compared to FLU-v alone. There were gross observations at the parenteral sites included inflammation, cystic spaces, fibrosis and necrosis and vacuolation and increase in paracortex cellularity was observed in the lymph nodes and the lungs. These effects were likely to be due to the adjuvant used in the studies, ISA 51, which contains a mixture of mineral oil and surfactant and is known to cause inflammation at the injection site. For more information on ISA 51, see ‘Adverse Reactions’ above and Investigator’s Brochure for Montanide® ISA 51, 2008. Treatment with FLU-v up to four doses of 263 µg/occasion or up to two doses of 553 µg/occasion with or without adjuvant, was well tolerated with no signs of systemic toxicity.

5.2.3 Clinical studies

5.2.3.1 Pharmacology and Safety

Two Phase I studies of FLU-v have completed and enrolled 82 subjects of whom 56 have been exposed to FLU-v.

Phase I study (FLU-v-001)

FLU-v-001 was a first-in-man, single-centre, randomised, double-blinded, placebo-controlled study. A total of 48 healthy adult males were to be enrolled, 51 subjects were enrolled. There were 2 groups with 24 subjects in each group. The first group received 250µg (Dose Level 1), and the second group received 500µg (Dose Level 2) FLU-v. 48 subjects were planned to be enrolled but 51 subjects were enrolled due to a dosing error with 3 of the subjects.

The study demonstrated a good safety profile as well as similar good immune responses in humans as those seen in animal models. No safety or tolerability concerns were identified at the doses administered to the subjects in the study. No safety or tolerability concerns were identified following administration of the adjuvant to the subjects in this clinical study.

Pharmacodynamics results:

FLU-v was shown to be immunogenic in humans, as measured by ex vivo INF-γ production. Strong IFN-γ responses were seen in six of nine subjects in the 250µg adjuvanted cohort and in eight of ten subjects in the 500µg adjuvanted cohort, indicating a potential dose-response to the vaccine. There were no significant responses in the unadjuvanted cohorts. No immunoglobulin production was seen in any of the vaccinated subjects. (See Section 6.3 in the IB v4.0 7th June 2016).

Safety results:

There were no deaths during the study. There was one SAE of appendicitis considered not to be related to study treatment.

Three subjects were excluded from the protocol and the total treated analysis sets as the dose of FLU-v they received could not be determined. A total of 129 TEAEs (treatment emergent adverse events) were reported by 36 subjects receiving 250 or 500 µg FLU-v and 20 TEAEs were reported by 8 subjects receiving placebo. There was no apparent increase in the incidence of TEAEs at Dose Level 2 (500 µg) compared with Dose Level 1 (250 µg).

The majority of TEAEs were reported in the system organ class of general disorders and administration site conditions; 15 TEAEs reported by subjects receiving 250 µg FLU-v without adjuvant, 29 TEAEs reported by subjects receiving 250 µg FLU-v with adjuvant, 15 TEAEs reported by subjects receiving 500 µg FLU-v without adjuvant and 34 TEAEs reported by subject receiving 500 µg FLU-v with adjuvant. Injection site pain was the most frequently reported TEAE and subjects receiving FLU-v with adjuvant reported more events of injection site pain (approximately 2-fold difference) than subjects receiving FLU-v without adjuvant. Within this system organ class, injection site pruritus (6 events), injection site swelling (6 events) and pyrexia (1 event) were only reported by subjects receiving adjuvant.

Adverse events of headache, malaise, fatigue, myalgia, nausea, vomiting diarrhoea, chills fever and other (as reported by the subject) were assessed for systemic tolerability. Neither vomiting nor chills were reported by subjects during the trial. The most commonly reported TEAEs by subjects receiving FLU-v were headache (11 events), fatigue (9 events), malaise

(7 events), myalgia (7 events) and nausea (4 events). One event each of diarrhoea and pyrexia were reported.

Assessment of local tolerability (itching, pain and tenderness) indicated that the majority of reactions were reported at the 0.08 h (5 minute) post dose time point. Most reactions were reported as mild and there were no reported severe reactions.

There were no clinically significant findings in either the individual or mean laboratory measurements, vital signs or ECG measurements.

In conclusion, no safety or tolerability concerns were identified at the doses administered (250µg and 500µg FLU-v) to the subjects in the study. No safety or tolerability concerns were identified following administration of the adjuvant to the subjects in the study.

The FLU-v vaccine candidate was demonstrated to be immunogenic in humans, as measured by ex vivo INF-γ production.

Phase Ib study (FLU-v-002)

FLU-v-002 was a single-centre, randomised double-blind, placebo-controlled, Phase Ib trial to evaluate the safety, tolerability and protective efficacy of the influenza vaccine candidate, FLU-v, in an influenza challenge model. 32 subjects were vaccinated, 28 were quarantined and 27 completed the study. 16 subjects FLU-v 500 µg + adjuvant and the other 16 received placebo + adjuvant. Subjects were healthy males aged 18 to 45 years, who had not had seasonal influenza vaccination since 2006 or a known influenza infection in the 12 months prior to study entry, who were serosusceptible to Influenza A/Wisconsin/67/2005 (H3N2) virus at screening, and in good health as determined by past medical history, physical examination, vital signs, electrocardiogram (ECG), and laboratory tests at screening.

Efficacy

The study demonstrated similar good immune responses to the previous Phase I study following FLU-v treatment (see Section 6.3.2 in the IB v4.0 7th June 2016). The dose of Challenge Virus used in the study was intended to cause mild symptoms in volunteers, and an attack rate of approximately 70% to 80% in the placebo group, which was not achieved.

It is possible that in this study, the low rates of infectivity in the placebo group and the resulting apparent lack of efficacy of FLU-v may have been related to pre-existing multi-strain (i.e., heterologous) cellular immunity arising from previous asymptomatic exposure of the population to viral infection during a recent Influenza pandemic between June 2009 and February 2010. In addition, pre-existing T cells responding to Influenza internal proteins may have been associated with lower virus shedding and a less severe illness.

As a result of these potential unforeseen circumstances, there was no statistical evidence that FLU-v conferred clinical protection to subjects or reduced illness subsequent to being challenged with intranasal Influenza A/Wisconsin/67/2005 (H3N2). However, when looking at the subjects in each group that did not have a pre-existing T cell response to the challenge

strain, the subjects in the control group were more symptomatic and 'ill' compared to the subjects in the vaccinated group who did not become significantly symptomatic. (see Section page 12 in the IB v4.0 7th June 2016).

Live virus challenge studies can play a pivotal role in developing novel vaccine such as this one. Through a well-controlled challenge study, the time and number of patients needed to evaluate such a vaccine can be greatly decreased, and a more thorough analysis of the immune response to such a vaccine in the presence of infection can be observed.

Safety Results:

16 FLU-v and 16 Placebo subjects were included in the safety analysis.

Adverse events:

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 and most were mild. 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. One FLU-v subject had an injection site lump removed 6 months following the study. It is likely that this was related to the site and volume injected which has been amended for this study. (see IB section 6.3.2)"

FLU-v appeared to be generally safe, although AEs associated with the adjuvant Montanide ISA-51-VG (Seppic) at the injection site were frequently reported. It was suspected that this is due to the large 1mL volume being injected into the forearm where there is little skin depth. In the current study the site chosen this time is the upper arm and with a reduced volume of 0.5mL.

- 4 FLU-v (25%) and 3 Placebo (18.8%) subjects reported rhinorrhoea 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 and resolved.

In conclusion, the dose of Challenge Virus used in this study was intended to cause mild symptoms in volunteers, and an attack rate of approximately 70% to 80% in the Placebo group, which was not achieved. It is possible that in the study, the low rates of infectivity in the Placebo group and the resulting apparent lack of efficacy of FLU-v may have been related to pre-existing multi-strain (i.e., heterologous) cellular immunity arising from previous

asymptomatic exposure of the population to viral infection during a recent Influenza pandemic between June 2009 and February 2010.

It is also possible that pre-existing T cells responding to Influenza internal proteins may have been associated with lower virus shedding and less severe illness. This was determined by looking at pre-vaccinated PBMC's from volunteers and looking for responses to pandemic strain flu viruses including the swine flu circulating at that time. It was found that the majority of volunteers in the placebo group had PBMC's against the pandemic while the minority in the vaccinated group had such pre-exposure. It has been clearly established by other researches that exposure to the swine flu produced cross protection against other strains of flu for a period of time.

5.3 Rationale for the study design

Current Phase IIb Study

The doses selected for the Phase IIb study have been chosen based on the best results seen from previous Phase I human trial studies.

This is a randomised, double-blind, placebo-controlled, single-centre, Phase 2b study of FLU-v administered at Day -43 and Day -22 prior to intranasal challenge at Day 0 with the Influenza A 2009 H1N1 human challenge virus. Subjects will be randomised 1:1:1 to three treatment arms:

- Group 1: adjuvanted placebo on Day -43 and on Day -22
- Group 2: 500 mcg adjuvanted FLU-v vaccine on Day -43 and 1 dose of adjuvanted placebo on Day -22
- Group 3: 500 mcg adjuvanted FLU-v vaccine on Day -43 and on Day -22

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment. Randomisation will be used to minimize bias in the assignment of participants to treatment groups, to increase the likelihood that known and unknown participant attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. Blinded treatment will be used to reduce potential bias during data collection and evaluation of endpoints. 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.

In this study, the main efficacy comparisons are for each of the FLU-v groups to placebo. The study will also compare the two FLU-v dosing arms but the study will only have power to detect a significant difference in MMID rates between the FLU-v groups and placebo and not against each other.

Assuming significant efficacy of at least one dose of FLU-v against placebo is demonstrated in the current protocol (Part A), when all the primary efficacy endpoint and key safety results are available, the protocol may be amended to include a second part (Part B) which will test the optimal FLU-v dose from Part A (in terms of efficacy and safety) versus current standard of care seasonal influenza vaccine. Details of Part B will be determined and documented in a future protocol amendment following the availability of the unblinded key efficacy and safety results from Part A. Approval will be sought via a substantial amendment from the competent authority before part B can commence

5.3.1 Overall benefit/risk assessment

Based on the available data and proposed safety measures, the overall risk/benefit assessment for this study is considered acceptable.

Influenza (flu) is an infectious disease of birds and mammals caused by an RNA virus of the family Orthomyxoviridae (the influenza viruses). In humans, common symptoms of influenza infection are fever, sore throat, muscle pains, severe headache, coughing, and weakness and fatigue. In more serious cases, influenza causes pneumonia, which can be fatal, particularly in young children and the elderly.

Typically, influenza is transmitted from infected people through the air by coughs or sneezes, creating aerosols containing the virus. Influenza can also be transmitted by saliva, nasal secretions, faeces and blood. Infections occur through contact with these bodily fluids or with contaminated surfaces. Flu viruses can remain infectious for about one week at human body temperature, over 30 days at 0°C (32°F), and indefinitely at very low temperatures. Most influenza strains can be inactivated easily by disinfectants and detergents.

Flu spreads around the world in seasonal epidemics. This was the situation during the influenza pandemic of 1918-19, when a completely new influenza virus subtype (influenza A/H1N1) emerged and spread around the globe in around four to six months. Several waves of infection occurred over two years, killing an estimated 40-50 million people. Since then there have been two subsequent influenza pandemics, in 1957 and 1968. In 2009, the swine flu (H1N1) was the cause of a pandemic that reached a phase 6 pandemic alert and was moderate in severity. Currently available influenza vaccines utilise a Quadrivalent killed vaccine, incorporating two A strains (H1N1 like and H3N2 like) and a two B strains. Whilst these vaccines confer a degree of immunity; antigenic drift, together with the emergence of newer strains, limit their usefulness to a single season, and the need for annual manufacture has considerable impact on cost, as well as the risk that an incorrect prediction of the virus types will lead to an unacceptable level of protection. There is a need for a “universal” vaccine that would be available on an annual basis for winter influenza, but would also have the ability to be rapidly deployed in the face of a pending pandemic, without the need to identify the novel strain and develop a novel vaccine.

PepTcell have developed a novel approach to this problem by developing a vaccination that contains four polypeptides, each of which contains multiple epitopes thought to generate a significant cell-mediated and antibody immune response to the influenza virus, named FLU-

v. Importantly, however, PepTcell have shown that these polypeptide sequences are relatively conserved between different strains and are therefore likely to provide a more consistent immune response, even in the face of a variety of viral strains.

FLU-v has undergone comprehensive testing in a number of non-clinical studies including safety pharmacology, repeat dose toxicology, four-week repeat dose toxicology and local tolerance tests conducted in rats, mice and rabbits. Overall, the vaccine and vaccine/adjuvant combinations were safe and had no significant toxicity at the doses tested. Safety pharmacology studies in rats showed only minor and transient changes in cardiovascular and respiratory parameters that were not considered biologically relevant. In addition, local tolerance of FLU-v administered subcutaneously with or without adjuvant (ISA 51), was assessed in rabbits each receiving a single subcutaneous injection of 502.7 µg FLU-v. Following observation for five days, the animals showed no sign of local reaction to treatment and no clinical signs or treatment-related bodyweight changes during the study period. Subcutaneous injection of FLU-v or FLU-v with adjuvant was well tolerated with no local or systemic sign of reaction to treatment. Although there were some histological findings, these were considered to be due to treatment with adjuvant or were procedural. There were no treatment related findings associated with FLU-v observed in the study. Two Phase I studies have demonstrated good safety , tolerability and good immunological responses in human subjects

In summary, the clinical and non-clinical studies completed with Flu-v suggest that this vaccine has an acceptable safety profile, well tolerated and suitable for ongoing development in human studies. In addition, PepTCells novel approach identifying conserved epitopes of the influenza vaccine differs from approaches used previously. Given these factors and the fact that a vaccination treatment is likely to represent an important option not only for seasonal influenza, but also as an important public health treatment in the event of a pandemic, the potential benefits of this study outweigh the risks.

5.3.1.1 Vulnerable populations

To reduce the risk of passing the Challenge Virus to others, subjects will be asked to avoid contact with vulnerable people for 2 weeks after they leave quarantine. For the purposes of this protocol, a vulnerable individual is a person who:

- Is a child under five years old
- Is elderly (aged 65 years or older)
- Pregnant or breastfeeding women
- Has known immunodeficiency
- Is receiving immunosuppressant medications
- Is undergoing or soon to undergo cancer chemotherapy within 28 days of Viral Challenge
- Has been diagnosed with chronic obstructive pulmonary disease (COPD) or other severe lung or heart disease
- Has received a bone marrow or solid organ transplant.

Should any new information relevant to the subjects' participation in the study and their safety become available, a member of the hVIVO medical staff will notify subjects in a timely manner. If appropriate, subjects will be asked to reconfirm their consent to their participation in the study.

6 STUDY OBJECTIVES

6.1 Primary objective

To determine the effect of FLU-v in reducing the incidence of Mild to Moderate Influenza Disease (MMID) defined as detectable viral shedding plus at least one symptom of influenza

Assuming significant efficacy of at least one dose of FLU-v against placebo is demonstrated in the current protocol (Part A) when all the primary efficacy endpoint and key safety results are available, the protocol may be amended to include a second part (Part B) which will test the optimal FLU-v dose from Part A (in terms of efficacy and safety) versus current standard of care seasonal influenza vaccine. Details of Part B will be determined and documented in a future protocol amendment following the availability of the unblinded key efficacy and safety results from Part A. Approval will be sought via a substantial amendment from the competent authority before part B can commence

6.1.1 Primary endpoint

Incidence of MMID.

6.2 Secondary objectives

To determine the overall effect of FLU-v on measurements of disease severity.

6.2.1 Secondary endpoints

- Viral shedding duration
- Viral shedding quantitation
- Symptom duration
- Total number of symptoms experienced
- Symptom severity score as measured by FLU-PRO Symptom Questionnaire
- Incidence of vaccine related adverse events (diary card for 21 days after each vaccine)

6.3 Exploratory objectives

To explore the immunological responses to FLU-v

6.3.1 Exploratory endpoints

- Immunogenicity of FLU-v as measured by
    - B-cell and T-cell responses to FLU-v both after vaccination as well as influenza challenge
    - Immunophenotyping

- Broadness of protection as measured by culturing PBMCs pre-influenza virus inoculation in study with PBMCs (same subject) infected with influenza and assess killing of infected cells by PBMCs from vaccinated subjects and use sera containing

non-neutralising antibodies and test killing of human influenza infected cells by complement activation or ADCC assays.

Exploratory endpoints may be reported separately from the clinical study report (CSR).

6.4 Safety objectives

To determine the safety and tolerability of FLU-v after vaccination in healthy subjects subsequently challenged with Influenza A 2009 H1N1 human challenge virus

6.4.1 Safety endpoints

- Incidence of treatment-emergent AEs (TEAE), severity, seriousness and causality
- Absolute values and change from baseline in routine clinical and laboratory parameters
- Vaccine Related Physical examination findings
- Concomitant medications.

7 STUDY POPULATION

Participants will be carefully selected using the inclusion and exclusion criteria described here to select the optimum participants for completing the study objectives and minimize the risk of adverse events (AEs).

7.1 Number of subjects

Up to 123 subjects will be inoculated with the influenza challenge virus at a dose of 10^7 TCID50 after being randomised 1:1:1, and dosed with FLU-v 1 dose plus placebo, 2 doses, or placebo

7.2 Inclusion criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

NO	INCLUSION CRITERIA
1	Healthy males and females aged ≥18 and ≤55 years of age at the point of enrolment.
2	Willingness to remain in isolation for the duration of viral shedding and to comply with all study requirements.
3	The following criteria are applicable to subjects in a heterosexual relationship and female subjects in a same sex relationship (i.e., the criteria do not apply to male subjects in a same sex relationship): - True abstinence- when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g. calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception). Or - Two forms of effective contraceptive methods among (between) the couple, which are defined as: o For males: condom with spermicidal foam/gel/film/cream, sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate. This applies only to males participating in the study). o For females: ▪ Women no longer of child bearing potential (post-menopausal females are defined as having a history of amenorrhea for at least 2 years, otherwise they should have documented status as being surgically sterile or post hysterectomy. The latter applies only to females participating in the study).

NO	INCLUSION CRITERIA
	▪ If of childbearing potential, then acceptable forms of contraception include: ▪ Established (a minimum of 2 weeks prior to admission) use of oral, injected or implanted hormonal methods of contraception. • Placement of an intrauterine device (IUD) or intrauterine system (IUS). • Barrier methods of contraception or occlusive cap (diaphragm or cervical/vault caps), both with one of the following - spermicidal foam/gel/film/cream/suppository. ○ The longevity of contraception is as follows: ▪ Males: • Comply with agreed contraception at entry to quarantine, and continuing until 90 days after the date of viral challenge/last dosing with IMP (whichever occurs last). • Must not donate sperm following discharge from quarantine until 90 days after the date of viral challenge/last dosing with IMP (whichever occurs last). ▪ Females: If of childbearing potential must have a negative pregnancy test at screening and just prior to the date of Viral Challenge, and must be using contraception consisting of two forms of birth control (one of which must be a barrier method) starting from at least 2 weeks prior to the first vaccination and continuing until 90 days after the date of Viral Challenge/last dosing with IMP (whichever occurs last).
4	Willing to have samples stored for future research.
5	Sero-suitable to the study challenge virus within 90 days of Day 0.
6	Agrees to abstain from alcohol intake 24 hours before admission on Day -2 or Day -1 and all other outpatient visits.
7	Agrees to not use prescription or over-the-counter medications (including aspirin, decongestants, antihistamines, and other NSAIDs), and herbal medication (including, but not limited to, Vitamin C, Vitamin D, immune booster products, herbal tea, St. John's Wort), within 14 days prior to study vaccine administration through the final follow-up visit, unless approved by the investigator and sponsor medical monitor.
8	An informed consent document signed and dated by the subject and the Investigator or delegate.

NO	INCLUSION CRITERIA
9	A history of childhood asthma before the age of 12 years is acceptable provided the subject is asymptomatic without treatment. Subjects with a single episode of wheezing (lasting less than 2 weeks) after the age of 12 years can be included at the Investigator's discretion provided the episode was more than 1 year ago and did not require a hospital admission and/or oral/intravenous steroids.
10	In good health with no history of major medical conditions that will interfere with subject safety, as defined by medical history, physical examination, and routine laboratory tests and determined by the Investigator at a screening evaluation. • A subject with a history of Herpes type 1 or 2 infection may be included if there are no active lesions present and the subject is not taking active medication. • A subject with or without any evidence of atopy including any history of allergic rhinitis, dermatitis, and conjunctivitis will be included as long as they do not conflict with exclusion criteria. Mild to moderate arthritis of non-inflammatory origin may be allowed if the subject is not at risk from relative immobility in the Quarantine Unit and does not require regular medication.
11	A documented medical history for a minimum of the last 2 years prior to inoculation.

7.3 Exclusion criteria

NO	EXCLUSION CRITERIA
1	Any subjects who have smoked 10 pack years at any time. Of those subjects that have smoked less than 10 pack years at any time, a subject will be excluded: If regular smokers (e.g., smoking every day) at the time of enrolment. If current casual smoker or use of smoking / nicotine-related products, they must agree to refrain from smoking during the in-patient stay
2	Presence of self-reported or medically documented significant medical condition including but not limited to: a. Chronic pulmonary disease (e.g., asthma (except what is stated in inclusion criteria 9), COPD) b. Chronic cardiovascular disease (e.g., cardiomyopathy, congestive heart failure, cardiac surgery, ischemic heart disease, known anatomic defects). c. Chronic medical conditions requiring close medical follow-up or hospitalisation during the past 5 years (e.g., insulin dependent diabetes mellitus, renal dysfunction, haemoglobinopathies). d. Immunosuppression, or immunodeficiency or ongoing malignancy.

NO	EXCLUSION CRITERIA
	e. Neurological and neurodevelopmental conditions (e.g., cerebral palsy, epilepsy, stroke, seizures). f. Post infectious or post vaccine neurological sequelae. g. Hyperlipidemia requiring medical therapy per current American College of Cardiology (ACC) and American Heart Association (AHA) guidelines published in 2013.
3	Individual with body mass index (BMI) <18 and >35.
4	Acute illness within 7 days of first vaccine administration day
5	Clinically significant abnormal electrocardiogram (ECG) and/or parameters, as determined by the Investigator
6	Subjects with clinically significant abnormal systolic and diastolic blood pressure or clinically significant abnormal pulse rate.
7	Subject has abnormal pulmonary function as measured by spirometry defined as a forced vital capacity or forced expiratory volume in 1 second (FEV1) < 80% of predicted or peripheral arterial oxygen saturation (SpO2) < 92% on room air.
8	Known allergy to treatments for influenza (including but not limited to oseltamivir, nonsteroidals).
9	Known allergy to 2 or more classes of antibiotics (e.g. penicillins, cephalosporins, fluoroquinolones, or glycopeptides). Known allergy to excipients in the challenge virus inoculum
10	Daily or household contact with vulnerable populations as defined by 5.3.1.1
11	a) Receipt of any investigational drug within 3 months prior to the planned date of Viral Challenge/first dosing with IMP (whichever occurs first). b) Receipt of three or more investigational drugs within the previous 12 months prior to the planned date of Viral Challenge/first dosing with IMP (whichever occurs first). c) Prior inoculation with a virus from the same virus-family as the Challenge Virus. d) Prior participation in another Human Viral Challenge study with a respiratory virus in the preceding 12 months taken from the date of Viral Challenge/first dosing with IMP (whichever occurs first) in the previous study to the date of expected Viral Challenge in this study.
12	Receipt of any vaccine within 6 months of enrolment.
13	Self-reported or known history of alcoholism or drug abuse (including marijuana) within 6 months prior to enrolment, or positive urine/serum test for drugs of abuse during the study

NO	EXCLUSION CRITERIA
14	Self-reported or known history of psychiatric or psychological issues that require treatment and are deemed by the PI to be a contraindication to protocol participation.
15	History of a previous severe allergic reaction with generalized urticaria, angioedema, or anaphylaxis.
16	History or evidence of autoimmune disease or known immunodeficiency of any cause – with the exception of atopic dermatitis/eczema and atopic rhinitis.
17	Subjects with any history of physician diagnosed and/or objective test confirmed asthma (except as per inclusion criteria 9), reactive airway disease, COPD, pulmonary hypertension, or chronic lung condition of any aetiology.
18	Positive human immunodeficiency virus (HIV) within 60 days of first vaccination visit, active hepatitis A (HAV), B (HBV), or C (HCV) test.
19	Any significant abnormality altering the anatomy of the nose or nasopharynx (including significant nasal polyps).
20	Venous access deemed inadequate for the phlebotomy and cannulation demands of the study.
21	Any nasal or sinus surgery within 6 months of Viral Challenge.
22	Recurrent history of fainting.
23	Those employed or immediate relatives of those employed at hVIVO or the Sponsor.
24	Any clinically significant history of epistaxis (nosebleeds) within the last 12 months and/or history of being hospitalized due to epistaxis on any previous occasion.
25	Females who: • Are breastfeeding, or • Have been pregnant within 6 months prior to the study, or Have a positive pregnancy test at any point during screening or prior to first dosing with IMP.
26	Presence of fever, defined as subject presenting with a temperature reading of > 38.0°C on Day -43

NO	EXCLUSION CRITERIA
27	Receipt of blood or blood products, or loss (including blood donations) of 470 mL or more of blood during the 3 months prior to the planned date of first dosing with IMP or planned during the 3 months after the final visit.
28	Receipt of systemic (intravenous and/or oral) glucocorticoids or systemic antiviral drugs within 6 months prior to the planned date of first dosing with IMP.
29	Any other finding that, in the opinion of the Investigator, deems the subject unsuitable for the study.

7.4 Discontinuation of a subject's participation

7.4.1 Subject withdrawal

A subject can withdraw from the study at any time, for any reason, without prejudice to his/her future medical care. Subjects do not have to give a reason for their withdrawal.

However, subjects would be counselled that early withdrawal from the challenge isolation portion of the trial is strongly discouraged, as it may pose a risk both to the subject and his/her contacts outside of the Quarantine Unit.

In the event of a subject insisting on early withdrawal following Viral Challenge, the subject would be encouraged to stay and would be advised of the potential risks of carrying an Influenza virus infection into the community, and to vulnerable groups (Section 5.3.1.1) in particular. The subject would be referred to his/her GP and wherever possible, followed up at home. Each subject would be counselled regarding their consent and asked to return for a follow-up visit.

All subjects will be advised that for 2 weeks following discharge from the Quarantine Unit they should avoid close contact with vulnerable people, as described in Section 5.3.1.1.

If a subject withdraws before completing the study, the reason for withdrawal should be documented in the electronic case report form (eCRF) and in the source document. Study drug assigned to the withdrawn subject will not be assigned to another subject. In case subjects withdraw from the study after receiving vaccination(s) but prior to influenza challenge, replacement subjects will be recruited.

In the case, that a participant is withdrawn from the study after receiving vaccination(s) but prior to influenza challenge, all efforts will be made to follow these patients at the outpatient visits as outlined in the T&E.

7.4.2 Subject discontinuation

A subject's participation may be stopped for any of the following reasons

- Withdrawal of subject consent.

- Clinically significant abnormal laboratory findings, which in the opinion of the Investigator(s) and/or Sponsor precludes further participation in the study.
- Consumption of homeopathic or pharmaceutical remedies for the symptomatic treatment of an upper respiratory tract (URT) infection (e.g., decongestants, antipyretics, etc.), unless prescribed by the Investigator after consultation with the Sponsor and Investigators.
- Development of inter-current illness which, in the opinion of the Investigator(s), would compromise the health of the subject or the study objectives.
- Pregnancy
- Investigator's decision that withdrawal from further participation would be in the subject's best interests.
- Non-compliance with study requirements.
- Termination of the study at the discretion of the Investigator(s) or Sponsor for safety behavioural or administrative reasons.
- The PI or the study-site personnel break the randomisation code.
- The subject experiences an acute systemic allergic reaction ≥ Grade 3.
- Lost to follow-up (every reasonable effort must be made by the study-site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented).

Suitability check will be conducted by the study physician to confirm that subject is still suitable to continue with study participation, on Day -22, Day -2 /-1 and Day 0. This will include but not limited to the following assessments:

- Acute illness within 7 days of any vaccine administration day or admission to quarantine. Subjects can be rescheduled for another Viral challenge admission day if possible and the protocol window permitting.
- Presence of fever, defined as subject presenting with a temperature reading of > 38.0 ◦C on Day -22 and/or Viral Challenge on Day 0. Subjects can be rescheduled for another Viral challenge day if possible and the protocol window permitting.
- Known close contact with anyone known to have a positive test for influenza in the 7 days prior to virus inoculation.

7.4.3 Pregnancy

In the event of a subject discontinuing the study prematurely due to pregnancy, or if pregnancy in a subject's partner becomes known, the procedure outlined in Section 15.4.4 should be followed.

7.5 Replacements and reserve subjects

If a subject is withdrawn from the study prior to influenza challenge, replacement subjects will be recruited. Once a subject is challenged with influenza virus he/she will not be replaced.

7.6 Subject numbering

hVIVO assigns a subject number to each volunteer in the hVIVO volunteer database. This subject number will be used to identify a volunteer up to the point of randomisation (Section 10.1), on source documents, on all study correspondence and in the study database.

For volunteers who subsequently do not enter the study, a reason will be documented in the source documentation and the subject's medical notes.

For subjects who are subsequently enrolled into the study, the PI will keep an identification code list and enrolment log, listing initials of each subject alongside the subject number assigned and the date enrolled. This log will remain in the ISF at the study site. Replacement subjects will be assigned a new, unique subject number (Section 10.1).

8 STUDY RESTRICTIONS

8.1 Concomitant therapies

All concomitant prescription medications (including vaccines but with the exception of post-exposure prophylaxis), over-the-counter medications, or herbal remedies taken during study participation must be approved by the PI and will be recorded in the participant's source documents. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorised/licensed clinician. Symptomatic treatment (antipyretics, antitussives, decongestants, etc.) in individuals not requiring an increased level of care will be administered at the discretion of the PI and recorded carefully in the source documents.

8.2 Strenuous exercise

Subjects will refrain from strenuous activities for the duration of the quarantine period and for 3 days prior to screening and follow-up visits. .

8.3 Tobacco

Subjects should abstain from smoking or using nicotine products or e-cigarette products prior to assessment visits to the research facility.

8.4 Alcohol, caffeine and xanthine-containing food and beverages

Subjects should abstain from alcohol, caffeine and xanthine-containing food and beverages (e.g., coffee, tea, energy drinks, cola, chocolate) between 24 hours prior to vaccine and dosing as well as prior to admission to quarantine. They will continue to abstain until after discharge from quarantine unit. Decaffeinated drinks will be provided during the quarantine period.

8.5 Contraception

Male and female subjects of childbearing potential who engage in heterosexual intercourse must agree to utilize protocol-recommended methods of contraception. The required contraceptive measures are outlined in the study eligibility criteria (Section 7.2).

Postmenopausal women (no spontaneous menses for at least 2 years), women with surgical sterilisation (documented GP evidence of a total hysterectomy, bilateral oophorectomy, bilateral tubal ligation/bilateral tubal clips without reversal operation), and women who are otherwise incapable of becoming pregnant must use a condom when having heterosexual intercourse from 2 weeks prior to entry to quarantine and continuing until 90 days after the date of Viral Challenge / last dosing with IMP (whichever occurs last).

Study approved contraceptive methods

Established use of oral, injected or implanted hormonal methods of contraception.

Placement of an intrauterine device or intrauterine system.

Barrier methods of contraception: Condom or diaphragm with spermicidal foam/gel/film/cream/suppository.

Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female subjects on the study, the vasectomized male partner should be the sole partner for that subject].

True abstinence: When this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Subjects should be advised that a male and female condom should not be used together due to risk of breakage or damage caused by latex friction.

8.6 Contact with vulnerable populations

Subjects should avoid close contact with people who are known to be vulnerable to Challenge Virus infection (Section 5.3.1.1) for 2 weeks following discharge from the Quarantine Unit.

9 STUDY DESIGN

This is a randomised, double-blind, placebo-controlled, single-centre, phase IIb study of FLU-v administered at Day -43 and Day -22 prior to intranasal challenge at Day 0 with the Influenza A 2009 H1N1 human challenge virus. Subjects will be randomised 1:1:1 to:

- Group 1: adjuvanted placebo on Day -43 and on Day -22
- Group 2: 500 mcg adjuvanted FLU-v vaccine on Day -43 and 1 dose of adjuvanted placebo on Day -22
- Group 3: 500 mcg adjuvanted FLU-v vaccine on Day -43 and on Day -22

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment. Randomisation will be used to minimize bias in the assignment of participants to treatment groups, to increase the likelihood that known and unknown participant attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. Blinded treatment will be used to reduce potential bias during data collection and evaluation of endpoints. 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 follow-up visit will be up to approximately 153 days or 22 weeks from screening within 90 days of viral challenge to the last follow up visit on Day 63 (± 5 days).

The total duration of the clinical phase of the study, from the start of volunteer screening to the last subject's last scheduled visit (LSLV) is expected to be approximately 18 months.

The timings of study procedures relative to each phase are shown in table 9.2 and the procedures and assessments which will be undertaken to evaluate safety and efficacy are described in Sections 13.

Subjects will:

- Attend study-specific screening (SSS) within -90 days prior to viral challenge
- Vaccination on Day -43
- Vaccination on Day -22
- Entry to quarantine on Day -2 or Day -1
- Challenge Virus inoculation on Day 0
- Subjects will be resident in the Quarantine Unit for a total of approximately 10 days (from Day -2/-1 to Day 7)
- End of quarantine phase starting from Day 7 when 2 negative diagnostic tests for influenza on two separate days are achieved
- Follow-up visits: Day 35 (+/- 3) and Day 63 (+/- 5) days

Table 9-2: Time & Events Schedule

Visits	Visit 1	Visit 2	21 days post vaccination 1	Visit 3	21 days post vaccination 2	Visit 4										Visit 5	Visit 6
Study Phase >	Screen	IMP DOSING		IMP DOSING		Quarantine Admission		Inpatient								Follow-Up	Follow-Up
Study Day >	~ DAY -- 90 to -43	D-43		D-22		D-2	D-1	D0 Challenge	D1	D2	D3	D4	D5	D6	D7	D8 - 15	D35 (+/- 3 days)	D63 (+/- 5 days) or Early withdrawal visit
Inpatient hospitalisation						X		X	X	X	X	X	X	X	X*			
Demographics	X																	
Medical & Medication History	X																	
Outpatient Visit	X	X		X													X	X
Eligibility Criteria	X	X																
Suitability check[4]				X		X[6]		X										
Written Consent	X																	
Adverse Events & Concomitant Medication review	X	X		X		X	X	X	X	X	X	X	X	X	X	(X)	X	X
Distribution and collection of Adverse Events diary card post vaccination visit		X		X		X												
Completion of Adverse Events diary card post vaccination visit			X		X													
Physician Assessment and CPE	X	X		X		X									X	(X)	X	X
Physician Assessment and DPE								X[1]	X	X	X	X	X	X		(X)		
Self-administered FLU-PRO Symptom Questionnaire[§]						X[6]		X	X	X	X	X	X	X	X	X	X	

Visits	Visit 1	Visit 2	21 days post vaccination 1	Visit 3	21 days post vaccination 2	Visit 4										Visit 5	Visit 6
Study Phase >	Screen	IMP DOSING		IMP DOSING		Quarantine Admission		Inpatient								Follow-Up	Follow-Up
Study Day >	~ DAY -- 90 to -43	D-43		D-22		D-2	D-1	D0 Challenge	D1	D2	D3	D4	D5	D6	D8 - 15	D35 (+/- 3 days)	D63 (+/- 5 days) or Early withdrawal visit
Physician assessment of Influenza Symptoms						X[6]		X	X	X	X	X	X	X	(X)		
Vital signs †	X	X		X		X	X	X	X	X	X	X	X	X	(X)	X	X
Pregnancy test‡	X	X		X		X										X	X
Urine drugs of abuse	X	X		X		X											
Urinalysis	X	X		X		X				X		X		X		(X)[2]	(X)[2]
Alcohol Breath Testing	X	X		X		X											
Nasal swab samples □	X					X[6]			X	X	X	X	X	X	X	(X)		
Respiratory pathogen screen						X[6]											
Spirometry [3]	X					X									(X)[2]		
Height, Weight and BMI (Height performed only at screening)	X	X				X											X
ECG†	X					X				X		X		X	(X)[2]	X	X
Randomisation		X															
Vaccination visit and clinic observation		X		X													
Challenge virus inoculation								X									
CBC + diff (Haematology)	X					X				X		X		X	(X)[2]	X	X
Biochem: Acute Care, Mineral, and Hepatic Panels	X					X				X		X		X	(X)[2]	(X)[2]	(X)[2]

Visits	Visit 1	Visit 2	21 days post vaccination 1	Visit 3	21 days post vaccination 2	Visit 4										Visit 5	Visit 6
Study Phase >	Screen	IMP DOSING		IMP DOSING		Quarantine Admission		Inpatient								Follow-Up	Follow-Up
Study Day >	~ DAY -- 90 to -43	D-43		D-22		D-2	D-1	D0 Challenge	D1	D2	D3	D4	D5	D6	D7	D8 - 15	D35 (+/- 3 days)	D63 (+/- 5 days) Or Early withdrawal visit
Biochem: Creatine Kinase, and Total Protein	X					X						X		X		(X)[2]	(X)[2]	(X)[2]
Serum / Whole Blood Collection[7]	X					X		X		X		X		X		(X)[2]	X	X
HIV, Hepatitis A/B/C	X					X[5]												

KEY NOTES FOR TIME AND EVENTS SCHEDULES

Symbol	Notes
Results of tests or examinations performed under other ethically approved hVIVO protocols, and within 90 days prior to admission to quarantine, may be used to determine eligibility without the need to repeat the assessment at study specific screening.	
*	If continued viral shedding is detected on Day 7, the participant(s) will remain in quarantine as an inpatient and will continue to have nasal swabs and physician assessment and exams. In addition FLU-PRO symptom questionnaires will continue up to day 15. Any other procedures will be performed if deemed necessary by the study physician.
†	Vital Signs and ECG's: participants must be supine for a minimum of 5 minutes prior to these procedures being performed; vital signs include blood pressure, mean blood pressure, heart rate, respiratory rate, temperature, pulse oximetry. During the quarantine confinement vital signs will be taken 3 times a day at the same time +/- 1 hour except on Day -2, when it will be conducted once and on discharge day when it will be performed once or twice depending on discharge time. ECG should be performed once daily
‡	Females only - Serum pregnancy testing at the screening visit and, urine pregnancy testing on Days -43, -22, -2, 35 and 63.
§	FLU-PRO Symptom Questionnaire(s) will be filled out daily in the evening on Days -2 or-1, 0 through to Day 15, and on Day 35
Δ	• NP nasal sampling will take place 2 times a day at the same time +/- 1 hour, except on Day -2 / -1 where there is one baseline sampling and on discharge day. No nasal sampling will take place on Day 0. • NPS - tolerance only at screening
(X)	Optional - Only for subjects kept in VCU after D7. If the subject is not discharged on day 7, they will continue to have vital signs, physical assessments, nasal swabs, adverse event s & concomitant medication check, and FLU-PRO questionnaires performed daily.
1	DPE to be performed before and after inoculation at D0
2	These assessments will be performed at the PI's discretion
3	Spirometry will be performed during the inpatient phase in presence of lower respiratory tract symptoms
4	A continued suitability check will be conducted by the physician to determine if subject is suitable to continue study participation
5	Repeat HIV test if screening results are more than 60 days prior to entry to Quarantine (on Day -2 or Day -1)
6	Assessments will be performed once, either on Day -2 or Day -1
7	Challenge virus Serology will be performed only at Screening visit. A repeat may be requested before admission if required, however, the serology test and result must be dated within 90 days of Day 0. PBMC & PAXgene samples will be collected from subjects, either at their screening visit or at a planned PBMC sampling visit prior to their vaccination.

9.1 Screening phase

9.1.1 Visit 1 - Study specific screening

Screening will be performed within 90 days of inoculation and prior to Day -43. The participants will sign the informed consent document prior to any study procedures. Study screening procedures will be conducted as detailed on the Time and Events, table 9.2.

9.2 Vaccination Phase

9.2.1 Visit 2 - Day -43

The first vaccine or placebo administration will take place on Day -43, 43 days prior to virus challenge. Study procedures will be conducted as detailed on the Time and Events, table 9.2

Once it is determined that the participant still meets Inclusion/Exclusion criteria for the research study, participants will receive the first of two injections of FLU-v 500mcg+adjuvant 2 doses, FLU-v 500mcg 1 dose + 1 dose placebo , or 2 doses of placebo. The study team will thoroughly discuss the consent with the volunteer. The study team will issue and explain the completion of self-reported adverse events in a 21-day diary card. This diary card will be used to record vaccine-related local and systemic events post first vaccination.

9.2.2 Visit 3 - Day -22 (minimum 21 days post first vaccination +7 days)

The second study drug administration will take place if the participant continues to meet all inclusion and none of the exclusion criteria. Study procedures will be conducted as detailed on the Time and Events schedule

The study team will issue and explain the completion of self-reported adverse events in a 21-day diary card. This diary card will be used to record vaccine-related local and systemic events post second vaccination.

9.3 Quarantine and Challenge

9.3.1 Visit 4 - Admission (Day -2 or Day -1 up to 40 days post second vaccination)

Subjects will be admitted to the Quarantine unit on day -2 or day -1 (minimum 20 days post second vaccination). If subject is not available to attend admission on the Day -2/Day -1 due date, the visit can be scheduled up to 40 days post second vaccination.

The admission procedures/assessments and testing will be performed after admission and before challenge virus administration. Study procedures will be conducted as detailed on the Time and Events schedule

As determined by the PI, results of standard procedures performed in the process of admission to the Quarantine unit may be used to determine suitability, including but not limited to vital signs, height, weight, etc. They can also be used to extend eligibility beyond 90 days from

screening if the participant has been vaccinated but his admission has fallen out of the 90 days window. A Viral Challenge serology test can be repeated if required before Quarantine if the participant has been vaccinated but the admission date has fallen out of the 90 days window. However, the serology test and result must be dated within 90 days of Day 0.

9.3.2 Virus Inoculation (Day 0)

All baseline procedures and tests will be performed prior to challenge virus administration. Once it has been determined that the participant still meets eligibility criteria for the study and that he/she is comfortable in the unit, administration of the challenge virus will proceed at a dose of 10^7 $TCID_{50}$ on Day 0. Once the influenza challenge virus has been administered, participants will remain hospitalized in isolation on the unit for a minimum of 8 additional days and will be discharged only after they meet the discharge criteria described below.

9.3.3 Day 1 through 7

While admitted to the Quarantine Unit, participants will remain in isolation in their room. Participants will undergo study procedures which will be conducted as detailed on the Time and Events table 9.2

Participants will be discharged when they meet the following discharge criteria: 2 consecutive negative morning nasal swabs (that occur on 2 different but consecutive days) for influenza A, are afebrile, show no signs of significant influenza symptoms, and are clinically and hemodynamically stable. It is expected that participants will shed virus for 3 to 5 days post inoculation; therefore, we expect most participants to have 2 consecutive negative nasopharyngeal swabs by Day 7 of the study.

The FLU-PRO questionnaires will be completed in paper form.

9.3.4 Discharge

Starting from Day 7 subjects will be discharged from the Quarantine Unit if the morning nasopharyngeal swab sample is negative (on two separate but consecutive days) for Challenge Virus. If the test is positive or the subject remains symptomatic, the subject will be asked to remain in the Quarantine Unit for further observation at the discretion of the PI.

9.3.5 Day 8 through 15 (if required)

All subjects will complete diary card up to Day 15 while at home.

All subjects remaining in quarantine unit will follow assessments as detailed in the T&E

9.4 Visit 5 & 6 - Follow-up phase

All participants will be followed for a minimum of 8 weeks after completing the inpatient portion of the study. Any participant who experiences complications due to challenge virus administration or study drug will be followed until resolution of such complications or appropriate referral to the necessary medical care has been made.

Follow-up visits will take place on Day 35 (+/-3 days) and Day 63 (+/-5) days or more often if deemed medically necessary; the procedures/evaluations to be conducted at these visits are shown in T&E schedule. Day 63 will be the final study visit unless the participant requires additional follow-up of study-related complications.

Participants will be asked to report to the study team if any close contacts become ill with an influenza-like illness. If a close contact becomes ill with an influenza-like illness during the follow-up period, the participant will be asked to contact that individual and have them contact the hVIVO study team. The possibly infected person will be asked to come to the hVIVO facility for an assessment.

9.4.1 Lost to follow-up

In the event that a subject does not return for the final follow-up visit post-Viral Challenge; the Investigator will make every reasonable effort to contact the subject to review all AEs. In the event that a subject drops out of the study at any time, the reason for discontinuation, if known, must be fully documented in the source data. The site personnel will document the AEs and any other assessments and will make every effort to complete all required end of study assessments.

9.5 End of trial

The end of the trial is defined as the last subject's last scheduled visit (LSLV). If an additional visit is required after the last scheduled visit, this will be at the PI's discretion, for example, repeat spirometry or laboratory tests. Such discretionary follow-up visits will not be considered part of the trial data unless they represent follow-up and closure on an AE or SAE identified during the trial period.

AEs that are unresolved at the follow-up visit may necessitate further follow-up visits if required.

9.5.1 End of Trial Report

It is the Sponsor's responsibility to send the End of Trial Report to the Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC) within 1 year of the end of the trial.

10 RANDOMISATION AND BLINDING

123 subjects will be randomised 1:1:1 to:

- Group 1: adjuvanted placebo on Day -43 and on Day -22
- Group 2: 500 mcg adjuvanted FLU-v vaccine on Day -43 and 1 dose of adjuvanted placebo on Day -22
- Group 3: 500 mcg adjuvanted FLU-v vaccine on Day -43 and on Day -22

10.1 Route of administration, dosage, dose regimen and treatment period

The route of administration was chosen based on the animal data and on the adjuvant manufacturer. The mechanism of action of and oil adjuvant-based vaccines includes the formation of a depot at the injection site, enabling the slow release of the antigen and the stimulation of antibody-producing plasma cells. These formulations promote antigen uptake by APCs. Formulated antigens are concentrated and protected against degradation, while phagocytosis is stimulated by different ligand/receptor interactions. Subcutaneous injection is ideal for that.

The dosage selection is supported by animal data and also previous clinical trial showing that 500ug was better than 250ug. In clinical trials we have only given a single dose of FLU-v but animal data (presented in IB), shows that a second dose boosts the response. The two doses are given 3 weeks apart because that is the necessary length of time required for the immune system to mature the response to the antigen administered.

10.2 Randomisation for the study

Potential subjects will be assigned a unique hVIVO subject number that will be used to identify them prior to entry to the study and during the study until the point of randomisation. Once a subject in randomised they will be allocated a randomisation number.

Randomisation will occur on Day -43 Randomisation numbers will be assigned sequentially in ascending order, and once assigned, a randomisation number shall not be reassigned. The study site will keep a log of the randomisation number assigned to each subject.

The assigned randomisation number and the randomisation schedule will determine which arm the participant will be in. Treatments will be assigned in a ratio of 1:1:1.

Randomisation numbers will follow a 3 digit format e.g., 101-1NN. A copy of the randomisation code list will be sent to the unblinded pharmacist preparing the vaccines, so that vaccine or placebo doses can be prepared for each subject as appropriate.

A designated unblinded statistician will be responsible for the computer generated randomisation schedule. Sealed copies of the randomisation code will be stored in a secure place. Following database lock, on receipt of authorisation from the Sponsor, a copy of the randomisation code list will be sent to the Study Statistician to conduct study unblinding.

10.3 Blinding

The PI will be provided with a tamper evident sealed envelope containing details of the treatment for each subject. All opened and unopened envelopes will be collected after the end of the subject's participation in the study.

With the exception of the manufacturer's pharmacy department, the independent statistician preparing the randomisation code list, the unblinded Clinical Research Associate (CRA) and the Quality Assurance (QA) auditors where necessary, the Investigator and all other clinical and non-clinical staff, including the Study Statistician, data management staff, and subjects, will remain blinded to the treatment allocation until after the database has been locked and approval for study unblinding has been given. The confidentiality of the randomisation code list and study blinding will be maintained.

10.4 Unblinding

Randomisation codes will be provided to the Pharmacist or designee at the start of the study. Individual emergency code break envelopes will be provided to hVIVO should it be necessary to break the blind for a subject. The Investigator will ensure there is an appropriate procedure in place to allow access to the code break envelopes in case of an emergency arising during the quarantine period, as per hVIVO's or HMR's standard operating procedures (SOPs). In the event of such an 'emergency', the relevant medical decision on the further care of a subject would depend on the actual identity of the study treatment that the subject received.

The emergency code break envelopes will contain information stating either 'This subject was on active drug' or 'This subject was on placebo'. Any premature code breaking will be explained and justified by the Investigator and hVIVO, and the Sponsor must be promptly informed. If possible, the Sponsor should be consulted before the code is broken.

When the code break envelope is opened, the Investigator must note the date, time, reason for unblinding and personnel details and record this information according to hVIVO SOPs. The Investigator must also immediately notify the Sponsor's Medical Expert (SME) that the code has been broken.

Even if the code is broken, blood samples for safety, efficacy, and other parameters assessments will continue to be drawn for the remainder of the planned study period following the last dose as long as doing so will not compromise subject welfare.

In the event of unblinding, the subject will still be followed up until resolution of any AEs.

If a subject's randomisation code is unblinded by the PI or study site personnel, the subject must be withdrawn from the study and followed up as appropriate. If the Sponsor breaks the code for safety reporting purposes, the subject may remain in the study.

11 CHALLENGE VIRUS

11.1 Description

Using reverse genetics, an investigational live Influenza A 2009 H1N1 human challenge virus similar to the wild-type strain A/Ca/04/2009 was manufactured in certified Vero cells under Good Manufacturing Practice (GMP) conditions by Charles River Laboratories in Malvern, PA from a seed stock produced by Dr. Matthew Memoli and his laboratory team in the Viral Pathogenesis and Evolution Section of the LID. It will be vialed at a maximum dose level of $10^{12}TCID_{50}$. This virus has been demonstrated to be sensitive to standard FDA-approved neuraminidase inhibitors such as oseltamivir and zanamivir. Similar to current seasonal H3N2 and all 2009 H1N1 pandemic viruses, it is resistant to the adamantanes.

11.2 Supply and accountability

hVIVO is obliged to establish a system for control of Challenge Virus, to ensure all supplies are received by a responsible person, and all deliveries and returns are documented and signed for.

The PI will maintain accurate records of receipt and condition of all Challenge Virus inoculum stock, including dates of receipt, and details and dates of the quantities dispensed and used in the study. Any departures from the protocol-dispensing regimen will be fully documented.

Challenge inoculum accountability records will be available for verification by the Study Monitor.

11.3 Storage

The Influenza A 2009 H1N1 human challenge virus will be stored at -60°C to -90°C in single-use vials. Multiple doses of virus may be prepared from the same vial after it is opened. Once opened, each vial will be discarded appropriately after use. Preparation and administration procedures will be documented in a laboratory analytical plan.

11.4 Preparation and administration

Challenge virus preparation and administration procedures will be documented in a laboratory analytical plan. This will be performed by trained personnel in the hVIVO laboratory.

12 INVESTIGATIONAL MEDICINAL PRODUCT

12.1 Description

12.1.1 FLU-v

FLU-v is a lyophilised product composed of four short peptidic active pharmaceutical ingredients (APIs) (FLU-5, FLU-7, FLU-8N and FLU-10). The APIs are manufactured by fluorenylmethoxycarbonyl (Fmoc) chemistry before reconstituting them in 50% acetic acid and mixed to prepare an equimolar solution of the four peptides. Glass vials are then filled with the solution under sterile conditions. Water and acetic acid are removed from the preparation by lyophilisation. The end product is a white to off white lyophilised solid that will need reconstitution prior administration by injection. It is subcutaneously administered and supplied in single-use vials as a dose of 500 micrograms suspended in 0.5ml volume of water and adjuvant.The intended application of the investigational medicinal product is as a prophylactic vaccine to prevent influenza virus infection by inducing an enhanced immune response.

12.1.2 Placebo

The placebo is adjuvant (Montanide ISA-51)

12.2 Supply and accountability

hVIVO external pharmacy vendor will only receive supplies of FLU-v and adjuvant once it has Qualified Person (QP) sign off by the GMP Pharmacy provider, and the IMP has been verified ready for dispatch. All IMP supplies will be used only for this protocol and for no other purpose.

Once received at hVIVO external pharmacy vendor, will perform stock level accountability. Vaccine accountability will be controlled by hVIVO external vendor and monitored by the Study Monitor throughout the study and at study closeout.

The Investigator will ensure that a responsible person receives all supplies, all deliveries and returns are documented and signed for, and the condition of the vaccine is monitored. Accurate records will be kept of when and how much vaccine is dispensed and used in the study. Any reasons for departure from the dispensing protocol will also be recorded.

FLU-v or placebo will be supplied only to subjects participating in the study. It must not be relabelled or reassigned for use by other subjects.

Accountability records will be available for verification by the Study Monitor at each monitoring visit. At the completion of the study, there will be a final reconciliation of all IMP.

12.3 Packing and labelling

FLU-v-004 and placebo will be packed under the responsibility of the Sponsor.

FLU-v-004 and placebo will be packed per subject according the randomisation list, assuring double-blindness for the FLU-v-004 versus placebo part of the study. All supplies will be packaged and received in accordance with Annex 13 of Eudralex volume 4 and the current version of the 'Rules and Guidance for Pharmaceutical Manufacturers and Distributors'[30]. Blinding, randomisation, and labelling will be performed at a GMP Pharmacy.

A release document signed by a legally authorised QP will be kept in the Trial Master File to document labelling and dispensing of the IMP to the subject. All documents required to perform GMP activities will be supplied as per the Technical Agreement.

Labelling and packing of study medication will be in accordance with current regulatory standards.[3,31-33]

No medication can be relabelled without prior approval from PepTcell (t/a SEEK).

Storage

FLU-v vials will be stored at -15 to -25^{o}C. The determination of the shelf life for the IMP is on-going. Stability studies of previous batches indicate that it should remain stable for 2 years when stored at -20^{o}C.

The vials ofwater for injection, Montanide ISA-51 should be stored at room temperature.

None of the materials can be dispensed beyond the expiry date.

Provision must be in place to store the IMP within the permitted temperature range. Temperature must be monitored regularly, ideally using a continuing electronic monitoring system (24/7) is expected. However, it is acceptable to use a well-maintained system of max min thermometer recording at least once per working day. Diligence is expected in checking that at the time of dispensing, the IMP has always been stored in the permitted range.

Whichever system is adopted the equipment must be regularly calibrated and a certificate of recalibration be available to the Sponsor / monitor to review.

Temperature logs should be maintained at all times and be available for inspection by the monitoring team, when requested at monitoring visits.

Should there be a deviation in the storage temperature of the IMP, it must be immediately quarantined. The deviation must be recorded on the Temperature Deviation Form, The Investigator should be immediately contacted and eventual IMP already dispensed recalled. Subject safety should be managed according to GCP.

12.4 Administration

The IMP will be administered in accordance with the study Pharmacy manual.

The investigator or delegate will be responsible for the administration of the vaccine to subjects enrolled into the study according to the procedures in the Pharmacy Manual.

Precautions:

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines.

Any temperature > 38.0 ºC or active infection is reason for delaying vaccination or according to eligibility and other criteria in section 7.4.2.

Standard immunization practices should be observed and care taken to administer the injection avoiding intravascular injection.

Subjects will be observed for 60 minutes after each injection for any immediate reactions. All subjects will be instructed to complete an adverse event diary card for 21 days for local symptoms (i.e., ecchymosis, erythema, induration, swelling and pain at the injection site) and systemic symptoms (i.e., chills, malaise, myalgia, arthralgia, nausea, headache, sweating, and fatigue) after each injection. Additional monitoring may be required for clinically significant reported events.

Appropriate medical treatment and supervision should be readily available in the case of rare anaphylactic reactions following administration of the study vaccine. Adrenaline 1:1000, Chlorpheniramine and Hydrocortisone, as per the Resuscitation Council (UK) should be readily available in case of any anaphylactic reactions.

12.5 Compliance

The exact times of dosing will be recorded in the relevant dispensing/ administration logs and subject's source notes. Any non-compliance or problems with the administration of the IMP will be recorded in the subject's source notes and reported to the Sponsor if appropriate.

12.6 Overdose

In the event of an IMP overdose, the Sponsor is responsible for notifying the MHRA and REC of the potential serious breach (see Section 18.3) within 7 days of becoming aware of it. The SME will be contacted before the next scheduled dose time (see the Pharmacy Manual for further procedural details). General supportive measures will be taken to manage any AEs associated with overdose and subjects will be clinically followed up until the AE has resolved.

12.7 Disposal

All study drugs will be disposed of according to the Sponsor's instructions. Study-site personnel must not combine contents of the study drug containers.

All used medication packages and unused study medication will be collected by the monitor and returned to the Sponsor unless other arrangements have been agreed to with the Sponsor. This must be documented on the Investigational Product Destruction Form.

13 CLINICAL ASSESSMENTS AND PROCEDURES

Assessments will be performed as detailed in Time and Events, Table 9.2 and in accordance with hVIVO's OIs, (unless stated otherwise). The Investigator may perform additional assessments in order to evaluate or manage clinical illness.

13.1 Clinical assessments

Where applicable, unless otherwise stated:

- Normal ranges will be identified in the Investigator Site File (ISF).
- Baseline measurements will be those taken pre-IMP administration

13.1.1 Complete physical examination

A complete physical examination will include an evaluation of the following body systems (these are not exclusive) and a skin examination:

- General appearance
- Eyes
- Ears
- Nose
- Throat
- Heart
- Lungs
- Chest (via stethoscope)
- Abdomen
- Peripheral pulses
- Lymph nodes
- Neurologic
- Musculoskeletal
- Head, neck, and thyroid

13.1.2 Directed physical examination

Directed physical examinations will be conducted as deemed appropriate by the Investigator and will include examination of the:

- Ear
- Nose
- Throat
- Chest (via stethoscope)

Assessment and grading of any URT (nasal discharge, otitis, pharyngitis, sinus tenderness) and LRT symptoms (abnormal breath sounds externally [e.g. stridor, vocal cord dysfunction] and on chest auscultation [new wheezing or rhonchi, crepitations] will be performed. Physician-reported assessments of viral challenge related illness will be graded in accordance with their intensity as absent, mild, moderate or severe documented in the source data.

Following viral challenge, URT and LRT symptoms (as described above) will be expected and presumed to represent virus infection consequent to viral challenge, and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs.

Following viral challenge all unexpected (in the opinion of the Investigator) directed physical examination findings will be captured as AEs, along with all other occurrences that meet the criteria for an AE.

13.1.3 Vital signs

Vital signs assessments will be recorded in accordance with hVIVO or HMRs operating instructions,:

Heart rate (HR) will be recorded in beats per minute (bpm).

Respiratory rate (RR): respirations will be counted and recorded as breaths per minute.

Blood pressure: systolic blood pressure and diastolic blood pressure will be measured in mmHg;

Peripheral arterial oxygen saturation (SpO_2 %) will be assessed using pulse oximetry.

In the event of a subject having an unexpected abnormal or out of normal range result, the assessment may be repeated after at least 2 minutes to exclude a technical fault and confirm the original reading. The assessment may then be repeated at the PI's discretion and in accordance with hVIVO or HMR's SOPs.

Study specific normal ranges and abnormalities are defined in Appendix 2: Vital signs: Study specific normal ranges and abnormalities, The Investigator will judge if the abnormalities are clinically significant. The severity of clinically significant out of normal range values will be assigned as per Appendix 4: Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table November 2007**.**

13.1.4 Temperature

Tympanic temperature will be recorded using a digital thermometer.

Temperature is related to definitions of illness (Section 4.2) and to symptomatic and specific therapy criteria; therefore temperatures > 38.0 ºC must be confirmed by a repeat measurement not less than 20 minutes and not more than 60 minutes after the first reading.

The first temperature measurement will be used if confirmed by the second reading; both readings will be recorded in the source document.

Pyrexia will be defined as temperature > 38.0ºC.

Following Viral Challenge, pyrexia will be presumed to represent Challenge Virus infection consequent to challenge. Section 15.2.6 describes the circumstances in which pyrexia would be recorded as an AE.

The study specific normal range for tympanic temperature is detailed in Appendix 2: Vital signs: Study specific normal ranges and abnormalities.

13.1.5 ECG

Twelve-lead electrocardiograms (ECG) will be obtained to evaluate cardiac function, and will be read on site by an appropriately qualified investigator. Wherever possible the same investigator will review subsequent ECGs from the same subject for the assessment of any change from baseline while in inpatient stay.

ECG abnormalities are detailed in Appendix 3: ECG: Study specific abnormalities. Any changes from screening during the study will be assessed for their clinical significance. Any clinically significant (in the Investigator's opinion) changes will be recorded as an AE and reported as described in Section 15.1.1.

13.1.6 Spirometry

Spirometry will be performed according to hVIVO's standard procedures.

Spirometry should meet the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines criteria (as per ATS/ERS guidelines for standardisation of spirometry[36]). For forced expiratory volume in 1 second (FEV_1), the highest value from 3 technically satisfactory attempts will be considered. The chosen value should not exceed the next one by more than 150 mL or 5%. If the difference is larger, up to 8 measurements will be made if appropriate. Predicted values will be calculated according to the formula of the European Coal and Steel Community (ECSC). For Caucasians of non-European descent and non- Caucasians, predicted values for FEV_1 and FVC will be adjusted for race as per the ECSC guidelines.

Any clinically significant changes from baseline will be assessed and recorded as described in Section 15.2.7.

Spirometry will be performed at baseline and may be repeated at any time in the inpatient phase of the study in the event of lower respiratory signs or symptoms (e.g. repeated coughing, dyspnoea, bradypnoea, tachypnoea, râles and rhonchi) or respiratory distress of any kind.

A 15% drop in FEV1 value (compared to baseline) will be a Grade 1 (mild) AE. The PI will use his/her clinical judgement to assign severity to drops in FEV1values of greater than 15%, based on evaluation of clinical signs and symptoms.

13.1.7 Adverse events

Adverse events will be recorded from the point of signature of the Informed Consent Form (ICF) to the last study follow-up visit (see Section 15).

Subjects will record any symptoms that they experience after administration of the IMP. These symptoms will be classified as AEs and graded by the Investigator/research nurse according to the tables provided in Appendix 4: Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table November 2007 as a reference**.**

13.1.8 Concomitant medications

All concomitant prescription medications (including vaccines but with the exception of post-exposure prophylaxis), over-the-counter medications, or herbal remedies taken during study participation must be approved by the PI and will be recorded in the participant's source documents. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorised/licensed clinician. Symptomatic treatment (antipyretics, antitussives, decongestants, etc.) in individuals not requiring an increased level of care will be administered at the discretion of the PI and recorded carefully in the source documents. The PI must consult with the sponsor medical monitor before an exclusion based on concomitant medication is made in order to determine level of deviation.

13.2 Efficacy assessments

Assessments to determine the efficacy of the IMP will be performed at the times detailed in T&E. Unless otherwise indicated, the value obtained at screening will be used as the baseline for efficacy assessments.

13.2.1 Directed physical examination

Directed physical examinations will be conducted as deemed appropriate by the Investigator.

Daily assessment of any upper and lower respiratory symptoms (nasal discharge, otitis, pharyngitis, sinus tenderness, new wheezes, râles and rhonchi) will be performed. All assessment outcomes including incidence and severity will be documented in the source data. Changes in physical examination will be reported as detailed in Section 15.2.

13.2.2 FLU-PRO Symptom Severity Assessment Tool and Physician assessment of Influenza Symptoms

Subjects will report and assess any Challenge Virus related signs and symptoms and assess their associated severity using the FLU-PRO Symptom Severity Assessment. The physician assessment will be performed daily to determine the presence or absence of influenza symptoms. The following mild (Grade 1) to moderate (Grade 2) signs or symptoms are induced by or associated with influenza, and any combination of these is expected to occur with virus challenge. These symptoms will not be reported as adverse events unless they are associated with administration of FLU-v and deemed possibly, probably, or definitely related to FLU-v by the PI. If deemed related to influenza by the PI they will be recorded as Adverse Events of influenza infection per protocol, only if deemed a risk to the participants' rights or wellbeing or as deemed appropriate by the Principal Investigator. The study team on a daily basis will check these events.

In the event of missing FLU-PRO Symptom Severity Assessment Tool data, the physician will review the data in retrospect, and complete the information as accurately as possible by asking the subject about any symptoms during the missing day. However, no protocol deviation will be recorded for missing FLU-PRO Symptom Severity Assessment Tool data.

- Arthralgia
- Chills
- Conjunctivitis
- Coryza
- Diarrhea
- Dry Cough
- Dyspnea/Shortness of Breath
- Fatigue/Tiredness
- Fever (>38.0°C)
- Headache
- Myalgia
- Nausea
- Oxygen Saturation Decrease by ≥3% from baseline
- Productive Cough
- Rhinorrhea
- Sore Throat
- Sweats

13.2.3 Adverse events diary cards (Diary report cards)

To look at expected (solicited) local and systemic signs of reactogenicity, 2 AE diary cards will be issued to all study subjects. The cards will need to be completed by the subjects from Day -43 and returned on D-22 and D-2 or D-1. In the event of missing diary card data, the physician will review the AE diary cards in retrospect, and complete the information as accurately as

possible by asking the subject about any symptoms during the missing day. No protocol deviation will be recorded for the missing diary card data.

13.2.4 Nasopharyngeal swab

Nasopharyngeal swab samples will be collected to evaluate the parameters described in Section 14.2.

14 LABORATORY ASSESSMENTS

The following assessments will be performed as detailed in Time and Events, table 9.2. The Investigator may request additional safety assessments in order to evaluate or manage clinical illness.

Any laboratory abnormalities will be determined according to the Division of Microbiology and Infectious Diseases (DMID) grading table and in accordance with the normal ranges of the clinical laboratory.

The PI or delegate must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the Adverse Event Section of the eCRF.

14.1 Blood

14.1.1 Blood volume

No more than 470 mL will be taken from each subject in a 6-month period (which includes the Screening visit to final scheduled follow up visit).

14.1.2 Routine blood analysis

The routine blood tests that will be performed as detailed Table 9.2 and in the laboratory analytical plan. The tests will include, but not limited to, haematology, biochemistry and cardiac enzymes. Additional safety assessments will be conducted at the discretion of the PI as required.

14.1.3 Whole blood and Serum analysis

1) These samples will be collected to perform research studies to better understand the pathogenesis of influenza, the immune response after influenza inoculation, and to further explore FLU-v's efficacy in vitro. Studies may include flow cytometric analysis, cytokine measurements, influenza antibody titre assays, host transcriptomics, and viral neutralization assays. Host genomic assays will not be performed as part of this study. At screening visit a serum sample will be collected to assess the Viral Challenge serology. A Viral Challenge serology test can be repeated if require before Quarantine if the participant has been vaccinated but the admission has fallen out of the 90 days window, however, the serology test and result must be dated within 90 days of Day 0. PBMC & PAXgene samples will be collected from subjects, either at their screening visit or at a planned PBMC sampling visit prior to their vaccination.

14.2 Nasopharyngeal swab

14.2.1 Respiratory virus screen

On admission to quarantine, a respiratory viral screen will be performed to detect the presence of a number of respiratory viruses that could potentially contraindicate a volunteer's participation in the study.

14.2.2 Viral shedding

Viral shedding will be confirmed by positive polymerase chain reaction (PCR) of nasopharyngeal swab samples.

RT-qPCR will use nucleic acids extracted from the samples taken at the times shown in T&E.

An infectivity assay might also be used to quantify the amount of virus in the sample.

14.3 Urine

14.3.1 Urinalysis

Clinical urine safety analysis will be undertaken using commercially available urine test strips that provide an instant result. Results will be documented in the source data.

The following parameters will be evaluated:

- Colour

Specific gravity

- Appearance
- pH.

and the presence of the following will be detected:

- Blood
- Glucose
- Leukocytes
- Ketones
- Nitrite
- Protein
- Bilirubin
- Urobilinogen.

If the dipstick yields abnormal results, a urine sample may be sent for microscopy, culture and sensitivity (MCS), at the Investigator's discretion. MCS will include but is not limited to RBC, WBC, epithelial cells, crystals, casts, and bacteria.

Urine safety analysis values will be evaluated by the Investigator. Those deemed to be clinically significant will be reported as AEs as defined in Section 15.1.1.

14.3.2 Pregnancy

During the study, females will undergo pregnancy testing of serum B-HCG/urine samples.

14.3.3 Drugs of abuse

Urine will be tested for drugs of abuse using commercially available kits that provide an instant result. The test will include (but is not limited to) amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines. A positive result must be repeated on a different sample to confirm or dispute a false positive before exclusion / screen fail. Results will be documented in the source data.

15 ADVERSE EVENTS AND TOXICITY MANAGEMENT

The PI is responsible for ensuring that all AEs, SAEs and pregnancies are identified, evaluated, recorded and reported in a timely manner as per hVIVO's, HMR'sor Sponsor's SOPs (as required), and also for ensuring that the medical management (including follow-up) of AEs, SAEs and, where appropriate, pregnancy symptoms/complications is provided by competent Investigator Site staff.

15.1 Definitions

15.1.1 Adverse event

Defined as 'any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product'[4].

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product', (or for the purposes of Human Viral Challenge studies, the Challenge Virus).

An AE includes:

- Exacerbation of a pre-existing illness.
- Increase in frequency or severity of a pre-existing episodic condition.
- A condition detected or diagnosed after IMP or inoculum administration even though it may have been present prior to the start of the study.
- Any malfunction or deterioration in the characteristics and/or performance of a device, and any inadequacy in the labelling or the instructions for use, which might lead to or might have led to endangering the health of a patient or the device user.
- A complication that occurs during a hospitalisation.

An AE does not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure are an AE.
- Pre-existing disease or conditions present or detected prior to start of IMP (when applicable) or Challenge Virus inoculation administration that does not worsen (including screening findings such as abnormal laboratory results).
- Hospitalisation for elective surgery, social and/or convenience admissions provided they are arranged before the start of IMP administration.
- Overdose of either study treatment or concomitant medication without any signs or symptoms.
- Typical/normal viral symptoms on FLU-PRO Symptom Questionnaire (see Section 15.2.1).

15.1.2 Adverse reaction

An adverse reaction (AR) is any untoward and unintended response in a subject to an IMP which is related to any dose administered to that subject.

'Response' in this context means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility[37].

All AEs judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey in general that there is evidence or argument to suggest a causal relationship.

15.1.3 Unexpected adverse (drug) reaction

An 'unexpected adverse (drug) reaction' is an 'adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out-:

(a) In the case of a product with a marketing authorisation, in the summary of product characteristics for that product,

(b) In the case of any other investigational medicinal product, in the Investigator's Brochure relating to the trial in question'[4].

15.1.4 Serious adverse event, serious adverse drug reaction and unexpected serious adverse (drug) reaction

A serious adverse event (SAE), serious adverse (drug) reaction (SAR) or unexpected serious adverse reaction is any AE, adverse reaction or unexpected adverse reaction, respectively, that -

(a) results in death,

(b) is life-threatening,

(c) requires hospitalisation or prolongation of existing hospitalisation,

(d) results in persistent or significant disability or incapacity, or

(e) consists of a congenital anomaly or birth defect

The above characteristics/consequences have to be considered at the time of the event, for example:

The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which, hypothetically, might have caused death if it were more severe[39].

'Important medical events' - some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered as 'serious' in accordance with the above definition.

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious. Important adverse events/reactions that are not immediately life threatening or do not result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above occurring, should also be considered serious. Details of the SAE must be provided.

15.1.5 Suspected unexpected serious adverse reaction

A suspected unexpected serious adverse reaction (SUSAR) is 'a serious adverse reaction, the nature and severity* of which is not consistent with the information about the medicinal product in question, as defined in the Summary of Product Characteristics for that product in the case of a product with authorisation, or in the Investigator's Brochure relating to the trial in question in the case of any other investigational medicinal product'.

*The term 'severity' is used here to describe the intensity of a specific event. This is not the same as 'serious' which is based on subject/event outcome or action criteria.

15.2 AE reporting

AEs will be captured from the time informed consent has been signed by the subject until the subject's last study follow-up visit.

Periodically during the study, the Investigator should enquire about the occurrence of AEs and use of concomitant medication.

The following are examples of open ended, non-leading questions that may be used to obtain this information:

How are you feeling?

Have you had any medical problems since your last visit/assessment?

Have you taken any new medicines, other than those given to you in this study, since your last visit/assessment?

Following the reporting of AEs and concomitant medication, the Investigator should assess the subject's eligibility to continue in the study.

The PI or delegated investigator will record all relevant information regarding an AE/SAE in the source documents and evaluate AEs using the following guidelines:

- Description of events (if the event consists of a cluster of signs and symptoms, a diagnosis should be recorded)
- Seriousness
- Severity (or grade)
- Onset date and time
- Frequency
- Date and time of resolution (or 'continuing' if unresolved)
- Action taken
- Concomitant medication
- Clinical outcome
- Relationship or causality (IMP/Challenge Virus/ study procedures/ concomitant medication/other).

Any clinically significant abnormal laboratory result, vital sign, or other measure will be followed until it returns to normal or baseline values, stabilises, or is judged by the Investigator to be no longer clinically significant.

If an AE is not resolved at the end of the study, the AE should be followed until it has resolved or (in the case of pregnancy) the pregnancy has been terminated (including spontaneous abortion)/resulted in a birth, or a decision has been made by the Sponsor that no further follow-up is required.

15.2.1 Challenge Virus symptoms

The Investigator will assess and review Challenge Virus related symptoms recorded on subjects' FLU-PRO Symptom Questionnaire. FLU-PRO Symptoms greater than Grade 0 will be expected and presumed to represent virus infection consequent to Viral Challenge, and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs.

Following Viral Challenge all unexpected (in the opinion of the Investigator), post-Viral Challenge symptoms will be captured as AEs, along with all other occurrences that meet the criteria for an AE.

15.2.2 IMP related adverse events symptoms

Following vaccination with IMP, diary card symptoms are not reported as individual adverse events unless they are deemed to be clinically significant (i.e. progress past 7 days) in the opinion of the investigator and / or SME.

15.2.3 Complete Physical examination

Any clinically significant change in complete physical examination findings during the study will be documented as an AE.

15.2.4 Directed physical examination

Following Viral Challenge, upper and lower respiratory symptoms (nasal discharge, otitis, pharyngitis, sinus tenderness, new wheezes, râles and rhonchi) will be expected and presumed to represent virus infection consequent to Viral Challenge and will not be additionally captured as AEs unless they meet the definition of an AE, and are deemed to be clinically significant (in the opinion of the Investigator) to be classed as AEs.

Following Viral Challenge all unexpected (in the opinion of the Investigator) post-Viral Challenge directed physical examination findings will be captured as AEs, along with all other occurrences that meet the criteria for an AE.

15.2.5 Vital signs

Deterioration in a vital sign (compared to baseline) should only be reported as an AE if the deterioration fulfils the criteria for an AE. If deterioration in a vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated vital sign will be considered as additional information.

15.2.6 Temperature

Following Viral Challenge, pyrexia will be expected and presumed to represent virus infection consequent to Viral Challenge, and will not be additionally captured as an AE unless it meets the definition of an AE, and is deemed to be clinically significant (in the opinion of the Investigator) to be classed as an AE.

Following Viral Challenge all unexpected (in the opinion of the Investigator) post-Viral Challenge pyrexia will be captured as an AE, along with all other occurrences that meet the criteria for an AE.

15.2.7 Spirometry

A 15% drop in a spirometry value (compared to baseline) will be a Grade 1 (mild) AE. The PI will use his/her clinical judgement to assign severity to drops in spirometry values of greater than 15%, based on evaluation of clinical signs and symptoms.

15.2.8 Laboratory values

Deterioration in a laboratory value (compared to baseline) should only be reported as an AE if the deterioration fulfils the criteria for an AE and it is clinically significant in the opinion of the

Investigator. If deterioration in a laboratory result is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result will be considered as additional information.

The Investigator will judge whether abnormal laboratory values are clinically significant or not clinically significant, and record this in the source document. This entry should be signed and dated by the relevant investigator. Laboratory abnormalities detected at screening will be considered as part of the medical history and will not be reported as AEs.

Challenge Virus associated laboratory abnormalities (e.g., elevated ALT, AST or GGT; decreased neutrophils) may be recorded as AEs (at the discretion of the Investigator).

Any laboratory abnormality that meet the criteria of a Grade 2 AE or above will be discussed with the SME. If a Grade 3 laboratory abnormality occurs (not related to Viral Challenge), a confirmatory test should be performed, preferably within 48 hours (if practically feasible) after the results have become available.

15.2.8.1 C-reactive protein

Any value above 5 mg/L but less than 60 mg/L will be a Grade 1 (mild) AE (unless deemed non clinically significant by the PI). The PI will use his/her clinical judgement to assign severity grades above Grade 1, based on evaluation of clinical signs and symptoms.

15.3 Classification of adverse events

15.3.1 Seriousness

The PI must record whether the AE meets the definition of serious. If the event is serious (Section 15.3.1), the PI must initiate reporting to the Sponsor and complete a SAE report form (Section 15.4).

15.3.2 Severity

The term ’severe’ is often used to describe the intensity (severity) of a specific event. This is not the same as ‘serious’ which is based on subject/event outcome or action criteria.

Appendix 4: Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table November 2007) will be used to standardise the severity and grading of AEs and anatomical/ pathophysiological terms.

The PI will use these tables as a reference when collecting, reporting and clarifying database queries of AEs, SAEs and ARs.

The severity of an AE that does not appear in the DMID tables should be determined according to the definitions in Table 15-1.

Table 15-1: Classification of adverse event severity

Grade	Classification	Definition
Grade 1	Mild	Mild level of discomfort, and does not interfere with regular activities
Grade 2	Moderate	Moderate level of discomfort and significantly interferes with regular activities
Grade 3	Severe	Significant level of discomfort and prevents regular activities

The DMID AE severity Grade 4 (life threatening) will not be used when assessing AE severity in this trial, because the term 'life threatening' does not relate to severity but to consequence. AEs can be classed, as 'life threatening', and all life threatening AEs must be reported to the Sponsor and NIH as SAEs (as per the definition of a SAE). DMID is a draft internationally recognised tool and is used as best practice guidance.

15.3.3 Frequency

The frequency of the AE should be categorised as one of the following:

- Single
- Intermittent
- Continuous.

15.3.4 Relationship

The relationship of an AE to the IMP will be categorised as shown in Table 15-2.

Table 15-2: Classification of adverse event relationship

Classification	Definition
Not related	The AE is related to an aetiology other than the IMP (the alternative aetiology must be documented in the subject's medical record).
Unlikely to be related	The AE is unlikely to be related to the IMP and likely to be related to factors other than IMP.
Possibly related	There is an association between the AE and the administration of the IMP, and there is a plausible mechanism for the AE to be related to the IMP, but there may also be alternative aetiology, such as characteristics of the subject's clinical status or underlying disease.

Probably related	A reasonable temporal sequence of the AE and the IMP administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the AE with the IMP seems likely.
Definitely related	A definite causal relationship exists between the AE and the administration of the IMP, and other conditions do not appear to explain the AE.

Unless an AE is 'definitely related' to the IMP, a causal relationship to one of the following should be considered, and full details provided on the AE reporting form as appropriate.

- Challenge Virus
- Study procedures
- Concomitant medication
- Other.

15.3.5 Action taken

The Investigator should ensure that adequate medical care is provided to subjects for any AEs, including clinically significant laboratory values related to the IMP. In addition, the Investigator will describe whether any treatment was given for the AE.

The Investigator will classify the action taken with regard to the AE. The action taken should be classified according to the following categories and full details provided as appropriate:

- None
- Non-drug therapy given
- Concomitant medication taken
- IMP dose not changed
- IMP dose adjusted
- IMP administration temporarily interrupted
- IMP administration permanently discontinued
- Subject withdrawn
- Subject hospitalised
- Other.

15.3.6 Outcome

An AE should be followed until the Investigator has determined and recorded the outcome or an alternative explanation. The outcome should be classified according to the categories shown in Table 15-3.

Table 15-3: Classification of adverse event outcome

Classification	Definition
Resolved	Resolution of the AE with **no** residual signs or symptoms
Resolved with sequelae	Resolution of the AE with residual signs or symptoms
Discharged to GP	AE considered stable or not anymore clinically significant and not requiring further follow up
Ongoing	Either incomplete improvement or no improvement of the AE, such that it remains on-going
Fatal	Outcome of the AE was death. 'Fatal' should be used when death was at least possibly related to the AE.
Unknown (Lost to follow-up)	Outcome of the AE is not known

15.3.7 Follow-up

Any AEs ongoing at the end of the study will be classed as ongoing and where appropriate the subject will be referred to the subject's GP or other healthcare professional.

Additional measurements and/or evaluations may be necessary to investigate the nature and/or causality of an AE or SAE - this may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. If the subject dies, any post-mortem findings (including histopathology) will be provided to the Sponsor if possible.

15.4 Serious adverse event reporting

All SAEs must be reported by the PI to the SME as soon as possible, and at the latest within 24 hours of becoming aware of the event. Upon becoming aware of a SAE, an initial phone call should be made as soon as possible by the PI to the SME in order to notify the Sponsor and NIH of the occurrence of a SAE.

Following the telephone notification, the PI must fully and accurately complete the SAE Initial Reporting Form. Only one SAE must be recorded on each form. The completed SAE Initial Reporting Form (including any supporting documents such as the subject's notes, concomitant medication logs and test results) must be e-mailed by the PI to the SME and the study Project Director/Project Manager at the latest within 24 hours of becoming aware of the event.

The PI must also complete the SAE Follow up Report Form.

The completed Sponsor Follow up Report Forms (including any supporting documents such as the subject's notes and test results) must be e-mailed by the PI to the SME and study Project Director/Project Manager within 5 days of the PI becoming aware of the event. Adherence to this timeline is crucial in order to ensure that any required reporting to the relevant REC and regulatory authorities takes place in a timely manner.

Further Follow Up Report Forms may need to be emailed to the SME and the study Project Director/Project Manager by the PI, for data/information collected later than 5 days of the PI becoming aware of the event, until:

The SAE has resolved, or the Sponsor has made a decision that no further follow up is required.

Table 15-4: Contact details for reporting SAEs

Contact	Details
Sponsor's Medical Expert (SME)	Dr Bryan Murray Email: bryan.murray@seekacure.com
SME Mobile Number	+ 44 (0)7833 204296

15.4.1 Reporting of SUSARs

The Sponsor is responsible for assessing SUSARs, unblinding potential SUSARs, and reporting SUSARs to the MHRA and REC as appropriate. NIH are responsible reporting to FDA if related to the virus.

The Sponsor shall ensure that all relevant information about a SUSAR that occurs during the course of a clinical trial in the UK and is fatal or life threatening is reported as soon as possible to the MHRA and the REC. This needs to be done within 7 calendar days after the Sponsor became aware of the event.

The Sponsor shall ensure that a SUSAR which is not fatal or life threatening is reported as soon as possible and in any event within 15 calendar days after the Sponsor became aware of the reaction.

Any additional relevant information should be sent within 8 days of the first report being sent.

15.4.2 Adverse reactions to non-IMPs

A SAE caused by a non-IMP would meet the stopping criteria for the study (Section18.4.1). In such a case Table 18.1and the procedure for early termination should be followed in line with Section 18.4.1)

A SAE caused by the Challenge agent would meet the stopping criteria for the study (Section 18.4.1). In such a case Table 18.1 and the procedure for early termination should be followed in line with Section 18.4.1.

15.4.3 Post-quarantine AEs and SAEs

All SAEs that occur during the follow-up period must be reported by the Investigator to the Sponsor as soon as possible, in accordance with the Sponsor's SOPs, and at the latest within 24 hours of becoming aware of the event.

15.4.4 Pregnancy

If a female subject or partner of a male subject becomes pregnant within 3 months after the study, the pregnancy must be reported by the Investigator to the Sponsor and Study Monitor by telephone as soon as possible, in accordance with the Sponsor's SOPs, and at the latest within 24 hours of becoming aware of the event.

Following the telephone notification, the Investigator must fully and accurately complete the appropriate pregnancy reporting form, which must be e-mailed to the Sponsor and the Study Monitor the latest within 24 hours of becoming aware of the pregnancy.

Subjects will be advised to contact their GP or a specialist, as appropriate.

Consent for follow-up of the pregnancy and pregnancy outcome will be sought from the pregnant study subject or the pregnant partner of the male study subject as applicable. Consent for follow-up will be documented on a Sponsor Pregnancy Follow-up ICF.

Provided that appropriate consent is in place, information related to the pregnancy will be collected as per the Sponsor's SOPs. The completed reporting form(s) will be sent to the Sponsor for review and assessment, and subsequent reporting as required.

A complete evaluation will be documented in the source data to permit transfer to the clinical database. The emergency code break envelopes will be requested (Section 10.4) to break the blind for the appropriate study subject to ensure that further care can be based on the actual identity of the study treatment that the subject received.

hVIVO will maintain contact with the subject for a protracted period of time, but certainly until after the birth, in order to assess for outcomes that may be reportable as related AEs, and for reporting to the Sponsor as appropriate.

hVIVO in consultation with the subject will keep the subject's GP informed.

All cases of foetal drug exposure via the parent as a study participant will be reported to the Sponsor and the REC.

16 STATISTICAL METHODS AND PLANNED ANALYSES

The unblinded statistician will perform the statistical analysis for this study.

16.1 Study Hypothesis

The hypothesis is that vaccination with 1 dose or two doses of FLU-v + adjuvant will reduce MMID when compared to individuals vaccinated with placebo.

16.2 Sample size

The primary objective of this study is to examine whether there is a difference in the MMID rates post inoculation between either of the 2 FLU-v arms and placebo. A sample size of 41 subjects per arm (total 123) will provide 80% power to detect a difference in MMID rates of .70 versus .40 using a one sided 0.05 significance level. The table below shows the power that the study would have with 41 patients per arm for other MMID rates. The sample size calculations do not adjust for multiple comparisons.

Table 1. Power with 41 subjects per arm with various MMID probabilities

Probability of MMID in placebo	Probability of MMID in Vaccinated arms	Power
.70	.40	.80
.70	.44	.70
.70	.47	.60
.60	.30	.80
.60	.34	.70
.60	.37	.59
.50	.21	.81
.50	.25	.68
.50	.28	.56

In this study the main comparisons are for each of the FLU-v dose groups to placebo. The study will also compare the FLU-v dosing arms but the study will not have adequate power to detect a significant difference in MMID rates at the predicted treatment rates. This study does not control for multiple comparisons since we are trying to maintain a small sample size but yet have high power to detect the pre-defined primary objective of efficacy versus placebo when FLU-v is truly beneficial. All comparisons will be made at the one sided 0.05 significance level.

16.3 Interim analysis

No interim analysis is planned for this study

16.4 FLU-v Statistical Analysis Plan

MMID rates in the 2 FLU-v groups and placebo group will be compared using a Fishers exact test. 95% confidence intervals around the difference in rates between the FLU-v groups will be presented. Adverse event rates between groups will also be performed using Fishers exact test. The secondary endpoints will be examined by calculating the median and interquartile range and then performing a two-sided Wilcoxon Rank Sum Test at the 0.05 significance level.

The SAP will be developed by the study team prior to locking of each relevant cohort. The SAP will give a more detailed description of the report presentations to be produced, expanding on the protocol specified analysis. Any deviation from the protocol specified analysis will be documented within a protocol or SAP amendment, as appropriate.

The SAP will describe and account for the occurrence of and extent of missing data, and its possible impact on the study analysis. All baseline assessments will be described in the SAP.

Analysis and reporting of exploratory endpoints may be separate to the clinical study report.

16.4.1 Subject accountability

The number of subjects randomised to FLU-v-004 or placebo and receiving FLU-v-004 or placebo, receiving Challenge Virus, withdrawing from (also split by reason for withdrawal), and completing the study, and the numbers in each analysis set will be summarised by treatment group and across all subjects.

16.4.2 Protocol deviations

Subject data will be reviewed for major protocol deviations prior to database lock at a planned blinded data review meeting (BDRM), and decisions will be documented within the meeting minutes. At this meeting, subjects will be reviewed for their inclusion/exclusion from the analysis sets.

16.4.3 Demographic and baseline characteristics

Demographics and characteristics for each of the three study groups will be summarised in a table.

16.5 Primary analysis

The primary analysis will be performed to evaluate the MMID rate in each of the three groups. This will be based on the physician assessment (presence or absence of symptoms) and the influenza PCR performed on nasal swab (demonstrating viral shedding). MMID rates in the 2

FLU-v groups and placebo group will be compared using a Fishers exact test. 95% confidence intervals around the difference in rates between the FLU-v groups will be presented.

16.6 FLU-v Secondary analysis

A secondary analysis will be performed to examine the secondary endpoints for this study. Adverse event rates between the three groups will be compared using Fishers exact test. The days of shedding, days of symptoms, number of symptoms, and FLU-PRO scores between will be examined by calculating the median and interquartile range for all three groups and then performing a two-sided Wilcoxon Rank Sum Test at the 0.05 significance level.

16.7 Safety Analyses

16.7.1 Adverse events

Adverse event terms will be coded using MedDRA. Treatment emergent AEs will be separately reported within summary presentations, by MedDRA system organ class (SOC) and preferred term, and by treatment group. An AE will be classified as treatment emergent if the onset date of the AE is on or after the start of IMP dosing. In the case of partial dates being recorded, a conservative approach will be adopted for inclusion of such events within these reporting periods. These definitions will be further detailed within the SAP.

If a subject experiences more than one AE with the same preferred term (within reporting period being considered) that preferred term will be counted only once in summary presentations. It will be assigned the worst observed severity and the strongest relationship to IMP among those events for summaries in which those characteristics are considered.

Summary presentations will be produced for the number and percentage of subjects reporting treatment emergent AEs, AEs by severity, and AEs related to IMP. In addition, SAEs and AEs directly resulting in withdrawal from study will be listed.

16.7.2 Laboratory parameters

Time point and treatment group for absolute laboratory parameters (haematology, biochemistry, coagulation, cardiac enzymes, and thyroid function tests) will tabulate summary statistics for absolute values and changes from baseline. Laboratory values outside the normal range will be identified in subject listings.

16.7.3 Physical examination

Physical examination findings (complete and directed examinations) will be included within subject listings.

16.7.4 Concomitant medications

Concomitant medication terms will be coded using the WHO Drug Dictionary Enhanced. Medications will be assigned as being prior to IMP dosing, or concomitant with IMP dosing based on start and stop dates of the medication and the timing of receiving IMP.

If the medication stop date is before the date of IMP (when applicable), the medication will be assigned as being prior to IMP. In all other situations, the medication will be assigned as being concomitant with IMP. Prior and concomitant medications (separately identified) will be included in subject listings.

17 STUDY FILES AND CLINICAL SOURCE DOCUMENTATION

17.1 Investigator's Study File

The PI will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents will be managed in accordance with hVIVO's SOPs.

17.2 Clinical source documentation

Study data management will be conducted in accordance with hVIVO or NIH/Sponsor SOPs (as required).

All data capture will be maintained in a manner compliant with the current regulations and guidance.

In cases where data are captured as hard copy e.g., ECG reports or spirometry read outs, the hard copy data will become part of the subject's source documentation. The original paper source will be maintained to enable source data verification by the Sponsor's monitor.

Source documents will be checked for accuracy and completion and will undergo quality control (QC) by hVIVO.

17.3 Data capture

The hVIVO external data management vendor (with input from the appropriate hVIVO site staff if required) will prepare and provide an electronic case report form (eCRF) for each study subject. hVIVO personnel will transcribe the study data from source documents into the eCRF in accordance with the data management vendor's eCRF completion guidelines and the agreed timelines. The eCRF will be transmitted in a secure manner to the Sponsor within the agreed timeframe.

hVIVO staff will ensure that data recorded in the eCRFs (i.e., when there is no prior written or electronic record of data) and the source data are available for review at each scheduled monitoring visit.

The Source Data Agreement and Source Data Management Plan will provide details of what data is deemed to be source and any specific information of the handling and transcription processes performed.

Data captured in electronic formats (e.g., laboratory results or electronic ECGs) will be transferred to the Sponsor's data management company via secure transfer as outlined in the Data Transfer Agreement (DTA).

All entries, corrections, and alterations of the eCRF or source data will only be made by the PI or other authorised study-site personnel.

17.4 Data quality assurance and quality control

Measures will be taken to ensure the accuracy and reliability of data and will include:

- Selection of qualified investigators and appropriate study sites
- Review of protocol procedures with the PI and study-site personnel prior to the study
- Periodic monitoring visits by the Sponsor
- Direct transmission of clinical laboratory data from a central laboratory into the Sponsor's database.

Written instructions will be provided for collection, handling, storage, and shipment of samples.

The Sponsor's monitor will review the study data for accuracy and completeness during on-site monitoring visits. Any discrepancies will be resolved with the PI or designee as appropriate. After upload of the data into the clinical study database, data will be verified for accuracy and consistency with the data sources.

17.5 Data coding

Adverse events and medical histories will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version: 19.0, March 2016.

Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary Enhanced version: March 1st, 2016.

Should a new version of either dictionary be released prior to any coding of the study data, the new version will be used for coding and the version number will be documented in the DMP.

If any coding of the study data has already been performed, the existing version of the MedDRA will be used and the new version will not be used.

17.6 Database lock

The Sponsor's database will undergo validation prior to database lock.

Following the Sponsor's locking of the database the final database will be transferred to the Study Statistician for statistical analysis and reporting.

17.7 Data protection

The study site will comply with the EU Data Protection Directive (95/46/EC)[40], as transposed into UK legislation via the UK Data Protection Act 1998[5]. The Sponsor agrees to comply by the principles of the US – European Safe Harbor Framework[41] to bridge the differences in the privacy approaches for data protection law.

18 STUDY MANAGEMENT AND ETHICAL RESPONSIBILITIES

18.1 Regulatory approval and Good Clinical Practice

This study will be conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki 1996[1], the principles of ICH GCP[2], current regulatory requirements as detailed in the Medicines for Human Use (Clinical Trial) Regulations (SI 2004/1031)[4] and all subsequent amendments, the UK Data Protection Act 1998[5], any other applicable laws and guidances, and any Sponsor requirements.

All ethical and legal requirements will be met before the first subject is enrolled in the study.

18.2 Deviations from the protocol

A protocol deviation log will be used to document any unplanned or unintended departures from the study protocol. Protocol deviations should be recorded as soon as possible after they occur, and if significant, reported to the Sponsor immediately.

The PI or delegate will complete the protocol deviation log.

18.2.1 Protocol amendments

The Sponsor will not alter the study protocol without obtaining written prior agreement from the PI. For all amendments, an evaluation will be made by the Sponsor as to whether the amendment is 'substantial' or 'non-substantial'.

Non-substantial amendments include for example, minor administrative or typographical changes to the protocol.

Amendments are 'substantial' where they are likely to have a significant impact on subject risk or the clinical trial objectives, specifically the:

1. Safety or physical or mental integrity of the subjects
2. Scientific value of the trial
3. Conduct or management of the trial
4. Quality or safety of the IMP used in the trial.

Substantial amendments must be reviewed and approved by the MHRA and REC prior to their implementation unless subject safety would be otherwise compromised (i.e., Urgent Safety Measures [USMs]).

When a protocol amendment substantially alters the clinical trial design or the potential risks or burden to subjects, the VIS/ICF will also be amended and approved by the REC, and all active subjects will be asked to reconfirm their continued willingness to participate in the trial.

A log of all protocol amendments will be maintained together with their designation as substantial or non-substantial.

18.2.2 Urgent Safety Measures

The Sponsor, NIH and the Investigator may take appropriate 'urgent safety measures' to protect clinical trial subjects from any immediate hazard to their health and safety.

Urgent safety measures should be taken immediately. There is no need to wait for MHRA approval before implementing urgent safety measures; however, the MHRA and REC must be informed in writing in the form of a substantial amendment within 3 days.

The Sponsor should telephone the Clinical Trial Unit at the MHRA and discuss the issue with a safety scientist immediately. Should further clarification be required the Sponsor will be contact by a medical assessor.

The Sponsor must notify the MHRA and the REC in writing, of the measures taken and the reason for the measures within 3 days. This notification should include a covering letter detailing the measures taken, the reason for them, an Annex II substantial amendment form and any supporting documentation.

If the PI (and not the Sponsor) has instigated the USM, the Sponsor should be notified immediately so that they can assess and report the USM within the timelines required.

18.2.2.1 Pandemic

For any period during which a disease is pandemic and a serious risk to human health or potentially a serious risk to human health, notice of USMs taken in order to protect the subjects of a clinical trial against any immediate hazard to their health or safety and the circumstances giving rise to those measures, may be given 'as soon as possible' to the MHRA and the REC.

Written notification to the MHRA and the REC should be provided within 3 days in the form of a substantial amendment and should describe the event, the measures taken and justification for the measures taken.

18.3 Serious breach of the protocol or GCP

Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [SI 2004/1031][4], as amended by SI 2006/1928[42], contains a requirement for the notification of 'serious breaches' of GCP or the trial protocol:

29A.

(1) The sponsor of a clinical trial shall notify the licensing authority in writing of any serious breach of -
(a) the conditions and principles of GCP in connection with that trial; or
(b) the protocol relating to that trial, as amended from time to time in accordance with regulations 22 to 25,

within 7 days of becoming aware of that breach.

(2) For the purposes of this regulation, a 'serious breach' is a breach which is likely to effect to a significant degree –
(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial'.

Any potential serious breach of the conditions and principles of GCP in connection with the study will be escalated within hVIVO as soon as an individual becomes aware of the potential breach. Rapid escalation will permit the urgent notification to the Sponsor and allow sufficient time for the Sponsor to fulfil the reporting timelines for the potential breach, as specified in UK Regulation 29A [42], should reporting be necessary.

The Sponsor will be responsible for notifying the MHRA within 7 days of becoming aware of any potential serious breach.

18.3.1 Protocol waivers

Protocol waivers are never acceptable as per all EU competent authorities' guidance (including the MHRA). It is not acceptable to include any subject by protocol waiver – i.e. included despite not meeting all inclusion criteria or fulfilling at least one exclusion criteria.

18.4 Discontinuation of the study

The Sponsor reserves the right to temporarily suspend or discontinue the study for any reason at any time. In addition, the study may be stopped at any time if, in the opinion of the Sponsor or the SME, safety data suggest that the medical safety of subjects is being compromised.

The Sponsor reserves the right to terminate the study for refusal of the PI to supply source documentation of work performed in this clinical study.

The Sponsor is responsible for informing the REC of the study termination. If the study is suspended or terminated for safety reason(s), the Sponsor will promptly inform the PI, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action.

If the study is prematurely terminated, all study data must be returned to the Sponsor. In addition, the site must conduct final disposition of all unused IMPs in accordance with the Sponsor's procedures for the study.

The study may also be stopped at any time if, in the opinion of the Investigator, or Sponsor, safety data suggest that the safety of subjects is being compromised. Study stopping criteria are detailed in Section 18.4.1.

These notifications will occur within 15 days of the termination/suspension. Routine termination of the study, in line with the end of trial as defined in Section 9.5, will occur within 90 days of the termination, with responsibilities for those notifications as outlined above. Termination of the clinical trial may also be initiated by the MHRA or the REC.

18.4.1 Stopping criteria

The study will be terminated if:

- One or more subjects experience any drug related SAEs or 2 or more subjects experience severe or clinically significant AEs considered to be at least possibly related to the FLU-v-004 therapy.
- One or more subjects experience any severe or clinically significant illness from Influenza inoculation.

The PI and the SME will perform safety reviews on available clinical and virology data as appropriate during the quarantine period.

Three clinical scenarios relating to the incidence of AEs and SAEs during the study and the procedures that should be performed in each case are presented in Table 18-1: Study stopping criteria.

Table 18-1: Study stopping criteria

Status	Criterion	Procedure
1	A report has been received of one (or more) IMP-related SAEs in any one (or more) subject(s).	If such a status occurs at any point during the study then further administration of IMP will not take place and the PI and the SME will review the data and make decisions on study continuation or termination. Subject follow-up should continue until resolution or stabilisation of AEs.
2	No IMP-related SAEs have been reported but an overall pattern of clinical changes or symptoms exists, attributed to the IMP, which may appear minor or moderate in terms of individual AEs but which collectively represent a concern for safety.	If such a status occurs at any point during the study then the PI and the SME will review the data and make a decision on study continuation or termination. Subject follow-up should continue until resolution or stabilisation of AEs.
3	Virus-related SAEs or virus-related AEs of clinical concern have been reported following Human Viral Challenge.	Subject follow-up should continue until resolution or stabilisation of all such AEs and final follow-up on Day 28 (± 5 d).

In any event, subject follow-up should continue until resolution or stabilisation of AEs and final follow-up on Day 63 (± 5 d). The exception to this would be suspension of the study for an USM (Section 18.2.2).

18.5 Study records retention and direct access to source documents

Data will be collected, reviewed and managed throughout the study as outlined in Section 17 and hVIVO's and Sponsor's SOPs. The PI shall keep a copy of the source data, the ISF and source documents, as specified in the Clinical Trial Agreement (CTA), until notified otherwise by the Sponsor.

hVIVO agrees to allow inspections of the study site and any source documentation by clinical research and audit personnel from the Sponsor, external auditors or representatives of the MHRA or REC, and will allow direct access to source data and documents.

Direct access to the subject's clinical records is necessary to verify and corroborate the data recorded in the process of source data verification.

During the review of records and documents, the anonymity of the subject will be respected with strict adherence to professional standards of confidentiality. All monitoring activities should be performed in accordance with the Clinical Monitoring Plan for the study.

Source documentation management is described in the DMP. The PI must retain all documents as listed in Section 8 of the ICH consolidated guideline on GCP (essential documents for the conduct of a clinical trial)[2].

18.5.1 Archiving

Paper and electronic records generated by hVIVO during the study will be archived as agreed in the CTA and in accordance with the applicable regulations and Sponsor requirements.

18.6 Sponsor responsibilities

18.6.1 General

The Sponsor agrees to adhere to the study protocol, and to comply with the principles of ICH GCP[2], the Declaration of Helsinki 1996[1], the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trial) Regulations (SI 2004/1031)[4], and all subsequent amendments, the UK Data Protection Act 1998[5] and any other applicable regional and local regulations.

The Sponsor has a legal responsibility to obtain MHRA approval to perform the study and to report the results of the study in full to the MHRA. A copy of the MHRA approval will be provided to the PI before the start of the study.

18.6.2 Ethical considerations

The Sponsor will submit the protocol, VIS/ICF, IB and any recruitment material to the REC for their review. Written approval of these documents must be received from the REC before the first subject is recruited.

The Sponsor will notify the REC of any protocol amendments as described in Section 18.2.1. Written approval must be obtained from the REC for all substantial amendments to the protocol, prior to their implementation, except when necessary to eliminate apparent immediate hazard to the subject (Section 18.2.2).

The Sponsor should also obtain a written statement of the composition of the REC. Appropriate reports on the progress of the study will be made in accordance with local regulatory practices.

IMP can only be supplied to the PI after documentation of all ethical and legal requirements for starting the study has been received by the Sponsor.

The REC and MHRA will be informed about the end of the trial with the required timelines.

In the event of early termination this is 15 days from trial completion and the reason for termination is required.

In the case of routine termination, this is 90 days from the protocol-defined end of trial (Section 9.5).

18.6.3 Laboratory certification and normal values

The Sponsor will provide the PI with the name and location of the clinical laboratory(ies) used for laboratory tests, plus a copy of the certification for all laboratory tests included in the protocol, certification number(s), date(s) of certification, and a list(s) of the normal values for all laboratory tests required by the protocol. These documents must be available prior to any subject being treated in the study. Updated versions of these documents must be provided as appropriate.

18.6.4 No-fault compensation and indemnity

The Sponsor will provide 'no-fault' compensation insurance against any risk incurred by a subject because of his/her participation in the study. The Sponsor will indemnify the PI (as detailed in a separate document). The indemnity will only apply where all study procedures have been carried out according to this protocol.

The PI confirms that he/she holds his/her own professional indemnity insurance, suitable for the activities of the Investigator(s) in relation to the study and, in addition, that this insurance satisfies any local insurance requirements, or he/she is a member of a medical union which provides such cover for its members.

The Sponsor has made the following arrangements for payment of compensation in the event of harm to the subjects, where no legal liability arises as follows:

If the subject suffers any side effect or other physical injury resulting directly from the study medication, the Sponsor will pay for the reasonable costs of necessary medical treatment to the extent permitted by the laws of the UK if:

- the subject took the study medication as directed by the Investigator
- the subject's injury was not deliberately caused
- the Investigator was immediately notified about the subject's injury; and
- the medical advice of the Investigator was followed.

If the subjects are caused any injury directly by their participation in the study, the Sponsor will compensate the subject in accordance with the guidelines laid down by the Association of the British Pharmaceutical Industry (ABPI)[43] in providing compensation for any harm or ill health incurred. The Sponsor has insurance to cover study-related injuries.

18.6.5 Monitoring

The Study Monitor will contact the site prior to the start of the study to discuss the protocol and data collection procedures with site personnel. In accordance with applicable regulations, ICH GCP, and the procedures of the Sponsor, the Study Monitor will also periodically contact the site and conduct on-site visits. The Study Monitor will ensure that all monitoring visits are conducted according to protocol and regulatory requirements.

During contacts with the study site, the Study Monitor's activities will include:

- Checking and assessing the progress of the study
- Reviewing study data collected to date for completeness and accuracy
- Conducting source data verification
- Identifying any issues and addressing resolutions.

These activities will be done in order to verify that the:

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

The PI will allow the Study Monitor direct access to all relevant documents and records, and allocate his/her time and the time of his/her staff to the Study Monitor to discuss findings and any relevant issues.

At study closure, study monitors will conduct all activities as indicated in Section 18.6.5.

18.6.6 Audits and inspections

At its discretion, the Sponsor may conduct a QA audit of this study. The Sponsor's auditing procedures will be followed in order to comply with GCP guidelines and ensure acceptability of the study data for registration purposes. If such an audit occurs, the PI will give the auditor direct access to all relevant documents, and will allocate his/her time and the time of his/her staff to the auditor as may be required to discuss findings and any relevant issues.

In addition, regulatory agencies (e.g., MHRA) may conduct an inspection of the study. If such an inspection occurs, the PI will allow the inspector direct access to all source documents and other study documentation for source data check and/or on-site audit inspection. The PI must allocate his/her time and the time of his/her staff to the inspector to discuss findings of any relevant issues.

18.6.7 Annual Safety Report and Development Safety Update Report

The Sponsor is required to submit an Annual Safety Report (ASR) and a Development Safety Update Report (DSUR) to the MHRA and the REC once a year throughout the clinical trial or on request. The DSUR will take into account all new available safety information received during the reporting period.

18.7 Investigator responsibilities

18.7.1 Informed consent procedure

Potential subjects are typically sent a copy of the volunteer information sheet (VIS) and informed consent form (ICF) when their screening visit is arranged, and are encouraged to read it prior to their appointment.

Upon arrival at the screening visit, subjects read the information sheet and consent form with a study nurse or the Investigator or delegate present. They are given the opportunity to ask any questions, and can take the information sheet away for at least 24 hours to consider their participation.

All subjects are required to have a good understanding of English and it is the Investigator or delegate's responsibility to ensure that the subject understands the information contained in the VIS/ICF. Once the Investigator or delegate has confirmed that the subject has understood the study, including the benefits and risks of participation, the subject and the Investigator are able to sign and date the ICF.

The ICF must be signed and dated by the subject and countersigned by the Investigator or delegate (whoever conducted the consent discussion). The VIS/ICF will be copied and filed within the subject notes; the original will be held in the ISF and a copy will be given to the subject.

Subjects will be assured that they can withdraw from the study at any time and for any reason without prejudice to their future medical care. They will also be informed in a timely manner if new information becomes available that may affect their willingness to continue participation in the study. The communication of this information must be documented. If the VIS/ICF is amended during the study, the PI must follow all applicable regulatory requirements pertaining to approval of the amendment by the REC. The site must use the amended VIS/ICF for all new subjects and repeat the consent process with the amended VIS/ICF for any on-going subjects, if required.

18.7.2 Delegation of Investigator responsibilities

The PI should ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, their study-related duties and functions, and the study medication. The PI should maintain a list of sub-investigators and other appropriately qualified persons to whom he/she has delegated significant study-related duties.

18.7.3 Information for General Practitioners

Confirmation of the volunteer's medical history will be requested from the volunteer's GP in order for the PI to judge the volunteer's suitability for the study. Volunteers will be required to consent to their GPs being contacted by the hVIVO team.

18.7.4 Payments

Subjects will be reimbursed for their inconvenience and out-of-pocket expenses, including travelling costs. All proposed payments to subjects will be approved by the REC prior to the start of the study and the amount of payment will be specified in the VIS.

18.7.5 Liability and insurance

Liability and insurance provisions for this study are specified in the CTA.

18.7.6 Investigator's Protocol Agreement

The Investigator's Protocol Agreement at the front of this document must be signed by the PI. The original or a copy must be kept on file by the Sponsor and the PI must retain the original or a copy. The completed Protocol Agreement signifies review and acceptance of the protocol by the PI prior to initiation of the study.

18.7.7 Quality assurance

hVIVO must implement and maintain QA and QC systems which may involve auditing the study.

Any contracted individual or group working on the study must implement QC systems in their work on the study.

18.8 Study termination

Upon completion of the study, the following activities, when applicable, must be conducted by the Study Monitor in conjunction with the PI, as appropriate:

- Review of site study records for completeness and accuracy
- Return of all study data to the Sponsor (excluding source data)
- Data clarifications and/or resolutions
- Accounting, reconciliation, and final disposition of used and unused study medication (study drug and/or placebo).

In addition, the Sponsor reserves the right to temporarily suspend or prematurely terminate this study for any reason (see Section 18.4).

19 DISCLOSURE OF DATA

19.1 Subject confidentiality

In line with EU Directive 95/46/EEC[40], as transposed into the UK Data Protection Act 1998[5], the PI and hVIVO have a legal obligation to protect at all times the confidentiality of subject personal data from the point of capture, through processing, dissemination in line with consent from the subject and to its final disposition.

The PI shall provide assurance to subjects that their confidentiality will be maintained during all audits and inspections of the study site and in any documentation by third parties.

hVIVO investigators, study nurses and other hVIVO personnel will record information about the subjects in a computerised database and in an hVIVO medical record. If the subject consents to participate in this study, any of their medical records may be reviewed by hVIVO staff and auditors for the purposes of checking that the study is being carried out correctly.

Subjects' medical records may also be reviewed by representatives from the REC, government agencies and the MHRA; all have the same legal obligation in respect of confidentiality. Subjects will be assigned a unique number prior to enrolment into the study (Section 7.6). Subjects' names will not be supplied to the Sponsor.

Any samples taken for analysis during the study will be labelled using the subject number. Some laboratories in the UK may require up to two-three identifiers for the accurate tracking and processing of samples, such as subject number, and date of birth. Under no circumstances will a laboratory involved in the analysis of samples in this study be given information regarding the identity of subjects, such as their full name.

19.2 Sponsor confidentiality

As detailed in the CTA, information concerning the IMP and any information such as clinical indications for the IMP, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the Sponsor and are unpublished, are confidential and must remain the sole property of the Sponsor. The PI will agree to use the information only for the purposes of carrying out this study and for no other purpose, unless prior written permission from the Sponsor is obtained.

19.3 Publication

By signing the study protocol, the PI agrees that the results of this study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals by the Sponsor.

If necessary, the authorities will be notified of the PI's name, address, qualifications, and extent of involvement. In order to allow the use of the information derived from this clinical study, the

PI understands that he has an obligation to provide complete test results and all data developed during this study to the Sponsor.

If the study is to be published, the Sponsor will jointly prepare and co-author manuscript(s) that may result from the clinical trial. The Sponsor agrees to allow the PI 40 days to review all manuscripts and abstracts prior to submission for publication. The Sponsor reserves the right to include the report of this study in any regulatory documentation or submission or in any informational materials. The Sponsor also reserves the right to delete any confidential information from any proposed manuscripts prior to submission for publication.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

20 REFERENCES

1. World Medical Association Declaration of Helsinki. 1996. http://www.birminghamcancer.nhs.uk/uploads/document_file/document/4f54bfed358e987415000194/Appx_III-VII.pdf.

2. ICH Harmonised Tripartite Guideline for Good Clinical Practice E6(R1). 1996.

3. Commission Directive 2003/94/EC of 8 October 2003, laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. 2003.

4. The Medicines for Human Use (Clinical Trials) Regulations. 2004: SI No. 1031.

5. Legislation.gov.uk. UK Data Protection Act. 1998. http://www.legislation.gov.uk/uksi/2008/1592/contents/made.

6. CDC. Estimates of deaths associated with seasonal influenza - United States, 1976-2007. 201059).

7. Nunes B VC, Machado A, Ringholz C, Rebelo-de-Andrade H, Nogueira P et al. Excess mortality associated with influenza epidemics in Portugal, 1980 to 2004. *PLoS One* 2011; **6**: e20661.

8. Thompson WW SD, Weintraub E, Brammer L, Bridges CB, Cox NJ et al. Influenza-associated hospitalizations in the United States. *JAMA* 2004; **NN2**: 1333-40.

9. Zucs P BU, Haas W, Uphoff H. Influenza associated excess mortality in Germany, 1985-2001 *Emerg Themes Epidemiol* 2005; **2**: 6.

10. Weinstock DM ZG. The evolution of influenza resistance and treatment. *JAMA* 2009; **301**: 1066-9.

11. Fiore AE FA, Shay D, Gubareva L, Bresee JS, Uyeki TM. Antiviral agents for the treatment and chemoprophylaxis of influenza - recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011; **60**: 1-24.

12. Dharan NJ GL, Meyer JJ, Okomo-Adhiambo M, McClinton RC, Marshall, al. Se. Infections with oseltamivir-resistant influenza A(H1N1) virus in the United States. *JAMA* 2009; **301**: 1034-41.

13. CDC. Oseltamivir-Resistant Novel Influenza A (H1N1) Virus Infection in Two Immunosuppressed Patients - Seattle, Washington. *Morb Mortal Wkly Rep* 2009; **58**: 893-6.

14. WHO. Influenza A (H1N1) virus resistance to oseltamivir - 2008/2009 season, northern hemisphere 2009.

15. Poland GA JR, Ovsyannikova IG. Influenza virus resistance to antiviral agents: a plea for rational use. *Clin Infect Dis* 2009; **48**: 1254-6.

16. Stephenson I DJ, Lackenby A, McNally T, Smith J, Pareek M et al. Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. *Clin Infect Dis* 2009; **48**: 389-96.

17. Hill DA, Baron S, Perkins JC, et al. Evaluation of an interferon inducer in viral respiratory disease. *JAMA* 1972; **219**(9): 1179-84.

18. Homan ER, Zendzian RP, Schott LD, Levy HB, Adamson RH. Studies on poly I:C toxicity in experimental animals. *Toxicology and applied pharmacology* 1972; **23**(4): 579-88.

19. Ichinohe T, Watanabe I, Ito S, et al. Synthetic double-stranded RNA poly(I:C) combined with mucosal vaccine protects against influenza virus infection. *Journal of virology* 2005; **79**(5): NN10-9.

20. Mendlowski B, Field AK, Tytell AA, Hilleman MR. Safety assessment of poly I:C in NZB/NZW mice (38565). *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine* 1975; **148**(2): 476-83.

21. Phillips BM, Hartnagel RE, Kraus PJ, Tamayo RP, Fonseca EH, Kowalski RL. Systemic toxicity of polyinosinic acid: polycytidylic acid in rodents and dogs. *Toxicology and applied pharmacology* 1971; **18**(1): NN0-30.

22. de Clercq E. Degradation of poly(inosinic acid) - poly(cytidylic acid) [(I)n - (C)n] by human plasma. *European journal of biochemistry / FEBS* 1979; **93**(1): 165-72.

23. Nordlund JJ, Wolff SM, Levy HB. Inhibition of biologic activity of poly I: poly C by human plasma. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine* 1970; **133**(2): 439-NN.

24. Zaas AK, Chen M, Varkey J, et al. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell host & microbe* 2009; **6**(3): 207-17.

25. Wilkinson TM, Li CK, Chui CS, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nature medicine* 2012; **18**(2): 274-80.

26. Woods C, McClain M, Chen M, et al. A host transcriptional signature for presymptomatic detection of infection in humans exposed to influenza H1N1 or H3N2. *PLoS One* 2013; **8**(1): e52198.

27. Florman AL, Poindexter A, Council FE. Use of an agglutination inhibition test in studying the effects of vaccination against influenza. *The American journal of the medical sciences* 1946; **212**(4): 409-17.

28. Spitzer RL WJ, Kroenke K, et al Patient Health Questionnaire-9 (PHQ-9). 2014. http://www.phqscreeners.com/pdfs/02_PHQ-9/English.pdf.

29. Spitzer RL WJ, Kroenke K, et al Generalised Anxiety Disorder Assessment (GAD-7). 2014. http://www.phqscreeners.com/pdfs/03_GAD-7/English.pdf.

30. MHRA. Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2014 - the 'Orange Guide'. 2014. http://www.mhra.gov.uk/Publications/Regulatoryguidance/Medicines/CON2030NN1.

31. Volume 4 GMP Annex 13, Manufacture of Investigational Medicinal Products, 31 January. 2010.

32. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. 2001.

33. Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use. In: EU, editor.; 2004.

34. Bazett H. An analysis of the time-relations of electrocardiograms. *Heart* 1920; **7**: 353-70.

35. Fridericia L. Die Systolendauer im Elektrocardiogramm bei nomralen Menshen und bei Herzkranken. *Acta Med Scand* 1920; **53**: 46-9.

36. Miller MR HJ, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Series ‘‘ATS/ERS Task Force: Standardisation of Lung Function Testing’’ ‘Standardisation of Spirometry’ *EurRespir J* 2005; **26**(2): 319–38.

37. Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on Clinical Trials on Medicinal Products for Human Use, and Repealing Directive 2001/20/Ec (Text With EEA Relevance) In: Union E, editor. 536/2014; 2014.

38. Communication from the Commission - Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use (‘CT-3’) In: Commission E, editor.; 2011.

39. ICH. Clinical safety data management: definitions and standards for expedited reporting. E2A; 1994.

40. 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 1995.

41. U.S.-EU Safe Harbor Framework. USA: Export.Gov; 2000.

42. The Medicines for Human Use (Clinical Trials) Amendment Regulations. 2006: SI No. 1928.

43. ABPI. Clinical trial compensation guidelines. 2013. http://www.abpi.org.uk/our-work/library/guidelines/Pages/ct-compensation.aspx.

44. Cox RJ. Correlates of protection to influenza virus, where do we go from here? *Human vaccines & immunotherapeutics.* 2013;9(2)

45. Stohr K, Esveld M. Public health. Will vaccines be available for the next influenza pandemic? *Science.* 2004;306(5705):2195-2196.

46. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA : the journal of the American Medical Association.* 2003;289(2):179-186

47. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA : the journal of the American Medical Association.* 2003;289(2):179-186.

48. Estimates of deaths associated with seasonal influenza --- United States, 1976-2007. *MMWR. Morbidity and mortality weekly report.* 2010;59(33):1057-1062.

49. Interim adjusted estimates of seasonal influenza vaccine effectiveness - United States, February 2013. *MMWR. Morbidity and mortality weekly report.* 2013;62(7):119-123.

50. Lin YL. Biological properties of an influenza A virus-specific killer T cell clone. Inhibition of virus replication in vivo and induction of delayed- type hypersensitivity reactions. *Journal of Experimental Medicine.* 1981;154(2):225-234.

51. Ajc. Cytotoxic T-cell Immunity to influenza McMichael A, Gotch F, Noble G, Beare P: New Engl J Med 309: 13, 1983. *Journal of Allergy and Clinical Immunology.* 1984;73(3):B9.

52. Lukacher AE. In vivo effector function of influenza virus-specific cytotoxic T lymphocyte clones is highly specific. *Journal of Experimental Medicine.* 1984;160(3):814-826.

53. Jaffe PA, Kuwano K, Yamada A, Scott M, Young JF, Ennis FA. Kinetics and Specificity at the Clonal Level of the Cytotoxic T Lymphocyte Response to Influenza Pneumonia. *Viral Immunology.* 1987;1(4):259-266.

54. Ennis FA, Wells MA. Recovery from a Viral Respiratory Tract Infection: III. Specificity of Protection Conferred by Immune Spleen Cells Stimulated In Vitro. *Genetic Variation Among Influenza Viruses*: Elsevier BV; 1981:577-585.

55. Yap KL, Ada GL, McKenzie IFC. Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza virus. *Nature.* 1978;273(5659):238-239.

56. Bennink JR, Yewdell JW, Smith GL, Moss B. Anti-influenza virus cytotoxic T lymphocytes recognize the three viral polymerases and a nonstructural protein: responsiveness to individual viral antigens is major histocompatibility complex controlled. *J Virol.* 1987;61(4):1098-1102.

57. Gotch F. Identification of viral molecules recognized by influenza-specific human cytotoxic T lymphocytes. *Journal of Experimental Medicine.* 1987;165(2):408-416.

58. Bennink JR. Murine cytotoxic T lymphocyte recognition of individual influenza virus proteins. High frequency of nonresponder MHC class I alleles. *Journal of Experimental Medicine.* 1988;168(5):1935-1939.

59. Reay PA, Jones IM, Gotch FM, McMichael AJ, Brownlee GG. Recognition of the PB1, neuraminidase, and matrix proteins of influenza virus A/NT/60/68 by cytotoxic T lymphocytes. *Virology.* 1989;170(2):477-485.

21 APPENDICES

Appendix 1: FLU-PRO Symptom Questionnaire

Participant ID: _____ **Participant Initials:** _____ **Date:** ____/_____/_____

FLU-PRO®

People experience the flu in different ways. We would like to know about the symptoms you have been experiencing during the past 24 hours. For each symptom, please mark one box ☐ under the response that best matches your experience. Mark the "Not at all" box, if you did not have that symptom in the past 24 hours.

What time is it? _____ **AM / PM (please circle)**

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Runny or dripping nose	☐	☐	☐	☐	☐
Congested or stuffy nose	☐	☐	☐	☐	☐
Sinus pressure	☐	☐	☐	☐	☐
Scratchy or itchy throat	☐	☐	☐	☐	☐
Sore or painful throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Teary or watery eyes	☐	☐	☐	☐	☐
Sore or painful eyes	☐	☐	☐	☐	☐
Eyes sensitive to light	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Felt nauseous (feeling like you wanted to throw-up)	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Lack of appetite	☐	☐	☐	☐	☐

August 20, 2015 FLU-PRO Version 2.0

Page 1 of 2

Research Tool in Development: Do NOT copy or distribute

Participant ID: _____ Participant Initials: _____ Date: ___/_____/_____

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Sleeping more than usual	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Felt cold	☐	☐	☐	☐	☐
Felt hot	☐	☐	☐	☐	☐
Sweating	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	Never	Rarely	Sometimes	Often	Always
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

Appendix 2: Vital signs: Study specific normal ranges and abnormalities

Vital signs parameters	Lower limit	Higher limit	Units
Tympanic temperature	35.5	38.0	^{o}C
Oxygen saturation	Normal is ≥ 95		%
Respiratory rate	10	20	breaths per minute
Heart rate	50	100	beats per minute
Systolic BP	90	140	mmHg
Diastolic BP	60	90	mmHg

[b] The classification of AEs related to hypotension and hypertension will be done according to the DMID grading scale.

Appendix 3: ECG: Study specific abnormalities

Ref: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. CHMP/ICH/2/04, May 2005.

ECG Parameters	Lower limit	Higher limit	Units
HR	50	100	bpm
QRS	60	109	ms
PR interval	120	200	ms
QT	320	450	ms
QTc	Normal for females is ≤ 450		ms
	Normal for males is ≤ 430		
QTcF	320	450	ms
QTcB	320	450	ms
RR	600	1200	ms

Appendix 4: Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table November 2007

ABBREVIATIONS (used in the table)

ULN = Upper Limit of Normal
R_x = Therapy
Mod = Moderate
ADL = Activities of Daily Living
LLN = Lower Limit of Normal
Req = Required
IV = Intravenous
Dec = Decreased

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild**	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required.
GRADE 2	**Moderate**	Mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention/ therapy required.
GRADE 3	**Severe**	Marked limitation in activity; some assistance usually required; medical intervention/therapy required; hospitalizations possible.
GRADE 4	**Potentially life threatening** [a]	Extreme limitation in activity; significant assistance required; significant medical intervention/therapy required; hospitalization or hospice care probable

[a] Revised by the Sponsor

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria [CTC], and World Health Organization [WHO]) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
Criteria are generally grouped by body system.
Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
CHEMISTRIES				
Hyponatremia	130 - 135 mEq/L	123 - 129 mEq/L	116 - 122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146 - 150 mEq/L	151 - 157 mEq/L	158 - 165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/L	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoalbuminemia[b]	<LLN to 3 g/dL	<3 to 2 g/dL	<2 g/dL	-
Hypoglycemia	55 - 64 mg/dL	40 - 54 mg/dL	30 - 39 mg/dL	< 30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL or abnormal calcium *with* life-threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)[a]	> 1.00 - ≤ 1.25 x ULN	> 1.25 - ≤ 1.50 x ULN	> 1.50 – ≤ 1.75 x ULN	> 1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)[a]	> 1.00 - ≤ 1.50 x ULN	> 1.5 - ≤ 2.00 x ULN	> 2.00 - ≤ 3.00 x ULN	> 3.00 x ULN
BUN[a]	> 1.24 - ≤ 2.50 x ULN	> 2.50 - ≤ 5.00 x ULN	> 5.0 - ≤ 10.0 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	12.1 – 15.0 mg/dL	>15.0 mg/dL
Creatinine[a]	> 1.0 - ≤ 1.5 x ULN	> 1.5 - ≤ 3.0 x ULN	> 3.0 - ≤ 6.0 x ULN	> 6.0 x ULN or dialysis required
Hypertriglyceridemia[b]	>ULN to <2,5 x ULN	2,5 – 5 x ULN	>5 – 10 x ULN	> 10 x ULN

[a] Revised by the Sponsor
[b] Added by the Sponsor

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
HEMATOLOGY				
Hemoglobin[a]	9.5 - 10.5 g/dL	8.0 - 9.4g/dL	6.5 - 7.9 g/dL	< 6.5 g/dL
Absolute Neutrophil Count	1000 - 1,500/mm^3	750 - 999/mm^3	500 - 749/mm^3	< 500/mm^3
Platelets	75,000 - 99,999/mm^3	50,000 - 74,999/mm^3	20,000 - 49,999/mm^3	< 20,000/mm^3
WBCs	10,000-<13,000/mm^3 or 1,000-<4,000/mm^3 [a]	13,000-<15,000/mm^3	15,000-30,000/mm^3	> 30,000 or < 1,000 /mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80 - < 90%[a]	90 – 95%	>95%	----------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: < 100 mg/dL High: > 600 mg/dL	Low: < 50 mg/dL ----------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/mL	41-50 mcg/mL	51-60 mcg/mL	> 60 mcg/mL
Prothrombin Time (PT)[a]	> 1.00 - ≤ 1.25 x ULN	> 1.25 - ≤ 1.50 x ULN	> 1.50 - ≤ 3.00 x ULN	> 3.00 x ULN
Activated Partial Thromboplastin (APPT)[a]	> 1.00 - ≤ 1.66 x ULN	> 1.66 - ≤ 2.33 x ULN	> 2.33 - ≤ 3.00 x ULN	> 3.00 x ULN
Methemoglobin	5.0 - 9.9%	10.0 - 14.9%	15.0 - 19.9%	> 20.0%

[a] Revised by the Sponsor

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
ENZYMES*				
AST (SGOT)	> 1.0 - < 2.0 x ULN	≥ 2.0 - < 3.0 x ULN	≥ 3.0 - ≤ 8.0 x ULN	> 8.0 x ULN
ALT (SGPT)	> 1.0 - < 2.0 x ULN	≥ 2.0 - < 3.0 x ULN	≥ 3.0 - ≤ 8.0 x ULN	> 8.0 x ULN
GGT	> 1.0 - < 2.0 x ULN	≥ 2.0 - < 3.0 x ULN	≥ 3.0 - ≤ 8.0 x ULN	> 8.0 x ULN
Alkaline Phosphatase	> 1.0 - < 2.0 x ULN	≥ 2.0 - < 3.0 x ULN	≥ 3.0 - ≤ 8.0 x ULN	> 8.0 x ULN
Amylase	> 1.0 - ≤ 1.5 x ULN	> 1.5 - ≤ 2.0 x ULN	> 2.0 - ≤ 5.0 x ULN	> 5.0 x ULN
Lipase	> 1.0 - ≤ 1.5 x ULN	> 1.5 - ≤ 2.0 x ULN	> 2.0 - ≤ 5.0 x ULN	> 5.0 x ULN
URINALYSIS				
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only < 10 rbc/HPF	gross, no clots >10 rbc/HPF	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion
CARDIOVASCULAR				
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg; treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by < 20 beat/min or decreased by < 10 mm Hg systolic BP; no treatment required	symptoms due to orthostatic hypotension or BP decreased by < 20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure < 60mm/Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

* Revised by the Sponsor

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
RESPIRATORY				
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring oxygen therapy
GASTROINTESTINAL				
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	> 6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
NEUROLOGICAL				
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, eg, vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, eg, vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (ie, upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures
MUSCULOSKELETAL				
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	muscle tenderness with moderate impairment of activity	severe muscle tenderness with marked impairment of activity	frank myonecrosis

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
SKIN				
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15 mm	15 - 30 mm	> 30 mm	
Erythema	< 15 mm	15 - 30 mm	> 30 mm	
Edema	< 15 mm	15 - 30 mm	> 30 mm	
Rash at Injection Site	< 15 mm	15 - 30 mm	> 30 mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	
SYSTEMIC				
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25 - 50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

For cholesterol and LDL cholesterol, the following severity gradings should be applied:

Cholesterol (fasting)

Adult ≥ 18 years
Grade 1: 200 – 239 mg/dL or *5.18 – 6.19 mmol/L*
Grade 2: 240 – 300 mg/dL or *6.20 – 7.77 mmol/L*
Grade 3: > 300 mg/dL or > *7.77 mmol/L*
Grade 4: NA

LDL cholesterol (fasting)

Adult ≥ 18 years
Grade 1: 130 – 159 mg/dL or *3.37 – 4.12 mmol/L*
Grade 2: 160 – 190 mg/dL or *4.13 – 4.90 mmol/L*
Grade 3: ≥ 191 mg/dL or ≥ *4.91 mmol/L*
Grade 4: NA