Flavivirus cross-priming potential of live attenuated Japanese encephalitis (JE) vaccine IMOJEV® in flavivirus naïve and flavivirus experienced participants

FlaviPrime Protocol
V.6, 08/07/2021

Study Sponsor(s):
University of Liverpool
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Liverpool
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Clinicaltrials.gov: NCT03920111

Research Ethics Ref: 19/NW/0330

Sponsor Ref: UoL001462

Protocol Approval

I, the undersigned, hereby approve this clinical study protocol:

Authorised by Chief Investigator:

Signature: _____ **Date:** Aug 9, 2021

Dr Lance Turtle
Senior Clinical Lecturer/Honorary Consultant in Infectious Diseases
Institute of Infection and Global Health
University of Liverpool

I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of Sponsor:

Signature: Neil French
Neil French (Aug 17, 2021 14:04 GMT+1)

Date: Aug 17, 2021

Dr Neil French
Head of Clinical Operations
University of Liverpool

I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of the Lead Statistician:

Signature: Richard Jackson
Richard Jackson (Aug 17, 2021 16:35 GMT+1)

Date: Aug 17, 2021

Richard Jackson
Study Statistician
University of Liverpool

General Information

This document describes the FlaviPrime study including detailed information about procedures and recruitment. The protocol should not be used as an aide-memoir or guide for the treatment of other patients. Every care was taken in its drafting, but corrections or amendments may be necessary. Any amendments will be circulated to the investigators participating in the study, but sites entering participants for the first time are advised to contact the coordinating centre (Liverpool Clinical Trials Centre) to confirm they have the most up to date version. Clinical problems relating to this study should be referred to the relevant Chief Investigator, Dr Lance Turtle, via the CTU.

This protocol defines the participant characteristics required for study entry and the schedule of treatment and follow-up. Participant recruitment will be undertaken in compliance with this document and applicable regulatory and governance requirements. Waivers to authorise non-compliance are not permitted.
Incidence of protocol non-compliance whether reported prospectively (e.g. where a treatment cannot be administered on a scheduled date as a result of public holidays) or retrospectively noted (e.g. as a result of central monitoring) are recorded as protocol deviations. These are monitored and reported to study oversight committees.

The template content structure is consistent with the SPIRIT (Standard Protocol Item: Recommendations for Interventional Trials 2013) and has regard for the Health Research Authority guidance. Regulatory and ethical compliance information is located in section 14.

The Liverpool Clinical Trials Centre has achieved full registration by the UK Clinical Research Collaboration (www.ukcrc.org) as their standards and systems were assessed by an international review panel as reaching the highest quality. The Liverpool Clinical Trials Centre has a diverse trial portfolio underpinned by methodological rigour, a GCP compliant data management system, and quality management system.

Contact Details: Institutions

Sponsor :	**Study Management, Monitoring and Analysis:**	**Clinical Laboratory:** (central)
Dr Neil French Head of Clinical Operations Clinical Directorate 4th Floor Thompson Yates Building Faculty of Health and Life Sciences University of Liverpool Liverpool L69 3GB Telephone: 0151 794 8373 E-mail: sponsor@liv.ac.uk	Liverpool Clinical Trials Centre (LCTC) The University of Liverpool 1st Floor, Block D Waterhouse Building 3 Brownlow Street Liverpool L69 3GL Telephone: 0151 794 9766 Email: flaviprime@liverpool.ac.uk	Dr Lance Turtle, PhD, MRCP University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Telephone: 0151 795 9668 Email: lance.turtle@liverpool.ac.uk
Statistics:	**Other Medical or Technical Departments and/or Institutions (1):**	**Other Medical or Technical Departments and/or Institutions (2):**
Richard Jackson Liverpool Clinical Trials Centre The University of Liverpool 1st Floor, Block D Waterhouse Building 3 Brownlow Street Liverpool L69 3GL Telephone: 0151 795 5288 Email: r.jackson@liv.ac.uk	Dr Lance Turtle, PhD, MRCP University of Liverpool Institute of Infection, Veterinary and Ecological Sciences, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Telephone: 0151 795 9642 Email: lance.turtle@liverpool.ac.uk	Prof Tom Solomon, PhD, FRCP University of Liverpool Institute of Infection, Veterinary and Ecological Sciences, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Tel: 0151 795 9626 Email: tsolomon@liverpool.ac.uk

Contact Details: Individuals

Individual Authorised to Sign the Protocol and Protocol Amendments on behalf of the Sponsor:	**Chief Investigator (CI):**	
Dr Lance Turtle, PhD, MRCP University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Telephone: 0151 795 9668 Email: lance.turtle@liverpool.ac.uk	Dr Lance Turtle, PhD, MRCP University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Telephone: 0151 795 9668 Email: lance.turtle@liverpool.ac.uk	

In cases where the CI is unavailable to respond to urgent queries the following individual/s will act as cover:

Medical Expert who will Advise on Protocol Related Clinical Queries:	**Medical Expert who will Evaluate SAE Reports:**	
Prof Tom Solomon, PhD, FRCP University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Tel: 0151 795 9626 Email: tsolomon@liverpool.ac.uk	Prof Neil French PhD, MRCP University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE Tel: 0151 795 9687 Email: french@liverpool.ac.uk	

Additional Contacts:

The contact details for the study oversight committee members and participating centres are detailed in documents supplementary to the protocol and stored in the Study Master File

1 Table of Contents

Glossary

AE	Adverse Event
CI	Chief Investigator
C	Core protein
CRF	Case Report Form
CTU	Clinical Trials Unit
DENV	Dengue virus
ELISpot	Enzyme linked immunospot
E	Envelope Protein
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRA	Health Research Authority
IB	Investigator's Brochure
IDSMC	Independent Data and Safety Monitoring Committee
IEC	Independent Ethical Committee
IFNγ	Interferon-gamma
IMP	Investigational Medicinal Product
IVIG	Immunoglobulin therapy
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
LCTC	Liverpool Clinical Trials Centre
LREC	Local Research Ethics Committee
LUHFT	Liverpool University Hospitals Foundation Trust
MAIMP	Manufacturing authorisation for investigational medical product
MCRN CTU	Medicines for Children Clinical Trials Unit
MREC	Multi-centre Research Ethics Committee
NS	Non-structural protein
PFU	Plaque forming unit
PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
prM	Pre-membrane protein
PV	Pharmacovigilance
QP	Qualified person
R&D	Research & Development
RNA	Ribonucleic acid
RUAE	Related Unexpected Adverse Event
RUSAE	Related Unexpected Serious Adverse Event
SAE	Serious Adverse Event
UTR	Untranslated region
WHO	World Health Organisation
WNV	West Nile Virus
YFV	Yellow Fever virus
ZIKV	Zika Virus

2 Protocol Overview

Full Title:	Flavivirus cross-priming potential of live attenuated Japanese encephalitis (JE) vaccine IMOJEV® in flavivirus naïve and flavivirus experienced participants. Human experimental medicine study.
Acronym:	FlaviPrime
Phase:	IV
Target Population:	Healthy adults, aged 18 – 70 years
Sample size:	Up to 25 participants in total to be vaccinated: Group 1; up to 3-4 participants of any previous flavivirus exposure state Group 2; up to 6-10 flavivirus naïve participants Group 3; up to 8-12 flavivirus experienced participants
Inclusion Criteria:	1. A male or female adult between 18 and 70 years of age at consent. 2. Written and informed consent obtained from participant and agreement of participant to comply with the requirements of the study 3. Able to attend regularly to donate study blood samples for the duration of the study (8 weeks), no planned re-location or travel to a flavivirus endemic area during the study period. 4. Satisfactory medical screen, as demonstrated by study screening document normal physical examination and normal screening blood tests 5. Group 1: Any flavivirus exposure status; Group 2: No previous flavivirus vaccination (JE, tick borne encephalitis or yellow fever (YF)), no residence in a flavivirus endemic area nor planned travel to a flavivirus endemic area during the period of the study; Group 3: JE vaccine and/or yellow fever vaccine or other proven flavivirus infection within the last 10 years or other proven flavivirus infection (lifetime). 6. An efficacious method of contraception must be used during the study for 28 days after vaccination for women of childbearing potential (see section 11).
Exclusion Criteria:	1. Use of any investigational or non-registered drug within 5 half-lives of the drug, or 30 days preceding administration of study JE vaccine, whichever is longer; or planned use during the study period.

	2. Receipt of any investigational biologic agents with mechanisms of action that might affect the immune system, at the discretion of the CI and local PI. 3. Administration of immunosuppressants or other immune-modifying drugs within a period of six months before vaccination or at any time during the study period; participants who have received these agents may also be excluded at the discretion of the CI and local PI. 4. Any confirmed or suspected immunosuppressive or immunodeficient condition. 5. A family history of congenital or hereditary immunodeficiency. 6. Any antiviral drug therapy within a period of 5 drug half-lives or 30 days before vaccination, whichever is longer, or at any time during the study period. 7. History of significant allergic reactions likely to be exacerbated by any component of the study vaccine, especially allergic disease or reactions to any previous dose of any vaccine. 8. History of having received JE vaccine, yellow fever vaccine, tick-borne encephalitis vaccine or experimental flavivirus vaccine (group 2 only). 9. Acute disease (for example acute infection) at the time of enrolment or vaccination, if symptoms are rated as anything more significant than a mild adverse event. Entry into the study and/or vaccination may be deferred until the illness has resolved for at least one week. 10. Acute or chronic, clinically significant in the opinion of the investigator, disease in any organ system, as determined by history, physical examination or laboratory testing. 11. Presence of any inflammatory condition that might require immunomodulatory therapy. 12. Recent blood donation (inclusion can be delayed under these circumstances; the participant should be enrolled 16 weeks after their last blood donation. Each participant should give no more than 470 ml per 16 weeks, so regular blood donation should be suspended during the study and can re-commence 1 month after the last study sample).

	13. Current or previous abattoir worker or sheep farmer in Scotland (risk of Louping ill virus exposure; group 2 only). 14. Administration of immunoglobulins and/or any blood products within the three months preceding administration of vaccine, or planned administration during the study period. 15. Seropositive for HIV. 16. Pregnancy or Lactation. 17. History of excessive alcohol consumption (>28 units per week), drug abuse or significant psychiatric illness. 18. Any other condition or consideration that, in the opinion of the Investigator, would pose a health risk to the participant if they were enrolled in the study, or would otherwise interfere with the evaluation of the study aims (e.g. difficult venesection).
Study Centres and Distribution:	Single site – Liverpool University Hospitals NHS Foundation Trust (LUHFT, Royal Liverpool site) Clinical Research Unit (CRU)
Participant Study Duration:	***Group 1***: 28 days after vaccination; ***Groups 2 and 3***: 8 weeks after vaccination
Study Duration	Participant duration: Group 1: 28 days Groups 2 and 3: 8 weeks
IMP / Intervention:	**Intervention:** Single dose live attenuated JE vaccine IMP: IMOJEV® Form: Intra dermal – injection Dose: Single dose, 0.5mL ($\geq 10^{4.0-5.8}$ PFU) Route: Subcutaneous No adjuvant or antimicrobial preservative has been added.

Objectives:		
Exploratory/ Translational; Primary:	Refer to section 8 for further details on endpoint/outcome measures	1. To establish a human model system of JEV infection in healthy adult volunteers using live attenuated JE vaccine IMOJEV®. 2. To sort and sequence individual responding B cells (plasmablasts) after vaccination with IMOJEV®, and

		to generate human monoclonal antibodies to JEV. 3. To generate JEV specific human monoclonal antibodies from the sequences derived in (2). 4. To describe the development, specificity, cross-reactivity and function of the T cell response to IMOJEV®. 5. To establish a sample bank for future work on cross-reactive and other responses to flaviviruses, flavivirus vaccines and other emergent viruses.
Exploratory/ Translational; Secondary:	Refer to section 8 for further details on endpoint/outcome measures	1. To examine the specificity and cross-reactivity of the antibody response after JE vaccination, using serum and human monoclonal antibodies. 2. To determine whether there are epitopes which can serve as the target of broadly cross-neutralising antibody responses.

2.1 Schematic of Study Design

Figure 1. Schematic of Study Design:

Prior to enrolment — screening, flavivirus immune assays, clinical history, as part of the Acute emerging Virus Immunity Study (AVIS).

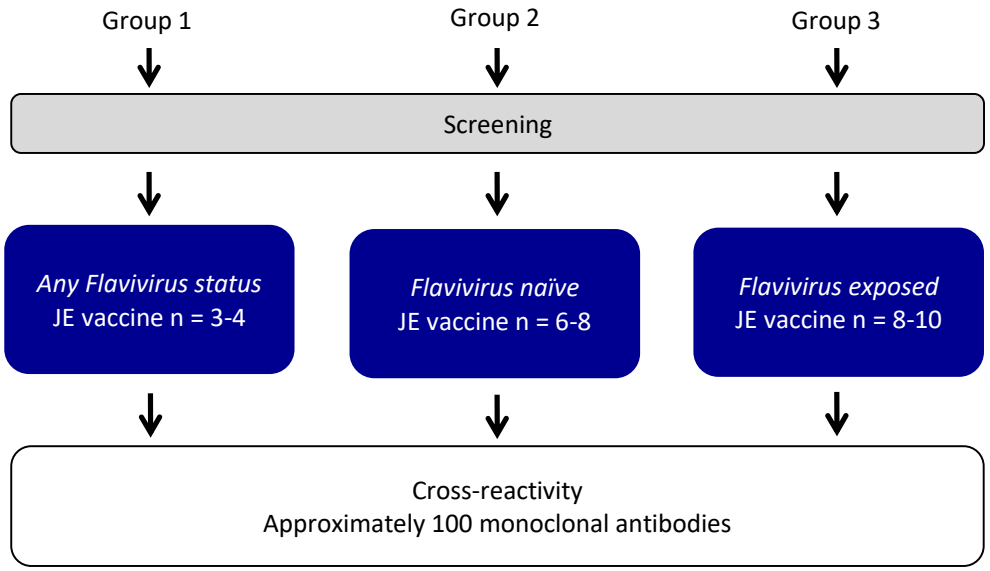
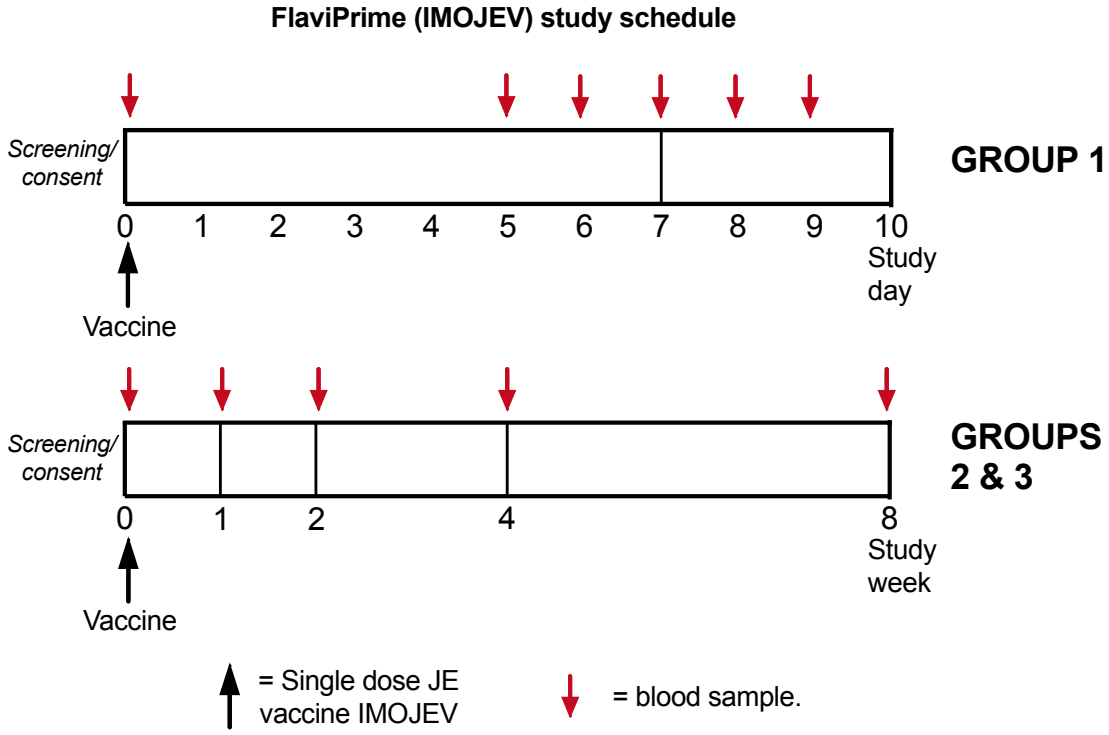


Figure 2. Schematic of Study Schedule:



3 Roles and Responsibilities

Sponsor

The University of Liverpool is the Sponsoring organisation and is legally responsible for the study. They will formally delegate specific Sponsoring roles to the Chief Investigator and Clinical Trials Unit.

Funder

This study is funded as part of a Fellowship Award by the Wellcome Trust and managed by the Chief Investigator and Research Support Office at the University of Liverpool.

Funder(s)	Financial and Non-financial Support Given	Role
Wellcome Trust	Up to £653,257	This funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results

Chief Investigator: Dr Lance Turtle is the Chief Investigator for the study and is responsible for overall design and conduct of the study in collaboration with other members of the study team.

Principal Investigators: In each participating centre a principal investigator will be identified to be responsible for identification, recruitment, data collection and completion of CRFs, along with follow up of study participants and adherence to study protocol at site. They will also be responsible for safety reporting and processing any applicable safety information.

Clinical Trials Unit: The Liverpool Clinical Trials Centre (LCTC) at the University of Liverpool in collaboration with the Chief Investigator, will have overall management responsibility and will be responsible for study management activities including (but not limited to) study planning, budget administration, Study Master File management, safety reporting, data management, registration, statistical analysis, participating site coordination and IMP management.

Oversight Committees

FlaviPrime study is subject to oversight from the following committee:

Study Management Group (SMG)
A Study Management Group (SMG) will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical) and members of the LCTC. The SMG are responsible for monitoring all aspects of the progress and conduct of the study and will be responsible for the day-to-day running and management of the study. The SMG will meet at least monthly at setup stage and then reduce to quarterly throughout the year unless more frequent meetings are required.

Study Oversight Committee (SOC)
The Study Oversight Committee will consist of an independent chairperson, 2/3 independent experts in the field of infectious diseases, biostatistician, including the CI and observers the role of the SOC is to provide overall supervision for the study and provide advice through its independent Chairman. The decision for the continuation of the study lies with the SOC and as such they will meet throughout the study (at least annually).

3.1 Protocol Contributors

Name	Affiliations	Contribution to protocol
Dr Lance Turtle	University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE	Development of protocol, clinical expertise
Prof Tom Solomon	University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE	Development of protocol, clinical expertise
Prof Neil French	University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE	Development of protocol, clinical expertise
Richard Jackson	The University of Liverpool Liverpool Clinical Trials Unit 1st Floor, Block D Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Statistical expertise
Kellie Platt	The University of Liverpool Liverpool Clinical Trials Unit 1st Floor, Block D Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Trial Management expertise
Hayley Hardwick	University of Liverpool Institute of Infection and Global Health, Ronald Ross Building, 8 West Derby Street, Liverpool, L69 7BE	Trial Management expertise
Priya Francis	Liverpool Clinical Trials Centre The University of Liverpool 2nd Floor Institute in the Park Alder Hey Children's NHS Foundation Trust Eaton Road Liverpool L12 2AP	Trial Management expertise
Mr Alex Astor	The University of Liverpool Research Support Office 2nd Floor, Block D Waterhouse building 3 Brownlow Street Liverpool L69 3GL	Sponsorship and protocol approval

Dr Mansour Yaïch	Vaxyn Consulting, Paris 75001, Île-de-France, France	Development of protocol

4 INTRODUCTION

4.1 Background

Japanese encephalitis and the genus Flavivirus

Japanese encephalitis (JE) is the most important epidemic encephalitis in the world. JE is caused by JE virus (JEV), a member of the genus *Flavivirus*, which contains the most important mosquito borne viruses of man, including dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV) and Zika virus (ZIKV). Japanese encephalitis (JE) remains the most common epidemic encephalitis globally with 68,000 cases per year[1]. DENV causes 100 million illnesses annually[2], and keeps spreading. Yellow fever virus (YFV) outbreaks in 2015-16 almost exhausted the World Health Organisation (WHO) vaccine stockpile at one point[3]. Recent ZIKV related neurological syndromes have shown us how vulnerable we are to the emergence of new flaviviruses[4, 5]. For several important flavivirus diseases there are no good vaccines available.

The flavivirus genome is 11 kb in size, positive sense single stranded RNA, and encodes a single 10.3 kb open reading frame flanked by untranslated regions. The open reading frame is translated as a single polypeptide which is then cleaved by host and viral proteases to produce three structural proteins (core, pre-membrane and envelope) and seven non-structural (NS) proteins, named NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (figure 3)[6].

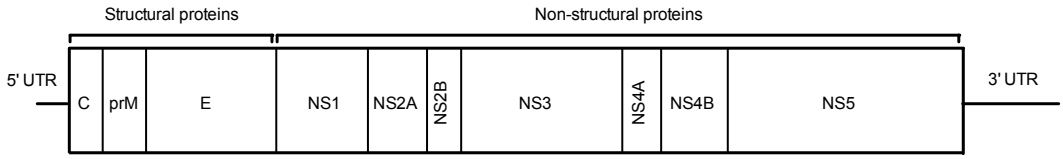


Figure 3. Flavivirus genomic organisation. *The structural proteins core (C), pre-membrane (prM) and envelope (E) proteins are present in the viral particle, and hence are found in inactivated vaccines. The non-structural (NS) proteins are made during replication, and therefore can be produced upon infection by a live attenuated vaccine. UTR = untranslated region.*

Flaviviruses are closely related[6], leading to a significant degree of immunological cross-reactivity. For example, DENV is in fact four closely related viruses with immunologic cross-priming potential[7]. Although cross priming may be protective[8] it can also be detrimental — interfering with vaccine development, diagnosis and resulting in dengue illness that is more severe than the first infection. Beyond dengue there is also clear evidence that flavivirus cross priming can affect disease potential. Several studies show a degree of partial protection against JE in people who have already experienced DENV infection and mount a secondary type of immune response to JEV resulting in higher antibody levels[7, 8]. JE vaccination may also make DHF less severe[9]. However, one study suggests that the presence of anti-JEV neutralising antibody (but *not* JE vaccination) might predispose to worse dengue illness[10], so the interactions are clearly complex and not completely understood.

Studies using human monoclonal antibody technologies (mAb)[11], have begun to give new insight into mechanisms of antibody cross-reactivity and protection against flaviviruses[11, 12]. Only a small fraction of antibodies against DENV are strongly neutralising[13], and these frequently recognise quaternary epitopes (where the antibody binds several E proteins)[11, 14, 15]. Many antibodies enhance infection[12], particularly highly cross-reactive antibodies against the prM protein if DENV[16] and the fusion loop of envelope (E) domain II (a conserved part of E protein important for cell entry)[11, 17].

To date, there has been only one study of human mAb to JEV, which made a modest number of antibodies from people receiving inactivated vaccine[18]. Murine and primate monoclonals have been more extensively studied[19-21] but no quaternary epitopes arising from productive infection in humans have been determined.

More efficiently neutralising murine mAb are often less cross-reactive[22], but, importantly, mice and humans mount quite different responses to flavivirus envelope proteins[23, 24].

Recent observations made during the Zika outbreak in South America have revealed at least one B cell epitope (called the envelope dimer epitope I, EDE I) that can serve as the target of human antibodies that can potently neutralise all four DENV serotypes and ZIKV[11, 25]. It is possible that by studying the response to a replication competent viral envelope protein in flavivirus experienced volunteers, we are able to uncover a similar epitope for the JE serogroup (JEV, St Louis encephalitis virus, West Nile virus, Murray Valley encephalitis virus, Usutu virus). Such an epitope could then be reverse engineered into a vaccine against multiple viruses, as is underway for DENV and ZIKV.

Vaccines against JE

Several different vaccines have been used to control JE since the 1950s[26] (table 1, reviewed in ref 17). Randomised controlled trials have shown the efficacy of JE vaccination, and showed that neutralising antibody correlated with protection[9]. Early JE vaccines were mostly derived from mouse brain, but this manufacturing method has now been replaced due to safety concerns and inferior immunogenicity[26].

IMOJEV® (Sanofi Pasteur) is a Chimeric JE vaccine of JEV SA14-14-2 envelope and pre-membrane protein inserted into a yellow fever backbone (figure 4). IMOJEV® (formerly known as ChimeriVax-JE) is licensed in Australia and New Zealand for the prevention of JE in travellers, it is also marketed in some parts of Southeast Asia.

Table 1. Vaccines against Japanese encephalitis.

Description	Type	Virus strain	Common name	Developer/country of manufacture
Older vaccines, no longer in use:				
Mouse brain	Inactivated	Nakayama	BIKEN	Japan, BIKEN
Mouse brain	Inactivated	Nakayama	Green Cross	Korea, Green Cross
Mouse brain	Inactivated	Beijing-1		Japan
Primary hamster kidney	Inactivated	P3		China
Vaccines currently available:				
Vero cell	Inactivated	P3		China
Primary hamster kidney	Live attenuated	SA14-14-2		Chengdu Biological Products, China
Vero cell	Inactivated	Beijing-1	JEBIK®V	Japan, BIKEN
Vero cell	Inactivated	Beijing-1	ENCEVAC®	Japan, Kaketsuken
Vero cell	Inactivated	SA14-14-2	IC51, IXIARO	Intercell, Valneva
Vero cell	Inactivated	Kolar-821564XY	JENVAC	Bharat Biotech Int. ltd, India
Yellow fever 17D recombinant vectored	Live attenuated	SA14-14-2 (Envelope)	Imojev, Chimerivax JE	Acambis, Sanofi Pasteur

4.2 Rationale

There is a pressing need for a better experimental system to understand flavivirus antibody responses, beyond dengue, to make sure we are using current vaccines to greatest effect and to inform the development of next-generation vaccines. This study will use live chimeric JE vaccine IMOJEV® as a tool for flavivirus epitope discovery. This will allow experimental JEV infection using replication competent, live, attenuated virus as a model, in a setting where the flavivirus infection history of humans can be tightly controlled.

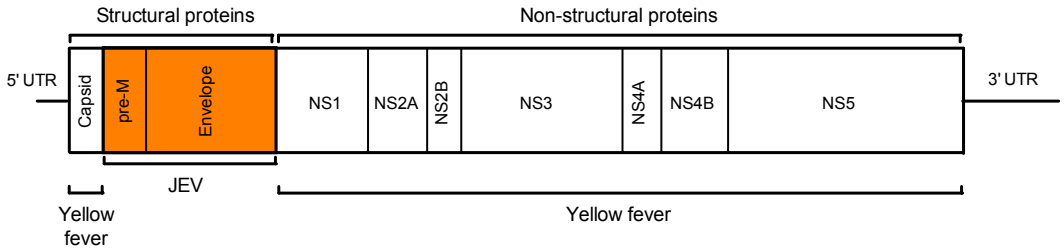


Figure 4. Schematic of IMOJEV® JE vaccine. *The pre-membrane (pre-M) and envelope (E) proteins of JEV (orange) are inserted into the attenuated yellow fever 17D virus.*

Importantly, this study must be conducted using a replication competent vaccine, and not the inactivated vaccine available in Europe, IXIARO. This is because a replicating virus particle undergoes several different changes of state during the replication cycle[27], exposing epitopes that may not be present in an inactivated vaccine.

The use of the live attenuated JE vaccine IMOJEV® in volunteers in the UK also permits the study of immune responses to JEV in completely flavivirus naïve subjects, something which is very difficult to achieve in flavivirus endemic areas where multiple viruses co-circulate and infection history is hard to determine accurately. By including a group of volunteers who have had previous flavivirus exposure (JE vaccine, yellow fever vaccine or dengue illness), it will also be possible to study the effect of previous flavivirus exposure on cross-reactive responses to JE vaccine in a highly controlled manner without the uncertainty of natural exposure arising from residence in an endemic area.

The specificity and cross-reactivity of antibody responses will be studied by cloning antibodies from plasmablasts (developing antibody secreting cells that appear early in an immune response). Plasmablast responses precede the development of antibody responses in blood, and a large antibody response usually implies a large plasmablast response. Giving a booster dose of live JE vaccine to children who have been immunized with a primary course of inactivated JE vaccine produces a marked (approximately 18-fold) rise in neutralizing antibody titre[28], therefore we expect this vaccine also to generate a significant plasmablast response after vaccination. The plasmablast population is very enriched for cells specific to the antigen of interest, and therefore makes ideal starting material to generate human monoclonal antibodies against specific pathogens[11].

These experiments are the first step in determining the nature of the immune response to sequential viral infections in known order using replication competent vaccines as models of viral infection in humans. This will have important implications for the sequence of use of future JE and dengue vaccines, and represents the first step towards combining flavivirus vaccines beyond the tetravalent dengue vaccines into a universal flavivirus vaccine.

4.3 Risk and Benefits

4.3.1 Potential Risks

The below side effects are stated in the patient information leaflet that accompanies IMOJEV®

Table 2 – side effects of IMOJEV®

How likely	Side Effect		
Very Common (1 in 10 persons)	Tiredness Feeling unwell Injection site pain Headache Muscular pain		
Common (more than 1 in 100 persons and less that 1 in 10 persons)	Feeling hot Chills Injection site redness Injection site itching Injection site swelling Injection site bruising Nausea	Vomiting Throat pain Shortness of breath Runny nose Cough Wheezing Rash	Dizziness Joint pain Diarrhoea Abdominal pain Nasal congestion
Uncommon (more than 1 in 1000 persons and less than 1 in 100 persons	Fever		
Rare (more than 1 in 10000 persons and less than 1 in 1000 persons)	Viral infections		

We have received clarification from the MHRA that this study is not a CTIMP and therefore does **not** require a CTA application to be submitted.

More detail regarding management of risks associated with this study are detailed in a separate Risk Assessment maintained in the Study Master File.

4.3.2 Potential Benefits

Participants will develop immunity to Japanese encephalitis 2 weeks after vaccination.

4.4 Objectives

Scientific hypotheses to be tested and experimental approach

This study aims to establish a unique human experimental medicine system which will be used to study flavivirus immune responses and for epitope discovery. As such, although safety will be monitored in the same way as a late phase clinical trial, there will be no primary or secondary endpoint in this study.

This study will test the hypothesis that in previously flavivirus-exposed individuals, the antibody response is more broadly cross neutralising, and that this will lead to the identification of conserved virion surface epitopes that could be the target of second generation vaccines. All the scientific objectives of this study are exploratory.

Exploratory/Translational/Mechanistic Objective(s)

4.4.1 Exploratory Primary Objectives

Specific aims:

1. To establish a human model system of JEV infection in healthy adult volunteers using live attenuated JE vaccine IMOJEV®.

2. To sort and sequence individual responding B cells (plasmablasts) after vaccination with IMOJEV®, and to generate human monoclonal antibodies to JEV.
3. To generate JEV specific human monoclonal antibodies from the sequences derived in (2).
4. To describe the development, specificity, cross-reactivity and function of the T cell response to IMOJEV®.
5. To establish a sample bank for future work on cross-reactive and other responses to flaviviruses, flavivirus vaccines and other emergent viruses.

4.4.2 **Exploratory Secondary Objectives**

1. To examine the specificity and cross-reactivity of the antibody response after JE vaccination, using serum and human monoclonal antibodies.

2. To determine whether there are epitopes which can serve as the target of broadly cross-neutralising antibody responses.

Experimentally, the fine specificity and cross-reactivity of the antibody response will be studied by cloning antibodies from plasmablasts (B cells responding to the vaccine) that have been single cell sorted by flow cytometry then sequenced at one week post vaccine. These human monoclonal antibodies will then be mapped on to the surface of the virus particle using established approaches[11], and tested to look for cross-reactive antibodies. T cell responses to the vaccine will be studied using custom pools of synthetic peptides by ELISpot and flow cytometry.

5 STUDY DESIGN

FlaviPrime is an open label, three arm, human experimental medicine study in healthy volunteers of any flavivirus background (group 1), flavivirus naïve (group 2) and flavivirus exposed (group 3) volunteers (figure 1). Each group will receive a single dose of JE vaccine IMOJEV®. Group 1 will be sampled daily from days 5 to 9 after vaccination, and groups 2 and 3 will follow an identical schedule (figure 2) with 4 sampling points over 8 weeks of follow-up. After the study, participants will be followed up in the observational Acute emerging Virus Immunity Study (AVIS), aiming for 5 years of follow up.

Group 1: up to 3-4 healthy adults.

Group 2: up to 6-10 healthy adults who have never travelled to a flavivirus endemic area and are negative in screening tests for flavivirus immunity.

Group 3: up to 8-12 healthy adults who have had JE vaccine and/or are previously flavivirus exposed, either through receiving yellow fever vaccine up to 5 years before the study, or from being diagnosed with a flavivirus illness (e.g. dengue or Zika).

Group 1 will allow confirmation of the kinetics of the plasmablast response which has never been addressed for any flavivirus vaccine. The inclusion of groups 2 and 3 will allow comparison of the adaptive immune response to JEV in flavivirus naïve individuals, verses flavivirus experienced individuals. Because the hypothesis under test is that after multiple exposure the response is focused on more conserved epitopes compared with single exposure, the exact virus on the previous exposure is not critical. Group 3 more faithfully mimics the reality of residence in flavivirus endemic areas where there is exposure to multiple viruses and it is hard to determine with certainty which viruses have infected any given individual.

Up to 25 participants will be studied (Group 1 = up to 3-4, Group 2 = up to 6-10, and Group 3 = up to 8-12). Groups can comprise male and/or female participants aged from 18 to 70 years at the time of consent. The group sizes do not need to be fixed because no sample size based comparisons are pre-specified. Any comparisons will be post-hoc and exploratory. We anticipate that antibodies made from group 3 will have wider virus specificity and therefore greater potential future application and interest; hence this group is allowed to be slightly larger.

Each group will receive a single dose of live JE vaccine IMOJEV®. All doses will be administered at the LUHFT CRU facility in accordance with a bespoke vaccination administration working instruction. Group 1 participants will be in the study for 28 days and will give 6 blood samples, one on the day vaccination and then daily on days 5 through to 9 post vaccination. Group 2 and 3 participants will be in the study for a total of 8 weeks and will be expected to attend study visits and provide six blood samples at baseline, and weeks 1, 2, 4, and 8 (see Figure 2, Study Schedule). All participants will have Adverse Events (AEs) recorded while they are in the study. Sample collection in the Acute emerging Virus Immunity study (AVIS) will be temporarily suspended during FlaviPrime, participants will then revert back to AVIS once the FlaviPrime sampling collection period has finished. Participants will be followed up, aiming for 5 years of follow up, and have already been consented for storage of contact details for future studies.

5.1 Blinding

This is an open label study with no blinding requirements. All researchers and participants know which treatment / intervention is being administered.

5.2 Study Setting

Participants will be identified and recruited from an existing observational study ("Immune responses to Zika virus and other flaviviruses," (Acute emerging Virus Immunity Study, AVIS) 16/NW/0170), by advertisement, and through Liverpool University Hospitals NHS Foundation Trust (LUHFT) Clinical Research Unit (CRU), for example using the consent4consent database. Follow up will occur at LUHFT.

5.2.1 Selection of Participating Sites

FlaviPrime is a single centre study that will undertake intensive laboratory investigation of the immune response to IMOJEV®. Therefore, the study site must be close to the necessary facilities (single cell sorting, sequencing, etc.) and will only be open at one site, the LUHFT CRU, which has the necessary expertise for this study.

The site will be opened to recruitment upon successful completion of all global (e.g. REC) and study-specific conditions (e.g. site personnel training requirements) and once all necessary documents have been returned to the CTU. Initiation of the site will be undertaken in compliance with LCTC internal processes. Conditions and documentation required will be detailed on a LCTC Green Light Checklist maintained in the TMF and must be fully completed prior to opening sites to recruitment.

5.2.2 Selection of Principal Investigators

Principal Investigators will be required to demonstrate equipoise, relevant experience and commitment during early stage feasibility assessment. All investigators will have the particular medical expertise necessary to conduct the study in accordance to the protocol and all regulatory and ethical requirements. Written agreement to conduct research as such will be obtained prior to site initiation.

A suitable co-investigator should be identified at each site to deputise in case of PI absence.

6 ELIGIBILITY CRITERIA

The FlaviPrime study aims to recruit up to 25 participants based on sample size calculations described in Section 12.2. All patients must provide written, informed consent before any study procedures occur (see Section 9.2 for more information regarding informed consent processes) and must meet all eligibility criteria as described below.

6.1 Inclusion Criteria

Patients eligible for the study must comply with all of the following at registration:

1. A male or female adult between 18 and 70 years of age at consent.
2. Written and informed consent obtained from participant and agreement of participant to comply with the requirements of the study
3. Able to attend regularly to donate study blood samples for the duration of the study (8 weeks), no planned re-location or travel to a flavivirus endemic area during the study period.
4. Satisfactory medical screen, as demonstrated by study screening document normal physical examination and normal screening blood tests
5. Group 1: Any flavivirus exposure status; Group 2: No previous flavivirus vaccination (JE, tick borne encephalitis or yellow fever (YF)), no residence in a flavivirus endemic area nor planned travel to a flavivirus endemic area during the period of the study; Group 3: JE vaccine and/or yellow fever vaccine or other proven flavivirus infection within the last 10 years or other proven flavivirus infection (lifetime).
6. An efficacious method of contraception must be used during the study for 28 days after vaccination for women of childbearing potential (see section 11).

6.2 Exclusion Criteria

Any patient meeting any of the criteria listed below at baseline will be excluded from study registration:

1. Use of any investigational or non-registered drug within 5 half-lives of the drug, or 30 days preceding administration of study JE vaccine, whichever is longer; or planned use during the study period.
2. Receipt of any investigational biologic agents with mechanisms of action that might affect the immune system, at the discretion of the CI and local PI.
3. Administration of immunosuppressants or other immune-modifying drugs within a period of six months before vaccination or at any time during the study period; participants who have received these agents may also be excluded at the discretion of the CI and local PI.
4. Any confirmed or suspected immunosuppressive or immunodeficient condition.
5. A family history of congenital or hereditary immunodeficiency.
6. Any antiviral drug therapy within a period of 5 drug half-lives or 30 days before vaccination, whichever is longer, or at any time during the study period.
7. History of significant allergic reactions likely to be exacerbated by any component of the study vaccine, especially allergic disease or reactions to any previous dose of any vaccine.
8. History of having received JE vaccine, yellow fever vaccine, tick-borne encephalitis vaccine or experimental flavivirus vaccine (group 2 only).

9. Acute disease (for example acute infection) at the time of enrolment or vaccination, if symptoms are rated as anything more significant than a mild adverse event. Entry into the study and/or vaccination may be deferred until the illness has resolved for at least one week.
10. Acute or chronic, clinically significant in the opinion of the investigator, disease in any organ system, as determined by history, physical examination or laboratory testing.
11. Presence of any inflammatory condition that might require immunomodulatory therapy.
12. Recent blood donation (inclusion can be delayed under these circumstances; the participant should be enrolled 16 weeks after their last blood donation. Each participant should give no more than 470 ml per 16 weeks, so regular blood donation should be suspended during the study and can re-commence 1 month after the last study sample).
13. Current or previous abattoir worker or sheep farmer in Scotland (risk of Louping ill virus exposure; group 2 only).
14. Administration of immunoglobulins and/or any blood products within the three months preceding administration of vaccine, or planned administration during the study period.
15. Seropositive for HIV.
16. Pregnancy or Lactation.
17. History of excessive alcohol consumption (>28 units per week), drug abuse or significant psychiatric illness.
18. Any other condition or consideration that, in the opinion of the Investigator, would pose a health risk to the participant if they were enrolled in the study, or would otherwise interfere with the evaluation of the study aims (e.g. difficult venesection).

6.3 Co-enrolment Guidelines

Participants can enter FlaviPrime if they are participating in other studies, provided that: i) no experimental drug is taken five half lives before the screening and for the 8 week period after vaccination, ii) no experimental vaccine is given within 4 weeks before screening or after vaccination in this study, and iii) the total volume of blood drawn in a 16-week period does not exceed 470 ml (the same volume as would routinely be collected during blood donation). Participants will remain in the Acute and emerging Virus Immunity Study (AVIS). Enrolment into any new study should take place >8 weeks after vaccination in FlaviPrime, unless there is a pressing reason for enrolment, and the discretion of the chief investigator. In any event, the FlaviPrime study team should be informed of any new enrolment into a study during the 28 period (group 1) or 8 week period (groups 2 and 3) following vaccination.

7 STUDY TREATMENT/INTERVENTIONS

7.1 Introduction

Eligible patients in each group will receive a single dose of live, attenuated, JE vaccine IMOJEV®.
IMP will be supplied by Sanofi Pasteur in accordance with all applicable guidelines.

7.2 Treatment Name / Description

7.2.1 Live attenuated JE vaccine IMOJEV®

Information on live attenuated JE vaccine IMOJEV® will be available to the investigators in the form of the Investigators Brochure (IB).

The vaccine is a live attenuated virus. Following administration, the virus replicates locally and elicits neutralising antibodies and cell-mediated immune responses that are specific to the Japanese encephalitis (JE) virus. Available results indicate that protection is mainly mediated by neutralising antibodies.

IMOJEV® is a monovalent, live attenuated viral vaccine. The virus was obtained via recombinant DNA technology. It is based on the 17D-204 yellow fever vaccine virus in which two genes have been replaced by the corresponding genes from Japanese encephalitis (JE) virus. These are the premembrane (prM) and envelope (E) coding sequences of the SA14-14-2 live attenuated JE vaccine virus. The immunising antigens are the prM and E proteins from the SA14-14-2 vaccine virus.

Brand name / Active ingredient:	IMOJEV® After reconstitution the active ingredients are: Live, attenuated, recombinant Japanese encephalitis virus: 4.0 - 5.8 $\log^{10}$ Plaque Forming Units, Propagated in Vero cells. Excipients to the above active ingredients are: • Mannitol • Lactose • Glutamic acid • Potassium hydroxide • Histidine • Human Serum Albumin • Sodium chloride • Water for injections No adjuvant or antimicrobial preservative is added.
Formulation:	Powder for reconstitution / diluent The powder is a white to creamy white homogeneous cake which might be retracted from the sides of the vial. The diluent is a clear solution. After reconstitution, IMOJEV® is a colourless to amber suspension.
Manufacturer:	Sanofi Pasteur

Packaging, storage and stability:	IMOJEV® is provided in separate packs, each pack contains: • One vial containing a white to creamy white vaccine powder • One vial (type I glass) each with a stopper (halo-butyl) and a flip off cap (aluminium/polypropylene) containing clear diluent • One disposable syringe (polypropylene) with 2 separate needles (stainless steel) The vaccine boxes are to be stored in a refrigerator (2°C – 8°C) not be frozen. When out of the fridge the vials should be kept in the outer carton in order to protect from light. Do not use IMOJEV® after the expiry date which is stated on the carton after EXP.
Supplier's name:	Sanofi Pasteur
Regulatory Status:	Licensed in Australia and New Zealand for the prevention of JE in travellers. It is also marketed in some parts of Southeast Asia.

7.3 Manufacturing and Distribution

IMOJEV® vaccine will be sourced from existing licensed stock in Australia where the vaccine is in routine use. The vaccine will be imported into the UK by Geryon Pharma, overseen by a Qualified Person (QP). The importation process will involve continual monitoring of temperature. After import, the vaccine will be stored by Geryon Pharma until the green light process is complete, at which point it will be transferred to the clinical trials pharmacy at LUHFT. The vaccine will be stored at 4°C in a monitored fridge in the clinical trials pharmacy. Upon prescription for use in the study, the vaccine will be dispensed by the pharmacy on a named participant basis to the CRU where it will be stored at 4°C in a monitored fridge until use.

After use, any materials that have come into contact with the vaccine will be soaked in virkon overnight to ensure inactivation of the vaccine, in accordance with the controlled release of a GMO.

7.4 Preparation, Dosage and Administration

Using aseptic technique, IMOJEV® vaccine is reconstituted by injecting all the 0.4% sodium chloride solution (clear of colour) into the vial of freeze-dried vaccine, using the syringe and one of the needles provided in the carton. The vial is gently swirled.
After complete dissolution, a 0.5 mL dose of the reconstituted suspension (colourless to amber) is withdrawn into this same syringe.

For the administration injection, the syringe is fitted with the second needle provided in the package. The product should be used once reconstituted and must be discarded if it is not used within one hour of reconstitution.

After use, any remaining vaccine and container must be disposed of safely, preferably by heat inactivation or incineration, according to locally agreed procedures.

7.5 Accountability Procedures

Further detail on shipping, Qualified Person (QP) and vaccine management processes will be detailed in a bespoke Pharmacy Operating Manual. In summary, shipping will be conducted under QP supervision. The vaccine will be shipped into the UK (destination specified in the bespoke Pharmacy Operating Manual) where it will undergo inspection including cold chain monitoring records, QP validation and release. It will then be transferred to the LUHFT CRU drug storage facilities and kept at monitored temperature according to standard protocols.

All vaccine vials will be signed for on entry. In LUHFT, the study vaccine vials will be QP labelled with the protocol number and Clinicaltrials.gov number In addition, each vial will be given an individual ID to facilitate tracking and accountability. This process will be recorded in the vaccine accountability file.

Upon use, the vaccine vial ID number, person using, time of removal from cold storage, time of reconstitution and fate of the vial will all be recorded in the accountability file.

7.6 Assessment of Compliance

The IMOJEV® vaccine dose will be administered by the PI or nominated members of the core clinical research team at the LUHFT CRU.

The following measures will be employed to ensure treatment compliance:

1. the vaccine will be administered under the supervision of suitably qualified LUHFT CRU staff or the study PI and delegated clinical staff

7.7 Concomitant Medications/Treatments and Specific Restrictions

This study is being conducted in healthy volunteers where the need for other medication is anticipated to be low. If an incidental diagnosis is made during the study (e.g. depression, hypertension) requiring drug treatment the CI/PI will review the individual situation and take a decision as to whether the participant should continue in a study. If the new medication required is not immunomodulatory, the presumption will be to continue in the study. Additional flavivirus vaccines should not be given during the study, but can be given once 8 weeks from the final dose of study vaccine has elapsed.

Medications Permitted

Permitted 'rescue' therapy (paracetamol, NSAIDs and anti-emetics) may be taken for either study related AEs or for any other reason, without the need to inform the investigators. All use of concomitant medication will be recorded throughout the study duration. The local PI and CI will need to be informed of any other medication needed during the study. The potential effects of concomitant medication will be reviewed by the investigators who will decide, along with the CI, whether the participant should remain in the study on a case by case basis.

Medications Not Permitted/ Precautions Required

Certain classes of medications, which would be expected to impair or alter immune responses, are not allowed to be taken during the study. These are:

- Corticosteroids, including inhaled steroids
- Colchicine
- Azathioprine, other cell cycle inhibitors, or chemotherapy agents

- Methotrexate or other anti-metabolites
- Any biologic immunosuppressive or immunomodulatory drug
- Insulin/OHGD
- Any vaccine other than those being given as part of the study

In the case of a participant needing such medication, the local PI and CI will review the case and decide whether to replace the participant or not. For example, if a participant has provided samples early in the study, replacement may not be necessary.

Data on Concomitant Medication

Data on Concomitant Medication

Participants will be asked to keep a diary of any other medicine use while they are taking part in the study. This will be reviewed by a member of the core clinical research team at each of the study visits.

Specific Requirements/Restrictions

Participants will be asked to follow some restrictions:

	Further details	**Why**
Medication	Not to take medication that will impair or alter their immune response, for example; - Corticosteroids, including inhaled steroids Colchicine - Azathioprine, other cell cycle inhibitors or chemotherapy agents - Methotrexate or other anti-metabolites Any biologic immunosuppressive or immunomodulatory drug, - Insulin or other oral anti-diabetic drug Any vaccine other than that being given as part of the study (COVID vaccines can be given)	To ensure the response seen is due to the body, and not other medication. If it is necessary to take certain medications for participant health, participants will be asked to inform the study team as soon as they can.
	Further details	**Why**
Travel	Not to travel to any country where Flaviviruses are found from screening till 8 weeks after the vaccination	To limit exposure to a flavivirus which could create another response
Alcohol	Not to drink more than **28 units** of alcohol a week e.g. - 16 bottles of larger, beer or cider - 2 and half bottles of wine	Alcohol may affect they body's response to the vaccine
Other studies	Not to take part in other studies until 8 weeks after receiving IMOJEV®	To ensure the response we see if due to IMOJEV® and not another study
Blood donation	Not to donate blood for 3 months after vaccination	There is a limit to the amount of blood a person can give in a 3 month period
Contraception	Female partners of childbearing potential of male participants in the study must use an acceptable method of contraception from the time of screening until 28 days after vaccination. Females of childbearing potential must use an acceptable method of contraception from the time of screening until 28 days after vaccination.	IMOJEV® is not suitable for use in pregnant women

7.8 Overdose

Overdose is not possible due to the single dose nature of the study, and that the vaccine will be administered under supervision.

Specific information on reporting adverse events can be found in Section 10.

8 OUTCOMES

There will be no primary and secondary endpoint to the study, which is not aimed at licensure of a product. The scientific objectives of this study are to describe the priming and cross-reactivity of B and T cell responses to JE vaccine IMOJEV®. This will include mapping of specific B cell epitopes of the vaccine virus, which is performed by cloning antibodies from plasmablasts (responding B cells) at week 1 post vaccine.

More details of the laboratory assays to be performed are given in section 9.5. In addition, the safety of study participants will be reviewed and monitored regularly by the study oversight committee.

9 PARTICIPANT TIMELINES AND ASSESSMENTS

9.1 Participant Identification and Screening

Participants will be identified from an existing observational study ("Acute emerging Virus Immunity Study," (AVIS) 16/NW/0170), by advertisement, and through the LUHFT CRU, for example using the consent4consent database. AVIS is aimed at recruitment of people who have had flavivirus clinical illness, positive laboratory assays or previous vaccination, and controls, allowing identification of a pool of flavivirus exposed subjects for group 3 recruitment.

Participants identified through AVIS will already have undergone the immunological profiling necessary for FlaviPrime. Therefore, the group allocation will already be known at the point of entry into FlaviPrime. Participants will be considered suitable for recruitment into FlaviPrime under the following conditions:

Group 1 *(any status):* No special exposure criteria apply

Group 2 *(flavivirus naïve):* No potential exposure to flaviviruses based on travel history

Group 3 *(flavivirus exposed):* Confirmed flavivirus exposure, illness or vaccination

Confirmed flavivirus exposure means travel history compatible with exposure (e.g. residence in a dengue endemic area) and positive neutralising antibody assay. Flavivirus illness means an illness compatible with a flavivirus infection (most commonly dengue), in combination with a positive PCR, or NS1 antigen test, or virus isolation, or IgM ELISA and neutralising antibody.

Potential participants will be contacted (by telephone and/or email) by a member of the core clinical research team and invited to take part in FlaviPrime (consent for this contact is taken in AVIS). Participants identified through the consent4consent database or otherwise at the CRU can be consented at RLUH by a member of the study team or CRU staff, or elsewhere, and then donate blood samples and have the necessary flavivirus immunological assays performed in the University as per the Acute emerging Virus Immunity Study (16/NW/0170). Once identified, participants will be given written and verbal information on the FlaviPrime study, as well as an opportunity to ask questions.

All potential participants will be given a unique screening ID which will subsequently be used to detail the reasons for the continuation or discontinuation at the screening stage.

9.2 Informed Consent

Informed consent is a process initiated prior to an individual agreeing to participate in a study and continues throughout the individual's participation. Written informed consent is required for all patients participating in CTU coordinated trials. The process should involve discussion between the potential participant and an individual knowledgeable about the research, the presentation of written material (e.g. information leaflet or consent document), and the opportunity for potential participants to ask questions and have these satisfactorily answered. In obtaining and documenting consent, the research team should comply with applicable regulatory requirements and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Informed consent should be re-affirmed throughout the study and all discussions and consent should be documented appropriately. If a potential participant does not want to provide consent they do not have to give a reason.

Prospective Informed Consent Process

Written informed consent will be sought from patients who will be approached by the study team and invited to consider participation.

Patients will be invited to visit the LUHFT CRU, where a nominated member of the clinical research team will discuss participation in the study with them. A written information sheet that forms part of the ethically approved Information Sheet and Consent form will be provided. This includes a detailed explanation of the study and makes clear that the rights and welfare of the participants will be protected; it will be emphasised that consent may be declined or withdrawn at any time in the future without the quality of care being adversely affected. The research staff will facilitate verbal discussions about the research and the consent process, as well as providing answers to any questions that arise.

After verbal and written information has been provided, the individual seeking consent will ensure that the patient has fully understood all the information and will ask if they are happy to consent to participation in the study.

Where this is the case, written informed consent will be obtained by means of a dated patient's signature on the consent form. This should be countersigned and dated by the person who obtained informed consent i.e. the PI or other appropriately qualified member of the research team who has been delegated this responsibility.

The original signed document will be retained in the study site's Investigator Site File (ISF) and copies will be made:

- One copy provided to the patients for their information,
- One copy transferred to the CTU via encrypted email / post
- One copy filed in the participant's medical records paper/electronic.

N.B. Details of the consent process (date, persons involved, version and type of information sheet and consent form used) must also be recorded directly into the participant's medical records.

9.3 Screening, Eligibility Assessment and Confirmation

Once the participant has signed the ICF, the interviewing member of the clinical research team will carry out a more detailed medical review, drug history and travel history (to ensure no new flavivirus exposure). A physical examination and the collection of screening blood samples to ensure participants meet the full study eligibility criteria a HIV (and hepatitis B and C) test will be conducted. Hepatitis B and C tests are included to rule out non-specific immune effects of these viruses; hepatitis C is related to flaviviruses so may also give rise to specific cross-reactivity. Also if female a pregnancy blood test will be conducted. Both tests are completed for safety reasons as the safety of IMOJEV® has not been established in this patient population.

Screening information recorded for all participants, regardless of their suitability for the study, will be retained and archived.

Eligibility can only be confirmed by an appropriately qualified medical professional who is named on the delegation log and must not occur until fully informed consent is documented. Eligibility criteria are described in detail in Section 6.

Eligibility confirmation must be documented in the participant's medical notes and then on the trial's Eligibility CRF. Details must include at a minimum who confirmed full eligibility, when this was confirmed, and when the participant was formally entered into the study (e.g. registration).

9.4 Enrolment/Registration and Baseline Assessments

When satisfactory clinical screening results are received, the participant will be contacted (by telephone and / or email) by a member of the core clinical research team and invited to attend the CRU where, prior to being registered on to the study, a final checklist of inclusion/exclusion criteria will be completed to ensure the potential participant's eligibility. This will include confirmation that the participant has not travelled to a flavivirus endemic area since they were identified to be suitable in AVIS. Once eligibility has been confirmed by the PI at the CRU, the participant will be registered onto the study using an electronic registration platform. The minimum time between screening/explanation of the study and enrolment is 24 hours, but in practice this will usually be longer as the time needed to process routine HIV is >24 hours. The screening window is 8 weeks as long as there is no change in the health of the potential participant. Documentation of reasons for non-inclusion is not necessary.

Travel to a flavivirus endemic area or other potential flavivirus exposure between immunological testing in AVIS and enrolment would render a group 2 participant ineligible for the study, though theoretically if such an individual experienced a confirmed flavivirus infection during travel they would be eligible for Group 3. Travel to a flavivirus endemic area would mean the immunological assays would need to be repeated for a group 3 participant, though if this were done the participant would still be enrolled.

Once a participant consents to taking part in the study, they will be registered on a bespoke electronic data capture system which will generate a unique screening ID for each participant. This system will be used to capture the progress of screening for all participants. Each participant's GP will be provided with a letter, notifying them of their patient's involvement in the research study.

At baseline, once screening is complete, participants will be invited to attend the CRU to confirm eligibility before vaccination. A baseline blood sample should be taken and peripheral blood mononuclear cell (PBMC) and serum cryopreserved. If a subject has not travelled to a flavivirus endemic area nor had any flavivirus vaccine, it is extremely unlikely that their immunological status will have changed.

All participants at this stage will be registered to take part in the study and receive the vaccination. Participants will be registered by authorised members of the research team at the LUHFT CRU using a bespoke electronic data management system. Registration using this system can take place 24 hours a day, 7 days a week.

9.5 Schedule for Assessments and Follow-up

Participants recruited into ***Group 1*** will remain on the study for 28 days post vaccination. Partipants recruited into ***Group 2*** and ***Group 3*** will remain on the study for 8 weeks post vaccination. Once consented to take part in the main study, participants will receive the first dose of the vaccine at baseline following the completion of final eligibility checks and the collection of a baseline blood sample. Following this, participants will be asked to attend further clinic visits at the CRU on days 5 through 9 after vaccination for group 1, and at weeks 1, 2, 4 and 8 for groups 2 and 3, so that blood samples can be taken (55 – 75ml per visit) and any AEs can be assessed and recorded. T cell IFNγ-ELISpot intracellular cytokine staining (ICS) and flow cytometry assays will be performed at baseline and at weeks 1, 2, 4 and 8 after the vaccine in ***Groups 2 and 3*** participants, and at baseline and one week after the vaccine in ***group 1***. Cells will be stained for plasmablast sorting for B cell ELISpot and single cell sequencing for assessment of the adaptive immune response at every timepoint for group 1, and at one week post vaccine for groups 2 and 3. Where possible, and where responses are identified, these will be mapped down to the peptide and later minimal epitope level and further characterised. Although the most crucial data regarding cross-reactivity are derived from the time point when responses are maximal (usually 1-2 weeks), the timing of responses can vary and these experiments have not been done in this population before, leading to uncertainty about when the maximal response is. For this reason, the assays will continue until week 8 post vaccine.

Details of the blood samples and volumes to be taken are shown in the Schedule of Assessments tables below. A slightly larger sample will be taken at week 1 post vaccine, to allow for single cell sorting of plasmablasts necessary to clone antibodies which will be performed at this time point. This time point can be revised based on the findings of group 1.

Schedule of Assessments:

Group 1

		Screening	Baseline*/ SV1	SV2	SV3	SV4	SV5	SV6
Timepoint				day 5	day 6	day 7	day 8	day 9
Procedures								
Signed Consent		X						
Assessment of Eligibility		X	X					
Review of Medical History		X	X					
Travel History		X	X	X				
Review of Concomitant Medications		X	X	X	X	X	X	X
Physical Exam	Complete	X						
	Symptom Directed		X			X		
	Vital Signs	X						
Assessment of Eligibility	Pregnancy blood test	X	X					
	HIV/HBV/HCV test	X						
Study Intervention – JE vaccine IMOJEV			X					
Assessment of Adverse Events			X	X	X	X	X	X
Samples/laboratory	Haematology (FBC)		3.7	3.7	3.7	3.7	3.7	3.7
	PAXgene vol - (for RNA)		2.5	2.5	2.5	2.5	2.5	2.5
	Serum tube (vol)		8.5	8.5	8.5	8.5	8.5	8.5
	Sodium Heparin (vol - for PBMC)		40	40	40	40	40	40
Laboratory Procedures:								
IFNγ-ELISpot JEV peptide pools			X			X		
Epitope mapping***							X	X
Intracellular cytokine staining/flow cytometry for activated cells			X			X		
Plasmablast cell sorting/B cell ELISpot			X	X	X	X	X	X
Cryopreserve PBMC/plasma/serum/PAXgene			X	X	X	X	X	X

Groups 2 and 3

		Screening	Baseline*/ SV1	SV2	SV3	SV4	SV5
Study Week			0	1	2	4	8
Timepoint				day 7** +/-1	day 14 +/-2	Day 28 +/-3	Day 56 +/-7
Procedures							
Signed Consent		X					
Assessment of Eligibility		X	X				
Review of Medical History		X	X				
Travel History		X	X	X	X	X	X
Review of Concomitant Medications		X	X	X	X	X	X
Physical Exam	Complete	X					
	Symptom Directed		X			X	
	Vital Signs	X					
Assessment of Eligibility	Pregnancy blood test	X	X				
	HIV/HBV/HCV test	X					
Study Intervention – JE vaccine IMOJEV			X				
Assessment of Adverse Events			X	X	X	X	X
Samples/laboratory	Haematology (FBC)		3.7	3.7	3.7	3.7	3.7
	PAXgene vol - (for RNA)		2.5	2.5	2.5	2.5	2.5
	Serum tube (vol)		8.5	8.5	8.5	8.5	8.5
	Sodium Heparin (vol - for PBMC)		40	60	40	40	40
Laboratory Procedures:							
IFNγ-ELISpot JEV peptide pools			X	X	X	X	X
Epitope mapping/functional assays***					X	X	X
Intracellular cytokine staining/flow cytometry for activated cells			X	X	X	X	X
Plasmablast cell sorting/B cell ELISpot				X			
Cryopreserve PBMC/plasma/serum/PAXgene			X	X	X	X	X

SV = Study Visit (tolerance in +/- days is indicated for each visit). *At baseline, all procedures should be done before study intervention. **To be confirmed based on group 1. ***Only done in response to positive assays from JEV peptide pools

Clinical Procedures

The timings of all measurements to be performed during the study may be subject to change based on the ongoing review of safety and tolerability. All changes will be agreed with the Sponsor and documented in the study management folder. Safety will be assessed through the reporting of Adverse Events (AE) and Serious Adverse Events (SAE) as detailed in Section 12.

Safety Assessments – Adverse Events (AE)

The condition of each participant will be monitored throughout the study, from the time of informed consent through to the final visit. Participants will also be encouraged to spontaneously report adverse events occurring at any other time during the study. Participants will be issued an emergency contact card with the CRU 24 hour emergency telephone number on it which they can use to contact the CRU on call doctor at any time during the study if they feel unwell. Any adverse events and remedial action required will be recorded in the participant's source data. The nature, time of onset, duration and severity will be documented, together with the Investigators opinion of the relationship to the study drug. Any clinically significant events identified during the course of the study will be followed up until they are resolved or can be clinically explained.

Adverse event definitions, assignment, causality and procedures for reporting serious adverse events are detailed in Section 10 of this protocol.

Vital Signs
Supine blood pressure, pulse rate and oral body temperature will be measured at the time indicated in the study schedule. Vital signs will also be performed at other times if judged clinically appropriate, if required by hospital policy or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

Clinical Laboratory Evaluations
Blood will be collected for clinical laboratory evaluations at the times indicated in the schedule. Additional clinical laboratory evaluations will be performed at other times if judged to be clinically appropriate, required by hospital policy (e.g. MRSA) or if the ongoing review of the data suggests a more detailed assessment of the clinical laboratory safety evaluations is required. An investigator will perform a clinical assessment of all clinical laboratory data.

Physical Examination
A full physical examination or abbreviated physical examination will be performed at the times indicated in the study schedule. The full physical examination will include the assessment of the following systems: general appearance, skin (site of vaccination), lymph nodes, HEENT (head, ears, eyes, nose and throat), neck, thorax / lungs, cardiovascular, abdomen, musculoskeletal and neurological. The abbreviated physical examination will only involve the assessment of symptoms as directed by the participant.

Special Assays or Procedures

All information relating to laboratory assays and procedures employed within this study will be detailed in a separate bespoke Laboratory Manual. This will include instructions for the preparation, handling and storage of specimens including required temperatures, aliquots of specimens, where they will be stored, and how they will be labelled. All procedures will be detailed in separate protocols / working instructions which will be included in the laboratory manual together with plans in terms of the long-term access and consent for the future use of specimens.

The aim of the experimental procedures will be to determine the epitope specificity, function and cross-reactivity of the human antibody response to JE vaccine IMOJEV®. Additional assays will be conducted according to sample availability for characterising white cell populations by immunophenotyping /flow cytometry after vaccination. T cell responses to the vaccine will also be studied using IFNγ ELISpot assays and flow cytometry, because T cell responses help in the generation of potent antibody responses.

PAXgene tubes will be stored for later RNA isolation. This will be used to track the evolution of specific B cell receptor sequences identified in the above experiments over time. If sufficient sample exists, transcriptomics and/or targeted RT-qPCR will be done to assess the relationship between early gene activation and the subsequent characteristics of the adaptive immune response[29]. PBMC samples will be collected for 8 weeks to enable deep analysis of the evolving B cell response over time.

PBMC, sera and PAXgene/RNA samples will be stored for 25 years. The future intended use of these samples is address key questions on adaptive immunity to flaviviruses. For example, if a novel or previously obscure flavivirus were to emerge as a major human pathogen, such as Zika virus in 2015, we would be able to rapidly test the ability of our participants' response to cross-react with such a novel threat. Therefore, the experiments that will be done using these samples cannot be pre-determined, as they will either be guided by previous results, or by new discoveries. However, they will be focussed on immunity to viruses/flaviviruses.

Samples will be stored in the liquid nitrogen facility (cells) and -80°C freezer (serum/plasma/PAXgene) in the Ronald Ross building, University of Liverpool, in compliance with the human tissue act. The ICF specifically addresses storage and future use of these samples, without the need for additional consent for experiments on immunity to viruses.

9.6 Sampling

Sample Collection

The blood volumes that will be taken from each participant are detailed in the schedule of study assessments. The maximum blood volume to be taken per participant will be approximately 300ml, or 330ml for group 1, which is less than the volume of a single blood donation.

People who are blood donors can participate in FlaviPrime, but they cannot give blood concomitantly to the study. The maximum amount of blood that can be given in blood donation is 470 ml per 16 weeks. Therefore, a participant who is a blood donor cannot join FlaviPrime until 16 weeks after their last blood donation. Four weeks after the last FlaviPrime study sample is taken, the participant becomes is able to donate blood again.

Sample Shipment/Custodianship

Laboratory safety samples will be analysed at the Royal Liverpool University Hospital Laboratories. Experimental blood samples relating to the outcomes of this study will be analysed within the Department of Clinical Infection, Microbiology and Immunology, University of Liverpool. Samples will be transported from the CRU to the University of Liverpool in line with the study specific Material Transfer Agreement (MTA). Details of sample transport are in the bespoke laboratory manual for the study. Serological analyses will be performed in the laboratory of Dr Sutee Yoksan, Mahidol University, and Bangkok. Depending upon the initial results of the study, it may be beneficial to send samples to other laboratories, as is the case in the Acute emerging Virus Immunity Study.

Sample Storage and Handling

Samples given as part of the study, if they are not used in meeting the scientific objectives, will be stored for a period of 25 years. The samples can be used in future studies addressing the development of immunity to flaviviruses, and the cross-reactivity to flaviviruses, and to other emergent viruses, but not for other purposes (control antigens/mitogens will also be included). An example of such a use might be, testing for markers of immunity to a flavivirus or another virus that is undiscovered at the time of the study, but causes an outbreak in the future.

These samples would be used for the purposes as outlined above in section 7.3.1, although these may apply to other viruses. Permission to send material to other laboratories, and the parameters of the purpose of this, will be included in the consent form at the start of the study.

9.7 Intervention Discontinuation and Participant Discontinuation/Withdrawal

In consenting to the study, participants are consented to study treatment, follow-up and data collection. If voluntary withdrawal occurs, the participant should be asked to allow continuation of scheduled evaluations,

complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the subject's condition becomes stable.

The critical study data in FlaviPrime is derived from experimental blood samples. Although participants can refuse a blood sample at any time, refusal to give crucial samples (weeks 1, 2 and 4 weeks post JE vaccine IMOJEV®) would result in significant compromise to the study. Therefore, any participant who does not give these samples for any reason will be replaced with a new volunteer and removed from the study sampling schedule thereafter. The participant will remain in the study for safety.

Premature Discontinuation of Study Intervention

Once the single dose of JE vaccine IMOJEV® has been given, there is, in practice, no intervention to be withdrawn from, and participants can remain in the study. Foreseeable reasons why a participant may withdraw from the study generally apply to the taking of study blood samples, and include:

i. Participant withdraws consent
ii. Participant is unable to attend regularly for assessment and to donate week 1, 2 and 4 post vaccination samples for any reason
iii. Loss of capacity during the study
iv. Any other change in the participant's condition that justifies the discontinuation of sample collection the study in the clinician's opinion.

A participant is free to withdraw from the study at any time. In addition, the CI may decide, for reasons of medical prudence, to withdraw a participant. In either event, the Sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the participant's source data. If a participant withdraws or is withdrawn, ideally they should remain in the study for collection of safety data and/or treatment of any AEs, until these have resolved.

Participant Withdrawal from Follow Up

If a participant wishes to withdraw from the study sampling schedule, they will be informed by the CRU staff of the importance of remaining on study follow-up for safety, or failing this, of allowing routine follow-up data to be used for study purposes. Generally, follow-up will continue unless the participant explicitly also withdraws consent for follow-up. The CI may also request that the participant return for an additional follow-up visit, for the purpose of collecting safety data, or ensuring resolution/adequate treatment of an AE.

Participants are free to withdraw from follow up at any time without providing a reason, though a reason should be recorded if one is given. Those who wish to withdraw from further follow-up will have the data collected up to the point of that withdrawal included in the analyses. The participant will not contribute further data to the study and the CTU should be informed via email to the CTU and via completion of a Withdrawal CRF to be returned to the CTU within 7 days.

If participants express a wish to withdraw from follow up, the research team at site should ascertain if this is for all elements of study follow-up, or if for example data from routine assessments can still be collected for the study. In the case of ongoing adverse events, participants should be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the participant's condition becomes stable.

Any SAEs will be notifiable to the CTU via processes detailed in Section 11 even if a participant has withdrawn from follow up.

Participant Withdrawal from Study Completely

Participants who withdraw from the study for other reasons have previously consented to follow-up in the study. Data up to this time can be included in the study if anonymised. They may need to reaffirm that they consent to follow-up through usual NHS mechanisms. If the participant explicitly states their wish not to contribute further data to the study, a withdrawal CRF should be completed.

Participant Transfer

For participants moving from the area, every effort should be made to encourage the participant to continue to attend follow-up if possible. If this is not possible, the participant will have to be withdrawn from the study. If the participant is withdrawn before the day 9 sample (group 1) / two-week sample (groups 2 and 3) has been donated, the participant should be replaced if possible (dependent on the availability of vaccine). This should be discussed with the Chief Investigator and LCTC.

Loss to Follow-up

If for any reason a participant does not complete the study they should attend a final visit / assessment with the study clinician as soon as possible. If the study treatment is stopped before the end of the scheduled assessment period, the clinician may contact the participant to request additional information up to the point where the study treatment would be complete.

Travel

Participants in the study will be required not to travel to a JEV and DENV endemic area until the sampling schedule has reached study week (SW) 8.

If any participant who does travel to a JEV and DENV endemic area before 8 weeks, consideration will be given to replacing them by the study oversight committee. They will remain in AVIS to gather additional data. For example, a participant who contracted a wild type flavivirus infection during the study would represent a natural experiment of great relevance to the principles under investigation in this study.

9.8 End of Study

The end of the study is defined to be the date on which data for all participants is frozen and data entry privileges are withdrawn from the study database (database is locked). Study enrolment may be stopped at a site when the total requested number of participants for the study have been recruited. The Study Oversight Committee may recommend that the study be stopped prematurely. In such circumstances of premature termination / suspension of the study, National Research Ethics Service (NRES) and HRA will be notified according to standard reporting guidelines

Site and closure activities will be centrally coordinated and conducted in accordance with CTU processes regardless of whether the study closes as planned or prematurely. This includes activities such as:

- End of Study notification to REC
- Study-related materials reconciled and returned/disposed of as appropriate see section 7.5 for IMP
- All site data entered onto the study database, discrepancies raised and satisfactory responses received

- Quality Control checks of the Investigator Site Files, Pharmacy Files and Study Master File as appropriate.

Study Discontinuation

If this study is prematurely discontinued (e.g., due to safety) all participants must be informed and the reason for the discontinuation should be written on the end of study form for each participant. If a participant has been withdrawn completely from the study whilst is still ongoing, an end of study form should be completed.

The need for stopping the study will be considered by the study Oversight Committee. The decision will be based upon participant safety. If any of the following scenarios occur with reasonable possibility of a causal relationship with the study vaccine, no further doses of study vaccine will be given.

1. Clinically relevant signs or symptoms or intolerable adverse events of similar nature occurring in 2 or more participants that in the opinion of the CI warrant stopping,

2. One or more participants report a serious adverse event considered by the CI, local PI, or delegated other physician who can assess causality and expectedness of AEs to be at least possibly related to the study drug.

10 SAFETY REPORTING

Safety reporting in clinical trials is a legal and ethical requirement and it is imperative that all applicable requirements detailed here are followed during the study.

10.1 Terms and Definitions

Adverse Event (AE)
Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the study vaccine.

Related Adverse Event (Related AE)
An AE which resulted from administration of any of the research procedures – i.e. assessed as "probably", "possibly" or "almost certainly" related to the study procedures or vaccine (see section 7)

Related Unexpected Adverse Event (RUAE)
A Related AE which is not expected, i.e. not consistent with the known effects of the study vaccine or research procedures (see section 7).

Serious Adverse Event (SAE)
An adverse event which meets the definition of "serious" (see section 10.2).

Related Serious Adverse Event (Related SAE)
A SAE which is assessed to be "probably", "possibly" or "almost certainly" related to the study vaccine or study trial procedures (see section 7).

Related Unexpected Serious Adverse Event (RUSAE)
A Related SAE which is not expected, i.e. not consistent with the known effects of the study vaccine or study trial procedures (see section 7).

Reference Safety Information (RSI)
The information used for assessing whether an adverse reaction is expected (see section 10.5). This is contained in the Investigators Brochure (IB) for the vaccine. The RSI used to assess the expectedness of a SAE/SAR must be the current approved version at the time of onset of the SAR. The RSI for this trial is defined in section 10.5.

10.2 Assessment of Seriousness

The assessment of seriousness of safety events should be performed by an appropriately delegated, medically qualified member of the site research team.

A #safety event (whether or not assessed as related to the study) is assessed as serious if it:

- Results in death;
- Is life-threatening (i.e. the investigator considers the event places the subject at immediate risk of death from the experience as it occurred (this does not include an adverse experience that, had it occurred in a more severe form, might have cause death);
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation);
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions);
- Consists of a congenital anomaly or birth defect (in offspring of study participants, or their partners, regardless of time of diagnosis), or

- Is otherwise considered medically significant by the investigator.

#safety event / reaction applies apply to either AEs, or ARs, or Related AEs,

10.3 Severity of Adverse Events

All adverse events should be assessed for severity. The assignment of the severity/grading should be made by the investigator responsible for the care of the participant using the definitions in the table below:

Table 1: Severity Grading

Severity	Description
Mild	Does not interfere with routine activities.
Moderate	Interferes with routine activities.
Severe	Impossible to perform routine activities.
Life-Threatening	
Death	

N.B. A distinction is drawn between **serious** and **severe** AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in Section 10.2. Hence, a severe safety event need not necessarily be a "serious" safety event.

10.4 Assessment of "Causality" - Relationship to Study Treatment/Intervention

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below:

Table 2: Definitions of Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship. N.B. An alternative cause for the AE should be given
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the study medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possibly	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the study medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probably	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Almost certainly	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Assessment of causality should be made based on known safety profile of IMOJEV®. In the case of discrepant views on causality between the treating investigator and others, the opinion of the treating investigator will never be downgraded and the REC will be informed of both points of view. Events that are assessed as being possibly, probably or almost certainly related will be reported as having a reasonable possibility of being

related, and events assessed as unrelated or unlikely will be reported as having no reasonable possibility of being related.

Assessment of causality should be made based on known safety profiles of IMP, or IB and known risk profiles of other drugs in the same class. If any doubt about the causality exists the local investigator should inform the LCTC who will notify the Chief Investigator. In the case of discrepant views on causality between the treating investigator and others, the opinion of the treating investigator will never be downgraded and the REC will be informed of both points of view.

10.5 Assessment of "Expectedness"

The Chief Investigator for the FlaviPrime study is responsible for determining whether a safety event is expected or unexpected, however a Chief Investigator will not assess their own participants, these participants will be assessed by the Medical Reviewer/Clinical Coordinator. There is no requirement for a reporting investigator to make an assessment of expectedness.

An event will be considered unexpected if it is not listed within the current and approved RSI for the study at the time of the event's onset. The nature, severity, or frequency of the event should be considered – if this is not consistent with that described for the type of event in the RSI the event should be assessed as unexpected.

Reference Safety Information / Information used to Assess Expectedness

Document name: Australian product information
IMOJEV® Japanese encephalitis vaccine (live, attenuated)

IMOJEV IB section "Adverse Effects" → "Data in adult populations"

The current version of the RSI documents applicable to this study will be made available to all clinicians involved in assessing the causality of adverse events, as well as the ethics committee and sponsor.

10.6 Time Period for Active Monitoring of Safety Events

IMPORTANT: Any safety events occurring after the end of the below described "active monitoring" period which meet the definition of serious and are recorded for this study must continue to be reported by sites to the LCTC in accordance with the timeframes and procedures described in section 11.3. The same processes established for SAEs within the active monitoring period should be followed for these events.

All new serious adverse events should be reported from the point of consent until 28 days after administration of the study vaccine. Depending on the nature of the event, the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to LCTC in the first instance. Group 1 participants, who have their last study visit 9 days after vaccination, will be encouraged to report any adverse events that occur up to 28 days to the LUHFT CRU by phone.

AEs that occur during this timeframe should be reported to LCTC via the AE form. When reporting an increase in severity for an existing AE, the AE form should be changed to the new grade and the reason why (increase in severity) included. For a decrease in severity, no action is to be taken unless the symptoms have fully resolved (in which case the AE/SAE should be updated until resolved.

Although the period of adverse event monitoring in this study is 28 days, if the site team become aware of an adverse event that occurs outside this time and there are reasonable grounds to suspect the event is related to the vaccine or to participation in the study, it would be good practice to report this event to LCTC.

10.7 Notes on Safety Event Recording

The following events must be recorded for the purposes of the study:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event/condition
- A condition (even though it may have been present prior to the start of the study) detected after study drug administration
- Continuous persistent disease or symptoms present at baseline that worsens following the administration of the study/study treatment
- Laboratory abnormalities that require clinical intervention or further investigation (unless they are associated with an already reported clinical event).
- Abnormalities in physiological testing or physical examination that require further investigation or clinical intervention
- Injury or accidents
- Pregnancy (See section 11 for more details)

Do not record:

- Medical or surgical procedures - the condition which leads to the procedure is the adverse event
- Pre-existing disease or conditions present before treatment that do not worsen
- Situations where an untoward medical occurrence has occurred e.g. cosmetic elective surgery
- Overdose of medication **without signs or symptoms***
- The disease being treated or associated symptoms/signs unless more severe than expected for the participant's condition

*N.B. If overdose occurred **with** resulting signs and symptoms that meet the protocol criteria for AE/SAE/SAR/RUSAE then they should be reported accordingly and the overdose highlighted to the LCTC team.

The events above do not need recording as the study population is healthy, and no disease is being treated by administration of this vaccine. The study is considered low risk. Any health event not related to the vaccine and not occurring during the study period (i.e. not an AE) is definitely unrelated to use of the vaccine, which is already in routine clinical use with a well understood safety profile.

11 Reporting of Pregnancy

If a participant or their partner becomes pregnant during study treatment or within 28 days following treatment, a Pregnancy Report Form must be completed and forwarded to the LCTC within 24 hours of learning of its occurrence and the investigational product will be permanently discontinued in an appropriate manner. (Should you need a paper copy of the Pregnancy Report Form please contact the Study Coordinator). Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy.

IMOJEV® has not been studied in pregnancy, so if a participant were to fall pregnant, there are no data upon which to base an opinion as to the likely effects. However, in order to have an effect, the participant would have to actually be pregnant at the time of vaccine administration, or when vaccine related viraemia is present. This lasts up to 6 days after vaccination[30], so a participant would have to fall pregnant very quickly after study enrolment for there to be any possibility of an effect on the pregnancy. The immune effects of the pregnancy could have an effect on the study data, but the risk of harm to the participant and the pregnancy would be extremely small. It should be noted that yellow fever vaccine (which is more toxic than IMOJEV) has been used in pregnancy during vaccination campaigns to contain outbreaks and there have not been reports of adverse effects on the fetus[31].

On pregnancy outcome, the final Pregnancy Report Form should be completed and forwarded to LCTC 28 days after the outcome. The final Pregnancy Report Form is used to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or new-born complications. Pregnancy follow-up information on this form also includes an assessment of the possible relationship to the study intervention of any pregnancy outcome. Pregnancy outcomes should also be collected for the female partners of male participants participating in the study. Infants should be followed for a minimum of 8 weeks. Consent to report information regarding these pregnancy outcomes should be obtained from the mother prior to completion and faxing of the final Pregnancy Report Form. Any SAE experienced during pregnancy must be reported on the SAE form.

The LCTC will report all pregnancies to the study Sponsor and National Research Ethics Service.

Please Report

All pregnancies using the Pregnancy Report Form.

This should be reported within 24 hours of becoming aware.

ALSO

Pregnancy Outcomes must also be reported by completing the Final Pregnancy Report Form.

This should be completed 28 days following the outcome.

Completed forms should be sent via SECURE email to lctcsafe@liverpool.ac.uk

Sexually active women of child bearing potential (WOCBP) must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimised.

Before study enrolment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy in accordance with the information contained in the Participant Information Sheet.

All WOCBP MUST have a negative pregnancy test within 72 hours before receiving treatment. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not be enrolled in the study. In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g. missed or late menstrual period) at any time during study participation.

Female participants who are of non-child bearing potential (i.e. due to being post-menopausal for at least 1 year (confirmed by FSH assessment) or permanently sterile following hysterectomy, bilateral salpingectomy, bilateral oophorectomy) will not be required to use contraception.

Female participants must be willing to use a highly effective method of birth control (i.e. contraceptive measure with a failure rate of <1% per year), from the time of Screening until 28 days after vaccination. Highly effective methods of contraception include:

- Placement of intrauterine device or intrauterine system
- Established use of oral, injected or implanted hormonal methods of contraception associated with inhibition of ovulation
- Additional barrier method (i.e. diaphragm or cervical vault cap used in conjunction with spermicide gel, foam, cream, film or suppository).
- Male sterilisation (with the appropriate post-vasectomy confirmation of surgical success). For female participants on the study, the vasectomised male partner should be the sole partner for that participant.
- Bilateral tubal ligation

Female partners of childbearing potential of male participants in the study must use an acceptable method of contraception from the time of screening until 28 days after vaccination. Other acceptable methods of contraception include:

- Any highly effective method of contraception listed above for female participants
- Progesterone-only oral contraception, where inhibition of ovulation is not the primary mode of action
- Cap, diaphragm, or sponge with spermicide

For participants who practice true abstinence, when this is in line with the preferred and usual lifestyle of the participant, contraceptive requirements do not apply. For participants who are exclusively in same-sex relationships, contraceptive requirements do not apply. Male participants with pregnant partners should use condoms from the time of vaccination and for two weeks afterwards.

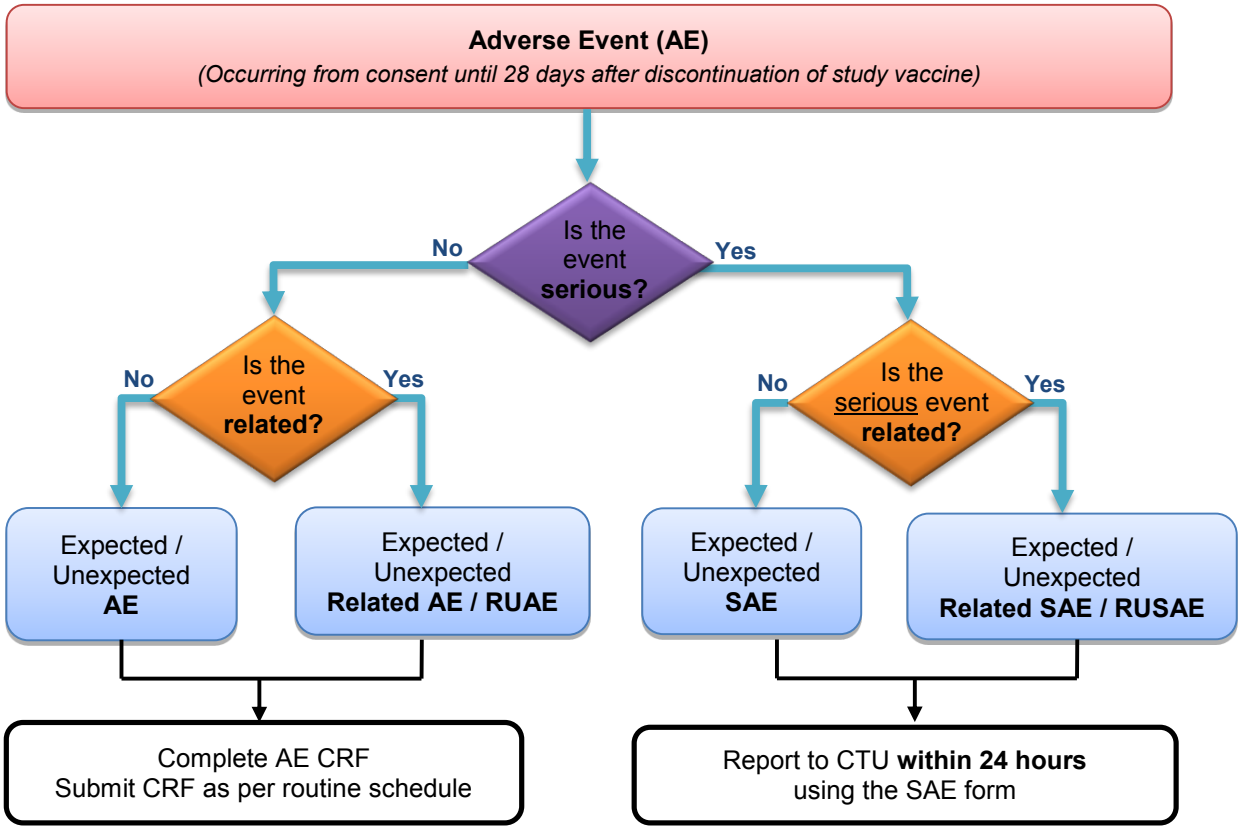
Notification of Deaths

If the research team become aware of the death of a participant (whether related to the study or not) this should be notified to the LCTC using the appropriate CRF within 24 hours of becoming aware.

11.1 Reporting Procedures

All safety events which are recorded for the study should be reported following the procedures detailed below. The occurrence of a safety event may come to the attention of research staff during routine study visits, from the participant's notes, directly from the participant or by other means. Note that reporting procedures vary dependent on the nature of the incident (i.e. "serious" events are to be reported to LCTC in an expedited manner). Any questions concerning adverse event reporting should be directed to the LCTC in the first instance. A flowchart is given below to aid in determining reporting procedures for different types of adverse events.

Flowchart for Site Reporting Requirements of Adverse Events



Reporting Safety Events to the LCTC

All safety events (whether or not assessed as serious / related / expected) should be recorded on an Adverse Event Form; multiple events can be recorded on one form.

Safety events which are assessed as "serious" must **also** be recorded in more detail on Serious Adverse Event Forms; a single form is used for each individual event (i.e. a single diagnosis), though multiple symptoms can be recorded. Each SAE should have a corresponding record on the participant's AE form. Where additional information is received by site after initial submission to LCTC, this should be provided on a follow-up form within 5 days. Serious Adverse Event Forms collect data regarding the nature of event, date of onset, severity, corrective therapies given, outcome and causality; all serious events reported to LCTC will be reviewed by the Chief Investigator or Medical Reviewer/Clinical Coordinator, and assessed for causality and expectedness.

Follow-up after Adverse Events

All reportable adverse events should be followed until satisfactory resolution or until the investigator responsible for the care of the participant deems the event to be chronic or the participant to be stable.

When reporting "serious" safety events the investigator responsible for the care of the participant should apply the following criteria to provide information relating to event outcomes:

- resolved
- resolved with sequelae (specifying with additional narrative)
- not resolved/ongoing
- ongoing at final follow-up
- fatal or unknown.

11.2 Investigator Reporting Responsibilities

The PI is responsible for ensuring that all safety events requiring recording on this study which the local research team becomes aware of are reported to LCTC. It is the responsibility of the PI/Co-PI as recorded on the Delegation Log (medically qualified person) to assess the seriousness and causality of events.

All safety events must be recorded on an AE form and transferred to LCTC **within seven days of the site team becoming aware of the event**.

Safety events which meet the definition of "serious" must be reported in more detail to the LCTC on an SAE form and reported **immediately and in no circumstances later than 24 hours from becoming aware** unless the SAE is specified in the protocol as not requiring immediate reporting where they will be appropriately processed.

The SAE form should be completed by an appropriately delegated member of the research team; the assessments of seriousness and causality must be performed by an appropriately medically qualified person. Minimum reporting information must be provided in initial reports for all studies.

Minimum information required for reporting:

- Study identifier
- Study centre
- Patient number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

N.B. In the absence of a delegated medically qualified person the form should be completed and signed by an alternative member of the research site study team and submitted to the LCTC. As soon as possible thereafter the responsible investigator should check the SAE form, make amendments as appropriate, sign and re-send to the LCTC. The initial report shall be followed by detailed follow-up reports as appropriate.

Safety events should be reported to the site R&D team in accordance with local policy.

11.2.1 Reporting an Initial or Follow-up SAE

The investigator should ensure the actions below are completed for all reportable SAEs:

1) Research sites should telephone the appropriate study co-ordinator / data manager on telephone number **0151 794 9766** to advise that an SAE report has been submitted as soon as possible.

2) **The SAE form should be transferred securely to lctcsafe@liverpool.ac.uk (within 24 hours) to the CTU**

3) The responsible investigator must notify their R&D department of the event (as per standard local governance procedures).

4) The participant must be identified by study number, age or month and year of birth and initials **only**. The participant's name **should not** be used on any correspondence.

5) SAEs must be subsequently followed up in line with the processes below:
    - Follow up must continue until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised (see Section 11.7.4). N.B. Follow-up may continue after completion of protocol treatment if necessary.
    - Follow-up information is noted on a new SAE form to be transferred securely to the CTU as soon as more information becomes available
    - Tick the appropriate box on the new SAE form to identify the type of report; this is dependent on resolution status of the SAE e.g. follow-up / final.

6) Extra, annotated information and/or copies of anonymised test results may be provided separately.

Participant safety incidents that take place in the course of research should be reported to the National Reporting and Learning System (NRLS) by each participating NHS Trust in accordance with local reporting procedures. This also includes reporting any NIMPs.

11.3 LCTC Responsibilities

The study Sponsor, University of Liverpool, have delegated to LCTC the duty of onward reporting of safety events to REC, regulatory authorities. SOPs will be followed to ensure appropriate reporting as detailed below.

All "serious" safety events will be forwarded to the Chief Investigator or Medical Reviewer/Clinical Coordinator by LCTC within 24 hours of receiving the minimum information from site. The CI or Medical Reviewer/Clinical Coordinator will review information provided by site and for all events assessed as "related" will provide an assessment of "expectedness".

Safety events which are assessed as "serious", "related" and "unexpected" (i.e. RUSAEs) will be onward reported by LCTC to the ethics committee **within 15 days** of the LCTC first becoming aware of the event.

Additionally, RUSAEs will be reported to the study Sponsor(s) and Principal Investigators of participating sites.

A list of all safety events recorded for the study will also be reported annually by LCTC to the ethics committee and Independent Data Safety & Monitoring Committee.

Any concerns raised by the TSC/IDSMC or inconsistencies regarding safety reporting noted at a given site may prompt additional training at sites, with the potential for the LCTC to carry out site visits if there is suspicion of unreported AEs and SAEs in participant case notes. Additional training will also be provided if there are unacceptable delays in safety reporting timelines.

Safety Reports

Safety reports will be generated during the course of the study which allows for monitoring of safety event including reporting rates and safety events by participant group. The LCTC will send Annual Progress Reports (APRs) containing a list of all SAEs to the IDSMC, regulatory authorities and main REC. If any safety reports identify issues that have implications for the safety of study participants, the PIs at all institutions participating in the study will be notified.

Urgent Safety Measures (USMs)

An urgent safety measure (USM) is a procedure to protect clinical study participants from any immediate hazard to their health and safety but has not previously been defined by the protocol. It can be put in place prior to authorisation by the REC.

The LCTC will notify the REC immediately and, in any event, within 3 days that such a measure has been taken and the reasons why it has been taken. The initial notification to the REC will be by telephone (ideally within 24 hours) and a notice in writing will be sent within 3 days, setting out the reasons for the USM and the plan for further action. After discussion with the REC, further action will be agreed, which may include submission of a substantial amendment, a temporary halt, or permanent termination of the study.

Following notification, if a substantial amendment is required this must be submitted as soon as possible to the REC. If the study is temporarily halted it may not recommence until authorised to do so by the REC. If the study is permanently terminated before the date specified for its conclusion (in the original applications to REC), the Sponsor should notify the REC within 15 days of the date of termination by submitting the formal End of Study Notification.

11.4 Contact Details and Out-of-hours Medical Cover

As IMOJEV® has a well-established safety profile, emergency and out-of-hours medical care will be in line with usual LUHFT CRU arrangements and local standard practice; no special provision is required for FlaviPrime participants. All participants will be provided with a CRU contact card with a 24-hour emergency telephone number for the CRU on call doctor, and a copy of the information sheet which includes information about their participation and contact details for the local research team who may be contacted if necessary. During office hours, the CI, LUHFT CRU, or delegate are able to provide medical advice in relation to participation using the contact details listed at the beginning of this document.

12 STATISTICAL CONSIDERATIONS

12.1 Introduction

In the following section a brief overview of the statistical considerations relating to the design and conduct of the study. No formally pre-specified statistical analyses will be conducted as part of the study.

12.2 Sample Size

As this is a study with an exploratory endpoint, there is no formal hypothesis to be tested or a formal sample size calculation based on powering a hypothesis to some degree of accuracy. In order to allow sufficient material for antibody cloning, the study aims to collect single B cell receptor repertoire sequencing data on > 1000 sorted plasmablasts from 20-25 participants. This sample size is broken down as:

- Group 1; up to 3-4 participants of any previous flavivirus exposure state
- Group 2; up to 6-10 flavivirus naïve participants
- Group 3; up to 8-12 flavivirus experienced participants

A total of 20 participants is expected to be vaccinated, though this figure can be increased up to a maximum of 25 if the critical B cell data are not obtained (e.g. the number of plasmablasts sorted for single B cell receptor repertoire sequencing is > 1000, or of there is technical failure of any kind). 20 participants was chosen as a pragmatic figure which would be feasible for generating sufficient antibody sequence data and monoclonal antibodies, which are extremely labour intensive in the laboratory, it being possible to make many hundreds of antibodies from a single individual.

12.3 Method of Registration

This study does not include participant randomisation. All participants who have given written informed consent and have been found to comply with the inclusion and exclusion criteria will be registered onto the electronic registration database (in accordance with the LCTC registration operating procedures) by authorised research staff. In the unlikely event that the back-up registration process is activated, participant registration will be performed by site staff with a second-line check being performed by authorised LCTC staff.

12.4 Outcome Measures

This study aims to establish a unique human experimental medicine system which will be used to study flavivirus immune responses and for epitope discovery and there will be no primary or secondary endpoint in this study.

12.5 Exploratory Objectives

The objective of this study is to describe the priming of B cells to JE vaccine IMOJEV® including mapping of specific B cell epitopes of the vaccine virus, by generating a panel of monoclonal antibodies from single cell sorted plasmablasts at one week post vaccine.

To determine the ability of B cell responses primed by JE vaccine IMOJEV® to cross-react with other flaviviruses, by neutralisation assay (B cells).

12.6 Interim Analyses

As there are no formal hypotheses being tested, there are no formal stopping rules (other than safety) or mechanisms defined here to stop the study prior to the planned end of the study. The study does have a formal Oversight Committee who will be able to review at regular intervals all accumulating data. The main responsibility of this committee will be to review the recruitment of participants, the collection of all essential data, to assess participant safety and to provide recommendations regarding the administration of a second dose of the JE IMOJEV® vaccine, based on the magnitude of the immune response to the vaccine.

12.7 Analysis Plan

Analyses for the study are under the remit of the CI and will not be performed by the LCTC. The analysis being carried out is exploratory in nature, the details in terms of the methodology for this analysis may be altered during the course of the study. The analysis will be largely descriptive including (but not limited to) summaries of

- % plasmablasts (defined as CD38hi, CD27hi) in the recently activated B cell population (CD19+, CD20lo).
- Number of plasmablasts sorted
- % of JEV specific cells, measured by B cell ELISpot
- Number of antibodies made that bind JEV
- Proportion of virion binding antibodies that neutralise
- Proportion that are specific for the fusion loop epitope
- JEV neutralisation/neutralisation of other viruses before and after vaccination (DENV1-4, ZIKV, WNV)

Reporting guidelines for analyses are detailed in section 12.7.1 to 12.7.6.

12.7.1 Participant Groups for Analysis

As this is not a study comparing an intervention on participant outcome, final analysis will take place on a per-protocol basis, removing any participants who have any major protocol deviations.

12.7.2 Significance Levels

As this is an exploratory study, no formal levels of significance are set. All statistics presented will be presented alongside 95% confidence intervals so as to give an indication of the level of precision only.

12.7.3 Missing Data

The likelihood of missing data are small given the small number of participants in this study. Final analysis will take place on a complete-case basis with no adjustments made (e.g. multiple imputation) in the case of missing data.

12.7.4 Exposure to Vaccine

Exposure to vaccine will be assessed by the assessment of compliance. Because the study involves a single dose of vaccine, only participants who have been vaccinated will be included in the subsequent sampling schedule and analyses.

12.7.5 Trigger for Final Analysis

Analysis of study data will take place once all participants have received the planned follow-up and all data are available for analysis.

12.7.6 **Data descriptions**

Continuous data shall be summarised as median, inter-quartile ranges and ranges. Categorical data shall be summarised as frequencies of counts and associated percentages.

13 DATA MANAGEMENT AND STUDY MONITORING

For the FlaviPrime study the responsibilities for Data Management and monitoring are delegated to the LCTC Separate Data Management and Study Monitoring Plans have been developed which provide detail regarding the internal processes that will be conducted at the LCTC throughout the study. Justification for the level of monitoring is provided within those documents and the study-specific risk assessment. All data will be managed as per local LCTC processes and in line with all relevant regulatory, ethical and legal obligations.

13.1 Source Documents

The case report form (CRF) will be considered the source document for data where no prior record exists and which is recorded directly in the bespoke CRF. A FlaviPrime source document list will be produced for each site to be kept in the ISF and provide detail of what constitutes FlaviPrime-specific source data.

Date(s) of informed consent processes (including date of provision of patient information, randomisation number and the fact that the patient is participating in a clinical study (including possible treatment arms) should be added to the patient's medical record chronologically.

13.2 Data Collection Methods

Participant eCRFs/ CRFs will be provided to sites for local completion by members of the research team trained and delegated the duty. Study staff named at each site will enter data from source documents corresponding to a participant's visit onto the relevant CRF in the participant's folder The CRF is the primary data collection instrument for the study so all data requested on the CRF **must** be recorded and all missing data must be explained. A copy of all CRFs should be retained at site. Any corrections should be made in accordance with GCP.

For participant registration: Data are to be entered into a bespoken LCTC electronic database by members of the research team at site. Training will be provided prior to any data entry.

13.3 Monitoring

Monitoring is conducted to ensure protection of patients participating in the study and all aspects of the study (procedures, laboratory, study intervention administration and data collection) are of high quality and conducted in accordance with sponsor and regulatory requirements.

A detailed Study Monitoring Plan will be developed and agreed by the SMG and CI to describe who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted. This will be dependent on the documented risk assessment of the study which determines the level and type of monitoring required for specific hazards. All processes may be subject to monitoring, e.g. enrolment, consent, adherence to study interventions, accuracy and timeliness of data collection etc.

Study Oversight Committees related to the monitoring of the study are detailed in Roles and Responsibilities see section 3.

Central Monitoring

There are a number of monitoring features in place at the LCTC to ensure reliability and validity of the study data, to be detailed in the study monitoring plan. Data will be entered into a validated database and during data processing there will be checks for missing or unusual values (range checks) and for consistency within

participants over time. Other data checks relevant to patient rights and safety will also be regularly performed as per LCTC processes. Any suspect data will be returned to the site in the form of data queries. Data query forms will be produced at the LCTC from the study database and sent either electronically or through the post to a named individual (as listed on the site delegation log). Sites will respond the queries providing an explanation/resolution to the discrepancies and return the data query forms to the LCTC. The forms will then be filed along with the appropriate CRFs and the appropriate corrections made on the database.

Site monitoring visits may be 'triggered' in response to concerns regarding study conduct, participant recruitment, outlier data or other factors as appropriate.

Clinical Site Monitoring

In order to perform their role effectively, monitors and persons involved in Quality Assurance and Inspection will need direct access to primary subject data, e.g. participant records, laboratory reports, appointment books, etc. Each PI therefore permits study related monitoring, audits, ethics committee review and regulatory inspections by providing direct access to source data/documents. As this affects the participant's confidentiality, this fact is included on the PIS and ICF.

13.4 Risk Assessment

In accordance with the LCTC Standard Operating Procedures and the requirements of the sponsor, a risk assessment has been completed in partnership with:

- Representatives of the Study Sponsors (University of Liverpool)
- Chief Investigator
- Members of the Study Management Group
- Study Coordinator
- Statistician
- LCTC Operational Director

In conducting this risk assessment, the contributors considered potential patient, organisational and study hazards, the likelihood of their occurrence and resulting impact should they occur.

The outcome of the risk assessment is categorised based upon the potential risk associated with the study intervention in accordance with MRC/DH/MHRA Project on Risk-adapted Approaches to the Management of Clinical Trials of Investigational Medicinal Products based on the following categories:

- Type A: No higher than that of standard medical care
- Type B: Somewhat higher than that of standard medical care
- Type C: Markedly higher than that of standard medical care
- **Non-CTIMP**

The FlaviPrime study is using a vaccine that is not licensed in an EU member State. However is licensed for use in Australia since August 2010, Thailand since October 2010 and Malaysia 2013. The MHRA have confirmed that this study is classified as a Non-CTIMP.

13.5 Confidentiality

This study will collect personal data (e.g. participant names), including special category personal data (i.e. participant medical information) and this will be handled in accordance with all applicable data protection legislation. Data (including special category) will only be collected, used and stored if necessary for the study

(e.g. evidencing provision of consent, for data management and central monitoring, statistical analysis, regulatory reporting, etc.). At all times, this data will be handled confidentially and securely.

CRFs will be labelled with a unique study screening number. Verification that appropriate informed consent is obtained will be enabled by the provision of copies of participant's signed informed consent forms being supplied to the CTU by recruiting sites. This transfer of identifiable data is disclosed in the PISC.
N.B. Consent forms must be transferred separately to any other study documentation to ensure the pseudonymisation of special category data is maintained.

Site-specific study-related information will be stored securely and confidentially at sites and all local relevant data protection policies will be adhered to.

The LCTC as part of The University of Liverpool will preserve the confidentiality of participants taking part in the study. The University of Liverpool is registered as a Data Controller with the Information Commissioners Office.

Breaches of data protection principles or regulations identified by LCTC will be notified promptly to the study Sponsor and The University of Liverpool's Data Protection Officer and appropriate processes followed.

13.6 Quality Assurance and Control

To assure protocol compliance, ethical standards, regulatory compliance and data quality, as a minimum, the following will occur:

- The PI and other key staff from each centre will attend initiation training, which will incorporate elements of study-specific training necessary to fulfil the requirements of the protocol.
- The SOC will determine the minimum key staff required to be recorded on the delegation log in order for the centre to be eligible to be initiated.
- The TC at the LCTC will verify appropriate approvals are in place prior to initiation of a centre and the relevant personnel have attended the study specific training. A greenlight checklist will verify all approvals are in place prior to study initiation at LCTC and the individual centre.
- The study will be conducted in accordance with procedures identified in the protocol.
- The Study Oversight Committee (SOC) will provide independent oversight of the study.
- SOC will monitor screening, consent rate and compliance with the protocol.
- Data quality checks and monitoring procedures will be undertaken in line with the study Data Management Plan.

13.7 Records Retention

The retention period for the FlaviPrime data and information is a minimum of 5 years after issue of the final report, or longer at Sponsor's request.

The PI at each investigational site must make arrangements to store the essential study documents (as defined by ICH GCP guidelines) including the Investigator Site File, the applicable participant medical records and Pharmacy Site File, for the full length of the study's retention period and will arrange for confidential destruction at the end of this period as instructed by the Sponsor / CTU.

The PI is also responsible for archiving all relevant source documents so that the study data can be compared against source data after completion of the study (e.g. in case of inspection from authorities). They must ensure the continued storage of the documents, even if they, for example, leave the clinic/practice or retire before the end of required storage period. Delegation of responsibility for this must be documented in writing.

All other persons and organisations involved in the study will be responsible for storing and archiving the parts of the TMF relevant to their delegated duties (e.g. laboratories, IMP manufacturers and distributors, third-party vendors providing randomisation and IMP allocation systems, etc.).

The LCTC undertakes to archive as per their contractual requirements; documents will be archived in compliance with the principles of GCP. All electronic CRFs and study data will be archived onto an appropriate media for long term accessible storage. Hard copies of data will be boxed and transferred to secure premises where unique reference numbers are applied to enable confidentiality, tracking and retrieval.

14 REGULATORY AND ETHICAL CONSIDERATIONS

14.1 Statement of Compliance

This study is designed to comply with the guideline developed by the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and will be conducted in compliance with the protocol, LCTC Standard Operating Procedures and EU Directive 2001/20/EC, transposed into UK law as the UK Statutory Instrument 2004 No 1031: Medicines for Human Use (Clinical Trials) Regulations 2004.

14.2 Ethical Considerations

The study will be conducted to conform to the principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly, 1964, and subsequent amendments (Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996).

The study will be conducted in accordance with the EU Directive 2001/20/EC, the Medicines for Human Use (Clinical Trials) Regulations 2004 and the principles of Good Clinical Practice.

Participants will be asked to consent that data are recorded, collected, stored and processed and may be transferred to other countries, in accordance with any national legislation implementing the EU Data Protection Directive (95/46/EC).

This study may be terminated at the request of the CI, Study Oversight Committee, or National Research Ethics Committee (REC) if, during the course of the study, concerns about the safety of further dosing emerge.

The CI will update the ethics committee of any new information related to the study vaccine when appropriate.

14.3 Approvals

The study protocol has received the favourable opinion of the North West – Greater Manchester Central Multi-centre Research Ethics Committee (MREC) but all participating sites must undergo site specific assessment of capacity and capability. A copy of all site approval documents and a copy of the PISC on local headed paper should be forwarded to LCTC before patients are entered. The LCTC should receive a confirmation of capacity and capability for each new centre via the site's R&D department.

14.4 Protocol Deviation and Serious Breaches

Deviations from, breaches or violations of, or non-compliance to either the protocol, the conditions or principles of GCP, and relevant regulatory and ethical e.g. REC requirements are handled based on their nature and severity.

Non-Serious breaches

Protocol deviations and other non-serious breaches of GCP etc. will be managed according to local site and LCTC procedures as appropriate. They will be reported to study oversight committee.

Serious breaches

A breach of the protocol or GCP is 'serious' if it meets the definition of being "likely to affect to a significant degree the safety or physical or mental integrity of the study participants, or the scientific value of the study". This assessment can only be determined by the Sponsor.

If any persons involved in the conduct of the study become aware of a potential serious breach, they must immediately report this to the LCTC who will in turn notify the Sponsor. The Sponsor will assess the breach and determine if it meets the criteria of a 'serious' breach.

The Sponsor may seek advice from medical expert members of the oversight committee in determining whether or not the breach is likely to affect to a significant degree the safety, physical or mental integrity of participants. The Sponsor may seek advice from the study statistician and CI in determining whether or not the breach is likely to significantly affect the scientific value of the study. However, the Sponsor retains responsibility for the assessment of whether or not a breach meets the definition of 'serious' and is subject to expedited reporting to REC.

Breaches confirmed as 'serious' will be reported to REC within 7 days by the CTU on behalf of the Sponsor and notified to the SOC at their next meeting. Any requests for additional information from the Sponsor, SOC, or REC, will be promptly actioned by the relevant member(s) of the research team and open communication will be maintained to ensure appropriate corrective actions are taken and documented. Incidents of protocol non-compliance will be recorded as protocol deviations, the incidence of which are monitored and reported to the study oversight committee.

15 INDEMNITY

FlaviPrime - IMOJEV® is sponsored by the University of Liverpool and co-ordinated by the LCTC in the University of Liverpool. The University of Liverpool does not hold insurance against claims for compensation for injury caused by participation in a clinical study and they cannot offer any indemnity.

As this is an investigator-initiated study, The Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation by the pharmaceutical industry do not apply. However, in terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical study and they are legally liable for the negligent acts and omission of their employees. Compensation is therefore available in the event of clinical negligence being proven.

Clinical negligence is defined as:

“A breach of duty of care by members of the health care professions employed by NHS bodies or by others consequent on decisions or judgments made by members of those professions acting in their professional capacity in the course of their employment, and which are admitted as negligent by the employer or are determined as such through the legal process”.

16 PUBLICATION AND DISSEMINATION

16.1 Publication Policy

The results from this study will be analysed together and published as soon as possible. Individual researchers must undertake not to submit any part of their individual data for publication without the prior consent of the Study Management Group.

The Study Management Group will form the basis of the Writing Committee and advise on the nature of publications. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/) will be respected. All publications shall include a list of participants, and if there are named authors, these should include the study's Chief Investigator(s), Statistician(s) and Study Manager(s) involved at least. If there are no named authors (i.e. group authorship) then a writing committee will be identified that would usually include these people, at least.

The members of the Study Oversight Committee should be listed with their affiliations in the Acknowledgements / Appendix of the main publication. All publications arising from the FlaviPrime Study will be open access, according to the conditions of the funder of the Study, the Wellcome Trust (https://wellcome.ac.uk/funding/managing-grant/open-access-policy).

16.2 Dissemination to Key Stakeholders

On completion of the research, a Final Study Report will be prepared and submitted to the REC. The results of FlaviPrime will be published regardless of the magnitude or direction of effect.

16.3 Data Sharing

At the end of the study, after the primary results have been published, the anonymised individual participant data (IPD) and associated documentation (e.g. protocol, statistical analysis plan, annotated blank CRF) will be prepared in order to be shared with external researchers. All requests for access to the IPD will be reviewed by an internal committee at the CTU and discussed with the Chief Investigator in accordance with the CTU policy on data sharing. Data will be shared with the AVIS study, which already collects many of the same data, to facilitate the investigation of how the response to IMOJEV is modulated by pre-existing immune responses to flaviviruses, which have been determined in the flavimmune substudy of AVIS.

17 CHRONOLOGY OF PROTOCOL AMENDMENTS

17.1 Version 6 (08/07/2021)

Summary of Amendments from Protocol V5.0 to Protocol V6.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Throughout	Throughout	Change to version and date of document.
Throughout	Throughout	Sample size amended from 'up to 20' to 'up to 25' Number of participants in group 2 amended from 6-8 to 6-10 Number of participants in group 3 amended from 8-10 to 8-12
Throughout	Throughout	'Patient' amended to 'participant' as necessary.
Throughout	Throughout	Study duration for group 1 clarified as 28 days.
Throughout	Throughout	Flavimmune study updated to AVIS study, as a reflection of study name change.
Throughout	Throughout	References to MedDRA/CTCAE removed – these were included in error.
N/A	Table of Contents and Glossary	Updated to reflect changes throughout protocol.
2	Protocol overview – Inclusion Criteria	Inclusion criterion 6 updated to clarify contraception must be used for 28 days after vaccination.
2	Protocol overview – Exclusion Criteria	Exclusion criterion 9 ('detectable anti-Flavirus neutralizing antibodies in screening tests, group 2 only') removed; all subsequent criteria re-numbered.
5	Study design	Text amended to clarify that sample collection in AVIS study will be temporarily suspended during participation in FlaviPrime.
6.1	Inclusion Criteria	Inclusion criterion 6 updated to clarify contraception must be used for 28 days after vaccination.
6.2	Exclusion Criteria	Exclusion criterion 9 ('detectable anti-Flavirus neutralizing antibodies in screening tests, group 2 only') removed; all subsequent criteria re-numbered.
6.3	Co-enrolment guidelines	Co-enrolment guidelines amended to confirm participants can join FlaviPrime provided certain certain criteria are met.

7.7	Concomitant medications/ treatments and specific restrictions	Clarification added that COVID vaccines can be given to participants, and participants should inform study team if they must take certain medications.
9.1	Participant identification and screening	'Negative neutralising antibody' removed as a condition for participants to enter group 2.
9.4	Enrolment/ registration and baseline assessments	Addition of GP notification added – participant GP will receive a letter to notify them of patient involvement in FlaviPrime.
9.5	Schedule for assessments and follow-up	Clarification of laboratory assessments and re-insertion of table showing correct schedule of assessments (note, these tables were used in a separate Schedule of Assessments document, as part of FlaviPrime Amendment 4; they have now been re-added to the protocol).
9.7	Intervention Discontinuation and Participant Discontinuation/ Withdrawal	Participant transfer section updated to clarify that replacing transferred participants is dependent on vaccine availability .
10.6	Time period for active monitoring of safety events	Clarficiation added that group 1 participants should report AEs that occur 28 after vaccination to site, by phone (group 1 last study visit to site is 9 days after vaccination). Clarification added that, if site become aware of AEs occurring outside of the 28 day AE monitoring window, these events can be reported to LCTC.
11	Reporting of pregnancy	Contact email address for reporting events updated to LCTC Central Safety Team (lctcsafe@liverpool.ac.uk). Error in birth control window corrected to 28 days after vaccination.
11.2.1	Reporting an initial or follow-up SAE	Contact email address for reporting events updated to LCTC Central Safety Team (lctcsafe@liverpool.ac.uk).
12	Statistical considerations	Section updated throughout to reflect: there are no formally pre-specificied statistical analyses conducted; analyses are under the remit of the Chief Investigator and not performed by LCTC; and statistical analyses plan will not be place due to the exploratory nature of the analyses.
12.2	Sample size	Sample size amended from 'up to 20' to 'up to 25' Number of participants in group 2 amended from 6-8 to 6-10 Number of participants in group 3 amended from 8-10 to 8-12 Information added to explain sample size figure chosen.
13.2	Data collection methods	Reference to screening log removed – this was not omitted previously, in error.
16.3	Data sharing	Statement added to clarify data sharing with AVIS study.

17	Chronology of protocol amendments	Chronology updated to reflect document history; history of previous protocol changes also added.

17.2 Version 5.0 (08/03/2021)

Summary of Amendments from Protocol V4.0 to Protocol V5.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Throughout	Throughout	Change to version and date of document.
1	Table of Contents	Updated to reflect current document.
9.5	Schedule of assessments (table)	Table removed from protocol due to typographical errors. This Schedule of Assessments table is now provided as a supplementary document to the study protocol.
17	Chronology of protocol amendments	Chronology updated to reflect document history.

17.3 Version 4.0 (18/12/2020)

Summary of Amendments from Protocol V3.0 to Protocol V4.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Throughout	Throughout	Change to version and date of document
N/A	Contact details	Sponsor Representative details updated. Institute of Infection and Global Health update to Institute of Infection, Veterinary and Ecological Sciences; contact number for Dr Turtle updated
Section 2	Overview	Study phase corrected. Study duration for group 1 specified (previously missing)
Section 2.1	Schematic of study design	Figure 2 updated to include the baseline blood sample for group 1 which was previously missing
Section 3	Roles and responsibilities – clinical trials unit	LCTC defined (Liverpool Clinical Trials Centre)
Section 3	Oversight committees	“Trial” corrected to “study” throughout

Section 4.3 and 4.4	Introduction	Minor typographical errors corrected
9.5	Schedule for assessments and follow up	Study duration for group 1 specified (previously missing)
9.5	Schedule of assessments (table)	Table updated to correct typographical errors - removal of X indicating 2nd HIV/HBV/HCV test (was never planned) and removal of travel history at followup visits for group 1 (never planned).
17	Chronology of protocol amendments	Chronology updated to reflect document history.

17.4 Version 3.0 (08/08/2020)

This version made major changes, merging the changes from v2.0 and v1.2, resolving version control issues and transferring to the current LCTC template.

Summary of Amendments from Protocol V1.2 and V2.0 to Protocol V3.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Throughout	Throughout	Change to version and date of document Complete overhaul of document due to the introduction of a new LCTC protocol SOP/template Correction of typographical errors Conflicts between versions 1.2 and 2.0 resolved (previously approved) 'Trial' replaced with 'study'

		Site name updated to Liverpool University Hospitals NHS Foundation Trust (LUHFT, Royal Liverpool site) (this is as a result of merger with Aintree) T cell and associated IFNγ-ELISpot and intracellular cytokine staining terms have been updated for clarification of the techniques that will be used to study the T cell response to the vaccine
N/A	Contact Details: Institutions	Update to contact details
N/A	Table of Contents and Glossary	Updated to reflect changes throughout protocol
2	Protocol Overview	Overview updated to reflect changes within protocol
2.1	Schematic of Study Design	New schedule for group 1 (newly added to protocol v3.0), no changes for groups 2 and 3, aside from group name
4.4	Objectives	Clarification that all objectives are exploratory, change to wording of primary objectives 3 and 4 (though scientific-meaning has not changed), addition of primary objective 5 ("To establish a sample bank for future work on cross-reactive and other responses to flaviviruses, flavivirus vaccines and other emergent viruses"). Addition of 2 secondary objectives ("To examine the specificity and cross-reactivity of the antibody response after JE vaccination, using serum and human monoclonal antibodies" and "To determine whether there are epitopes which can serve as the target of broadly cross-neutralising antibody responses.") Clarification of which objectives are primary and which are secondary
5	Study Design	Addition of another patient group (now 3 groups in total; group 1 = participants of any previous flavivirus exposure, group 2 = flavivirus naïve participants, group 3 = flavivirus experiences participants) Increase in the sample size from 6 to 'up to 20.' Change to timelines as a result of new group.
6	Eligibility Criteria	Inclusion criterion 3 – typographical error corrected regarding study duration Inclusion criterion 5 – due to the introduction of another patient group, group-specific criteria amended to reflect new group numbers Exclusion criteria 8, 9 and 14 – due to the introduction of another patient group, group-specific criteria amended to reflect new group numbers
7.3	Manufacturing and Distribution	Information added that omitted from the previous protocol
7.7	Specific Requirements/ Restrictions	Table of study-specific restrictions added (this was omitted from previous versions of the protocol in error, but was present in the PISC)

9.1	Participant Identification and Screening	Clarification text added confirming that participants identified through consent4consent database at LUHFT CRU can be consented at site, donate blood and have flavivirus immunological assays performed as per the Flavimmune study
9.3	Screening, Eligibility Assessment and Confirmation	Hepatitis B and C tests added at screening to reduce non-specific effects from these viruses, and text added to explain the rationale behind this
9.5	Schedule of Assessments	Schedule of Assessments added to reflect the 3 study groups
9.5	Adverse Events	Added in the use of an emergency contact card that participants will received so they can contract the CRU doctor on call
9.6	Sampling	Slight increase in blood volumes collected Change in name of University of Liverpool department who will analyse blood Added in use of blood samples for other emergent viruses (specifically proposed up front consent for experiments relating to newly emerging viruses, based on experience with SARS-CoV-2)
9.7	Participant Transfer	Correction of error to process for participant transfers – now clarified that participants will have to be withdrawn if they are unable to attend follow-up visits, and if withdrawn before the day 9 sample (group 1) two-week sample (groups 2 and 3) has been taken, participant should be replaced.
10	Safety Reporting	Overhaul of Safety Reporting section to refer to non-CTIMP processes, and new LCTC reporting processes
11	Reporting of Pregnancy	Information added regarding pregnancy, the possibility of vaccine viraemia, and the low likelihood of these coinciding has been resolved between protocol v2.0 and v1.2 (previously approved)
11.4	Contact Details and Out-of-hours Medical Cover	Information added regarding out-of-hours medical cover (this was omitted from previous versions in error)
12.7	Statistical Analysis Plan	Removal of 'proportion binding virion versus NS1' from list of descriptive statistics that will be calculated
13	Data Management and Study Monitoring	Change from central monitoring to on-site monitoring (LCTC will only carry out monitoring if triggered)
17	Chronology of Protocol Amendments	Chronology of protocol amendments updated for clarification of document history
18	References	Additional references added

17.5 Version 1.2 (15/01/2020)

This version was created from v1.1 in error. V1.2 was submitted as part of Amendment 1.

Summary of Amendments from Protocol V1.1 to Protocol V1.2		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Section 4.2 (v1.2)	Exclusion criteria	Lymphocyte count < 1.0 x 10^9/L left in the protocol, in error
Section 5.3 (v1.2)	Screening	Minor wording changes relating to pregnancy inserted at the request of the REC upon review of PISC
Section 9.2.3 (v1.2)	Reporting of pregnancy	Clarification of the likely effects on pregnancy inserted at the request of the REC upon review of PISC

17.6 Version 2.0 (27/06/2019)

This was the original approved version of the protocol (REC favourable opinion received 25/07/19).

Summary of Amendments from Protocol V1.1 to Protocol V2.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
4.2 (v1.1, now section 6.2)	Exclusion criteria	Lymphocyte count < 1.0 x 10^9/L removed (had been left in accidentally from a previous version of the protocol that used a different vaccine)

17.7 Version 1.1 (17/04/2019)

This was the original version of the protocol submitted to the REC and considered on 21st May 2019.

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19 DOCUMENTS SUPPLEMENTARY TO THE PROTOCOL

Documents referenced within the protocol are separately maintained and version controlled. Any of the supplementary documents subject to CA and / or Ethical review are submitted as separate version controlled documents.