An Observational, Longitudinal, Natural History, Feasibility Cohort Study to Evaluate the Characteristics of Cytomegalovirus (CMV) Shedding in CMV Seropositive Women Throughout Pregnancy (cCHIPS)

Sponsor's Project Number: 2018.0296
REC Reference Number: 19/LO/0161
IRAS ID: 257710
Protocol Version and Date: 3.0, 25/03/2020

CHIEF INVESTIGATOR (CI):
Professor Paul Heath
Professor of Paediatric Infectious Diseases
Institute for Infection and Immunity
Paediatric Infectious Diseases Research Group
St. Georges University of London
Jenner Wing, Level 2, Room 2.216F, Mail Point J2C
London
SW17 0RE

Phone: 02087255980
Email: pheath@sgul.ac.uk

PRINCIPAL INVESTIGATOR (PI):

Dr Shari Sapuan
Clinical Research Fellow
Institute for Infection and Immunity
Paediatric Infectious Diseases Research Group
St. Georges University of London
Jenner Wing, Level 2, Room 2.216F, Mail Point J2C
London
SW17 0RE

Phone: 02087254851
E-mail: ssapuan@sgul.ac.uk

SPONSOR: St George's, University of London

SPONSOR REPRESENTATIVE:

Name Subhir Bedi
Address: St George's Joint Research and Enterprise Services
St George's, University of London
Hunter Wing, Ground Floor,
Cranmer Terrace SW17 ORE
Phone: 020 8266 6397
E-mail: sbedi@sgul.ac.uk

SPONSOR CONTACT:

Name Ms Nadia Azzouzi
Address: St George's Joint Research and Enterprise Services
St George's, University of London
Hunter Wing, Ground Floor,
Cranmer Terrace SW17 ORE
Phone: 020 8266 6488
E-mail: nazzouzi@sgul.ac.uk

Funding Source: This study is funded by the Merck Investigator Studies Program (MISP No. 58414).

Statement

The Chief Investigator (CI) and the Sponsor representative have discussed this protocol version. The investigators agree to perform the investigations and to abide by this protocol except where departures from it are mutually agreed in writing.

The Investigator agrees to conduct the trial in compliance with the protocol, GCP, the Data Protection Act (2018), the Trust Information Governance Policy (or other local equivalent), the UK Framework for Health and Social Care Research (2018), the Sponsor's SOPs, and other regulatory requirements as appropriate.

This protocol has been written in accordance to the Sponsor's procedure identified as: JRESSOP0039 "Protocol Design" and is intended for use at UK sites only

Chief Investigator Paul Heath Professor of Paediatric Infectious Diseases St George's, University of London	**Signature**	**Date**
Sponsor Representative Subhir Bedi Head of Research Governance and Delivery St George's, University of London		
Sponsor Contact Nadia Azzouzi St George's, University of London		

Acknowledgements and Protocol Contributories

Professor Paul Heath and Dr Shari Sapuan conceived the study and Professor Paul Heath, Professor Asma Khalil, Dr David Carrington, Mrs Caroline Star and Dr Shari Sapuan, initiated the study design. Professor Paul Heath and Dr Shari Sapuan wrote the protocol and Professor Paul Heath, Professor Asma Khalil, Dr David Carrington, Dr Christine Jones and Mrs Caroline Star are grant holders. Dr Charlotte Jackson provided statistical expertise in the study design. All authors contributed to refinement of the study protocol and approved the final manuscript.

The study was funded by the by the Merck Investigator Studies Program (MISP No. 58414). The funder has no role in the design, collection, management, analysis and interpretation of data, writing of report or the decision to submit for publication. The sponsor has the final decision about the decision to submit for publication.

Contents

List of abbreviations

CI	Chief Investigator
CMI	Cellular mediated immunity
CMV	Cytomegalovirus
cCMV	Congenital cytomegalovirus
CRF	Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immunospot
GW	Gestational weeks
GCP	Good Clinical Practice
ICF	Informed Consent Form
IFN-γ	Interferon Gamma
IGRA	Interferon Gamma Release Assay
ISF	Investigator Site File
NHS R&D	National Health Service Research & Development
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PIS	Participant Information Sheet
RCT	Randomised Control Trial
REC	Research Ethics Committee
SDV	Source Document Verification
SNHL	Sensorineural hearing loss
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

1 Roles and Responsibilities

Chief Investigator (CI):
Professor Paul Heath
E-mail: pheath@sgul.ac.uk
Phone: 02087255980

Principal Investigator (PI):
Dr Shari Sapuan
E-mail: ssapuan@sgul.ac.uk
Phone: 02087254851

Statistician:
TBC
E-mail: TBC
Phone: TBC

Laboratory:
Dr David Carrington
E-mail: dr.d.carrington@gmail.com

Trial Management Group:
Membership:
Professor Paul Heath
Dr Shari Sapuan
Professor Asma Khalil
Dr David Carrington
Project Manager Suzannah Wright
Statistician TBC
Study midwife- *to be appointed*

2 Study synopsis

Brief title:	cCHIPS
Official title:	An Observational, Longitudinal, Natural History, Feasibility Cohort Study to Evaluate the Characteristics of Cytomegalovirus (CMV) Shedding in CMV Seropositive Women Throughout Pregnancy
Short title:	Cytomegalovirus Shedding Characteristics in Pregnant Women
Sponsor reference number:	2018.0296
Public database identifier	TBC
Study type & Phase	Feasibility study
Study design	Observational cohort, longitudinal, natural history study
Study Population/disease condition	CMV seropositive pregnant women with at least one child of less than four years of age
Eligibility criteria:	***Inclusion criteria:*** • Pregnant • CMV seropositive • Willing and able to provide informed consent • Living with child(ren), at least one of whom is less than four years old • Willing to have saliva, urine and vaginal secretion sampling to be tested for CMV PCR • Willing to be followed up until the postpartum period
	E*xclusion criteria:* • Age less than 18 years • Evidence of acute maternal CMV infection at the time of screening • Documented immunodeficiency of any aetiology including the use of oral steroid therapy equivalent to >1mg/kg of prednisone per day for more than one week • In the opinion of the investigator is unlikely to comply with the study procedures • In the opinion of the investigator does not have adequate understanding or verbal and written English
Target number of participants	200
Study Aim	Assessment of feasibility of future large scale studies to evaluate the relationship between CMV shedding in pregnancy with congenital CMV
Criteria for evaluation	**Primary outcome measure(s)** • The prevalence of CMV shedding in saliva, urine and vaginal secretions of CMV seropositive women throughout pregnancy
	Secondary outcome measure(s) • The quantity of CMV shedding in saliva, urine and vaginal secretions of CMV seropositive women throughout pregnancy • The fold-differences in the prevalence and quantity of CMV shedding in saliva, urine and vaginal secretions in CMV seropositive women throughout pregnancy • The fold-differences in the prevalence and quantity of CMV

	shedding in saliva, urine and vaginal secretions between different gestational stages of pregnancy in CMV seropositive women • The risk factors for CMV shedding in CMV seropositive pregnant women • The acceptability of the study procedures to participating pregnant women • The proportion of women approached who are recruited into the study • The proportion of participants completing the study • The relationship between cellular mediated CMV-specific immunity and CMV shedding in CMV seropositive pregnant women
	Tertiary outcome measure(s) • The fold-differences in CMV-specific T cell immune responses, as measured by QuantiFERON-CMV and CMV-ELISPOT assays, in the evaluation of CMV-specific T cell responses with CMV shedding in CMV seropositive pregnant women
Sources of funding	Merck Investigator Studies Program
Anticipated start date:	March 2018
Anticipated primary completion date:	April 2021
Sponsor/Co-Sponsor	St George's, University of London
Key Contact names	Sponsor contact: Nadia Azzouzi Email: nazzouzi@sgul.ac.uk Tel: 02082666488 Chief Investigator: Paul Heath Email: pheath@sgul.ac.uk Tel: 02087255980 Principal Investigator: Shari Sapuan Email: ssapuan@sgul.ac.uk Tel: 02087254851

3 Background

Congenital cytomegalovirus (cCMV)

cCMV is the most common congenital infection in the UK. With an estimated incidence of 3 per 1000 live births (approximately 2400 cases in the UK annually) [Peckham 1983; Griffiths 1991], it is more common than many better known congenital conditions, such as Down's syndrome, spina bifida and cystic fibrosis [Dollard 2007].

Up to 15% of infants infected with cCMV have symptoms at birth. Of those infants who are initially asymptomatic, up to 15% will be symptomatic later in life. About one-fifth of children with cCMV will have life-long problems, of which sensorineural hearing loss (SNHL) is the most common. Other problems include visual impairment, learning difficulties, movement problems and epilepsy. The most severely affected children need life-long support with everyday activities, placing emotional and financial strain on their families and requiring significant input from health and social care services [Dollard 2007].

Despite the burden of adverse health outcomes, most pregnant women are not aware of CMV infection or the simple measures that can be taken to prevent it. Advice about avoidance of CMV infection is not routinely offered in the NHS. There is no licensed CMV vaccine and no routine treatment of antenatal CMV in the NHS. This may be a reflection of the limited knowledge on CMV infection in pregnancy and its relationship with cCMV.

The natural history of CMV in pregnancy is complex, because maternal primary infection, maternal reinfection with a new strain or reactivation of latent infection can all result in intrauterine transmission of CMV [de Vries 2013; Mussi-Pinhata 2018].

CMV shedding

Shedding of CMV in those with latent infection may be common and has been demonstrated in different samples (blood, saliva, urine, cervical secretions, breast milk) in pregnant women during primary infection, reactivation of endogenous latent virus, or following reinfection with a new strain of CMV [Barbosa 2018; Atkinson 2014; Kimberlin 2003; Cannon 2011]. However, the pattern and intensity of CMV shedding and its association with vertical transmission and cCMV is not well defined [Williams 2015; Marty 2017; Pass 2009; Sabbaj 2011; Griffiths 2011].

In a longitudinal study looking at the prevalence of CMV shedding throughout pregnancy of seropositive women in a high seroprevalence setting [Barbosa 2018], shedding was detected at least once in 35% of the pregnant women, with saliva being the most common (20%), followed by urine (13.3%), vaginal secretions (12.5%) and blood (0.8%). This study also found that mothers living with or providing daily care to young children were twice as likely to shed CMV at least once compared to women with less exposure to young children (58% vs 26%).

With the exception of urine, no other bodily fluid of pregnant women has been studied as a surrogate of vertical transmission. In a study looking at HIV-infected pregnant women who were

CMV seropositive, urinary CMV shedding was a significant risk factor for vertical transmission and cCMV [Adachi 2017].

Cellular mediated immunity (CMI) to CMV

It has been recently shown that the measurement of CMV-specific T cell activity might reflect a patient's ability to control CMV infection and predict the risk for post-transplant viral replication. It has therefore been proposed that immunological monitoring of CMV specific T cell immunity might be an effective strategy [Lee 2017]. In particular, interferon gamma (IFN-γ) appears to play a critical role in controlling CMV infection. Studies suggest that pre-existing CMV-specific T cell immunity may be poor prior to CMV reactivation [Revello 2006] and this is supported by animal studies in which CD4+ T cell depletion in infected pregnant rhesus macaques leads to poor outcomes when compared to immunocompetent primates [Bialas 2015].

There is limited evidence of the importance and the natural history of CMI in CMV seropositive pregnant women, and its association with vertical transmission.

One study has described the association between CMI and congenital infection in the context of maternal primary infection. As expected, CMV specific T cell responses were not detected before infection and were high following infection [Saldan 2015].

Currently, there are two IFN-γ release assays (IGRAs) which are commercially available to measure CMV-specific T cell immunity; QuantiFERON-CMV assay (manufactured by Cellestis, a QIAGEN Company, Australia), a commercially available enzyme-linked immunosorbent assay (ELISA) to detect IFN-γ released in whole blood by ex-vivo stimulation with human leukocyte antigen (HLA) class I restricted CMV peptides from pp28, pp50, pp65, IE-1, IE-2, and gB; and CMV-ELISPOT, an enzyme-linked immunospot assay which measures IFN-γ production in peripheral blood mononuclear cells (PBMCs) isolated from whole blood following stimulation with CMV antigens pp65 and IE-1. Although both assays measure IFN-γ to detect T cell responses upon stimulation by CMV antigens, their characteristics in terms of methods (ELISA vs. ELISPOT) and principles (CMV-specific CD8+ vs. CD4+ plus CD8+ responses, respectively) are different. In one study, a CMV specific T cell ELISPOT assay was able to predict the risk of CMV viraemia post-transplant in CMV seropositive kidney transplant recipients but a QuantiFERON-CMV assay was not [Lee 2017]. There are no studies which have compared the performance of the 2 assays in the context of CMV shedding in pregnancy.

3.1 Study rationale

CMV infection is difficult to diagnose in pregnancy because it does not produce a characteristic illness. The first indication of clinical consequences of maternal infection may be seen on routine ultrasound of foetal growth, on clinical examination at birth, following abnormal hearing tests in infancy, or when older children present with hearing loss or unexplained developmental delay. This means that the opportunities to diagnose, treat and potentially improve the outcome of infection, whether by treating in pregnancy or by treating an affected infant in a timely way, are currently very limited.

Prevention of cCMV in babies born to mothers who are seropositive, or at least measures to ameliorate its consequences, is therefore a high priority.

A better understanding of the characteristics, natural history and factors associated with CMV shedding in seropositive pregnant women will help define the risk factors for horizontal as well as vertical transmission, and could provide insight into sources and routes of virus acquisition in maternal populations. Collecting samples during pregnancy to detect shedding may not only be a means of diagnosing CMV infection in a timely and convenient way, but it may also be essential for developing prevention strategies.

CMV shedding may be a suitable proxy (surrogate) for cCMV infection. If this is shown, then detection of shedding might be used as a convenient (and much more common) endpoint for trials of prevention or treatment strategies.

Additionally, measurement of CMV-specific T cell immunity may be a suitable surrogate for either CMV shedding or cCMV, or both.

If CMV virus reactivation (as a cause of shedding and / or as a cause of cCMV) is dependent on the pre-existing levels of CMV-specific T cell immunity, it is likely that altered levels can be detected earlier in CMV seropositive women in whom reactivation is going to occur. Thus, CMV-specific T cell responses may be predictive of CMV reactivation, as evident by shedding and / or vertical transmission (and cCMV). As immunomodulation is expected in pregnancy, and may vary over the course of pregnancy, longitudinal measurement of T cell immunity may provide valuable insight into the changes in immune function as pregnancy proceeds.

The significance of CMV shedding in pregnancy and its relationship with congenital transmission is not well defined. This study aims to lay the groundwork for future studies that will fully assess this relationship.

This study also aims to better characterise the significance of cellular mediated CMV-specific immunity in CMV seropositive pregnant women and its relationship with CMV shedding.

This study will also assess which of the two commercially available IGRAs is best able to measure CMV-specific T cell immune responses in CMV seropositive pregnant women and their association with CMV shedding.

4 Study objectives

4.1 Primary objective

- To evaluate the prevalence of CMV shedding in saliva, urine and vaginal secretions of CMV seropositive women throughout pregnancy.

4.2 Secondary objectives

- To evaluate the quantity of CMV shedding in saliva, urine and vaginal secretions of CMV seropositive women throughout pregnancy
- To compare the prevalence and quantity of CMV shedding in saliva, urine and vaginal secretions in CMV seropositive women throughout pregnancy

- To compare the prevalence and quantity of CMV shedding between different gestational stages of pregnancy in CMV seropositive women
- To identify risk factors for CMV shedding in CMV seropositive pregnant women
- To evaluate the acceptability of the study procedures to the participating pregnant women
- To evaluate the proportion of women approached who are recruited into the study
- To evaluate the proportion of participants completing the study
- To evaluate the relationship between cellular mediated CMV-specific immunity and CMV shedding in CMV seropositive pregnant women

4.3 Tertiary objectives

- To compare the measurement of CMV-specific T cell immune responses between QuantiFERON-CMV and CMV-ELISPOT assays in CMV seropositive pregnant women

5 Trial design

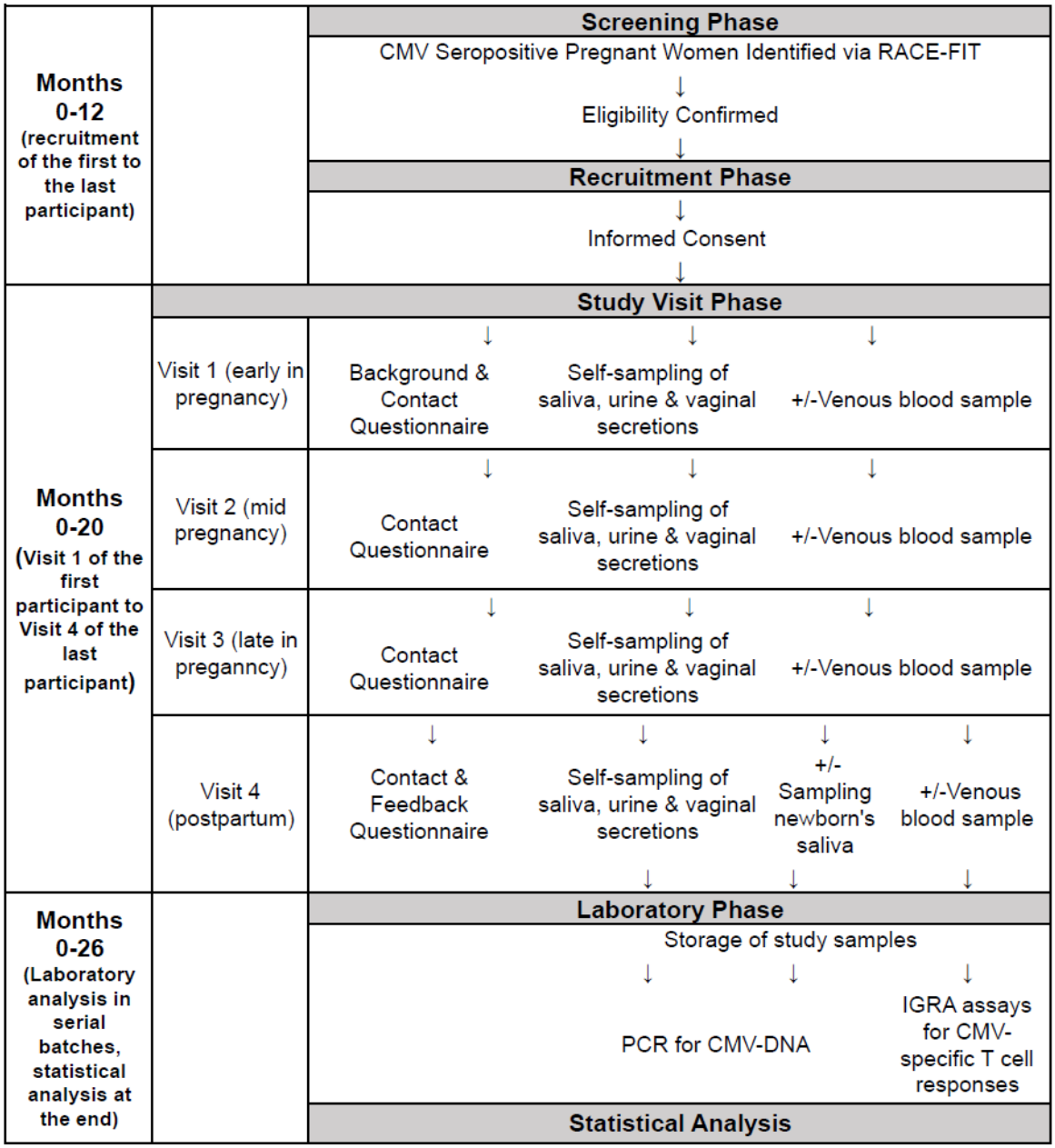
5.1 Overall design

This is a feasibility study for larger multi-centre studies and is designed as a single-centre observational cohort, longitudinal, natural history study.

5.2 Treatment/intervention plan and rationale

The overarching aim of this study is to evaluate the feasibility of performing larger scale, multi-centre studies to evaluate the relationship between CMV shedding in pregnancy with cCMV. There is no randomisation involved in this study and all participants will have the same study procedures and receive treatment as usual.

5.3 Schematic of study design



6 Participation selection criteria

There will be no exceptions (waivers) to eligibility criteria prior to participant inclusion into the study. Any questions raised about eligibility should be addressed prior to entering the participant.

The eligibility criteria have been carefully considered and are standards used to ensure the study results can be appropriately used to make future treatment decisions for other people with a similar disease or medical condition. It is therefore vital that exceptions are not made to the following detailed selection criteria.

All participants that are screened for inclusion into the study must be entered onto the Sponsor screening log JRESLOG0001 and will be assigned a sequential number. Participants will be considered eligible for enrolment into this study if they fulfil all of the inclusion criteria and none of the exclusion criteria as defined below.

Eligible participants will be entered onto the Sponsors Subject ID log JRESLOG0002 and assigned a study specific identification number in a pre-agreed format in accordance with site identifier and next sequential numerical value e.g. SG001.

6.1 Inclusion criteria

- Pregnant
- CMV seropositive
- Willing and able to provide informed consent
- Living with child(ren), at least one of whom is less than four years old
- Willing to have saliva, urine and vaginal secretion sampling
- Willing to be followed up until postpartum period

6.2 Exclusion criteria

- Age less than 18 years
- Evidence of acute maternal CMV infection at the time of screening
- Documented immunodeficiency of any aetiology including the use of oral steroid therapy equivalent to >1mg/kg of prednisone per day for more than one week
- In the opinion of the investigator is unlikely to comply with the study procedures
- In the opinion of the investigator does not have adequate understanding or verbal and written English

7 Participant Recruitment process

Patient recruitment at a site will only commence once evidence of the following approval/essential documents are in place:

1. REC approval and HRA approval;

2. Final sponsorship and Confirmation of capacity and capability.

All participants who wish to enter the study will be fully screened and consented by the Chief Investigator, or an appropriate delegate.

The study will be undertaken in parallel with a separate study called RACE-FIT (Reducing Acquisition of CMV through antenatal Education: A feasibility study to assess an educational intervention to prevent cytomegalovirus infection in pregnancy, ClinicalTrials.gov Identifier Number NCT03511274) which will have ethical approval to screen pregnant women with children less than 4 years of age

identified during the antenatal combined screening bloods appointment or the antenatal booking appointment, for their CMV antibodies on a sample of blood collected as part of the screening process. The pregnant women screened and found to be CMV seronegative will be eligible for recruitment into the RACE-FIT study. Those screened and found to be CMV seropositive will be eligible and approached for recruitment into the cCHIPS study; as part of the informed consent for the screening process information will be provided about both the RACE-FIT and cCHIPS study and written consent will be provided to share their identifiable details and CMV seropositive results with both study teams.

Once the screening period has ended for the RACE-FIT study (31st August 2019), the screening process will continue just for the cCHIPS study to achieve the recruitment target; the pregnant women screened and found to be CMV seronegative will end with their participation and the pregnant women screened and found to be CMV seropositive will be eligible and approached for recruitment into the cCHIPS study where as part of the informed consent for the screening process, information will be provided for the cCHIPS study. The cCHIPS study team will then make contact with the potential participant and invite them to participate in this study via telephone, email, post and/or face-to-face. The study team who are not part of the direct care team will not have access to identifiable patient data prior to consent. The eligibility criteria of the potential participant will be checked during the initial contact with the participant by the study team as part of the recruitment process.

There will be information about the study displayed on hospital and university notice boards, in community clinics and on institutional websites (both university and hospital) so women may already be aware of the study before a contact is made by the study team.

8 Study procedures

8.1 Informed consent

Please note, it is essential that all trial personnel/staff undertaking the informed consent process have signed the Sponsor's Delegation of Responsibilities Log JRESLOG0004 to ensure that the person has been delegated the responsibility by the study CI/PI. All personnel taking informed consent must be GCP trained. Refer to Sponsor SOP JRESSOP0027.

Informed consent from the participant must be obtained following explanation of the aims, methods, benefits and potential hazards of the trial and before any trial specific procedures are performed. The only procedures that may be performed in advance of written informed consent being taken are those that would have been performed on all participants in the same situation as routine clinical practice. The investigator or designee will explain that the patients are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

Any appropriately trained member of the research team who has received study specific training may take consent. There will be no minimum time period between being provided with information

about the study and informed consent being taken, provided the woman feels that she has had adequate time to consider participation in the study.

If the study team makes the initial contact with the potential participant face to face, informed consent can be sought at this point followed by the study procedures related to the first study visit. If the initial contact between the study team and potential participant is not face to face, a face to face appointment either at a clinical setting or at the participant's home will be arranged for the informed consent. The participant will be asked to sign an informed consent form for participation in the study.

A copy of the signed Informed Consent Form (ICF), along with a copy of the most recent approved Patient Information Sheet (PIS), will be given to the study participant. The original copy of the ICF will be placed in the Investigator Site File (ISF). In addition, a copy of the ICF will be sent to the General Practitioner (GP).

If new information results in significant changes to the risk–benefit assessment, the consent form will be reviewed and updated if necessary. All participants, including those already being treated, will be informed of the new information, given a copy of the revised consent form and asked to re-consent if they choose to continue in the study.

8.2 Randomisation procedure

This section does not apply.

8.3 Emergency unblinding

This section does not apply.

8.4 Discontinuation/withdrawal of participants and stopping rules

In consenting to the study, participants are consenting to study interventions, study follow up and data collection. However, an individual participant may stop study participation early or be stopped early for any one of the following reasons:

Any change in participant's situation that in the investigator's opinion justifies the discontinuation of study participation.

As participation in the study is entirely voluntary, the participant may choose to discontinue participation at any time without penalties or loss of benefits to which they may be entitled. Although not obliged to give a reason for discontinuing their participation a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant's rights.

If a participant chooses to withdraw from the study, we will ask if they would be willing to complete the study feedback questionnaire at the time of withdrawal. If no follow up is possible data that has already been collected should be kept and analysed according to the ITT principle for all participants who stop follow up early.

As this is a feasibility study, once a participant has been recruited into the study and completed the first study visit (Visit 1), if a participant chooses to withdraw after this point due to non-compliance to part of the study procedures (not all), for instance non-compliance to vaginal secretion sampling or venous blood taking, we will ask if the participant is willing to continue participating in the study and complete the rest of the study procedures that the participant is willing to comply with.

9.5 Lost to Follow up

We will seek consent from all participants for permission to contact their GP if we lose contact with them during the study. We will ask for additional consent to use NHS number to trace the GP if the GP has changed since the start of the study.

9.6 Definition of the End of Trial

The end of the clinical phase of the study will be the last follow up visit of the last participant enrolled in the trial. The end of the study will be after the last sample has been assayed and analysed and database has been locked. The REC requires notification of the end of trial within 90 days of its planned completion or within 15 days if the study is terminated early. Refer to JRESSOP0015 and inform the JRES to facilitate assistance and compliance with requirements.

9 Study Procedures

9.1 Screening Phase

The screening process for cCHIPS will be conducted alongside the RACE-FIT study which will have ethical approval to screen pregnant women with children less than 4 years of age for CMV antibodies on a sample of blood collected at the time of antenatal combined screening bloods appointment or the antenatal booking appointment. Information about both the cCHIPS and RACE-FIT studies will have been provided to women at screening and the contact details of those women screened and their results can therefore be used by both study teams. Women who are CMV seropositive will be invited to participate in the cCHIPS study. Once the screening period has ended for the RACE-FIT study (31st August 2019), the screening process will continue just for the cCHIPS study to achieve the recruitment target. They will be asked if they are willing to consent for CMV serology being tested in addition to the combined screening bloods. If they are willing to consent, they will complete the contact details and consent sections of the form which will be printed on carbon copy paper. When the woman proceeds to have the combined screening bloods taken, a member of staff will check that the woman is willing to consent and that the informed consent has been properly completed and will give the woman the carbon copy of the form and retain the original for the study team. Information will be given to women about how they will receive results and the expected timescale and will be provided with contact details for the study team.

At the time of blood sampling for the combined screening bloods an additional clotted sample will be taken for serological testing for CMV. A sample form will be completed with the participant details and a pre-filled sticker will be placed in the clinical details section. Participant details will be

completed on the sample bottle. The sample will be transported to the lab for serological testing for CMV. In most instances this will not require a separate blood test, just an additional 5mls volume of blood at the time of routine blood tests. However, where this has not been possible, it is permissible for an additional blood test to be performed with verbal consent from the participant.

9.2 Recruitment Phase

The study team will be sent a full list of results each week from the laboratory and they will then contact women with their results. Women who are seropositive (have previously had infection) will be sent a letter with this result and be invited to take part in the cCHIPS study alongside information about the study. The GP will also be informed about women who are seropositive using appropriate letters. A very small minority of women may be found to have recent infection and as receiving these results may generate anxiety in the women and their families these will be communicated by a clinical member of the team by phone. A letter may subsequently be sent outlining what was said for reference. This group of women will be referred to the Fetal Medicine Unit (FMU) for further investigation and counselling. To expedite this process all such results will be discussed with Dr Asma Khalil (or a designated specialist in her absence) to arrange a timely clinic appointment at the FMU. A letter will also be sent to the GP to inform them of the result and the referral to FMU.

Contact will be made with the potential participant screened via telephone, email, post and/or face-to-face. Eligibility criteria will be checked at this point. If the initial contact with the potential participant is face to face, informed consent can be sought at this point followed by the study procedures related to Visit 1. If the initial contact between the study team and potential participant is not face to face, a face to face appointment either at the participant's home or at a clinical setting will be arranged for the informed consent. The participant will be asked to sign an informed consent form for participation in the study and a unique identification number will be assigned to the participant.

As part of the eligibility inclusion criteria, all participants must provide written consent to the self-sampling of saliva, urine and vaginal secretions to enable them to participate in the study. Written informed consent will also be sought for the study team to collect a venous blood sample from the participants at each study visit, however if consent is not given to this the participant can still take part. Similarly, informed consent will be sought for leftover study samples to be stored and used in future ethically approved research in CMV infection but if consent is not given to this the participant can still take part.

9.3 Study Visit Phase

There will be four study visits (Visit 1, Visit 2, Visit 3, and Visit 4) for each participant:

- Visit 1: as soon as possible following screening, aiming up to 16 weeks and 6 days gestational age (early in pregnancy)
- Visit 2: 20 gestational weeks or any time in 2nd trimester from 17 weeks and 0 days gestational age to 26 weeks and 6 days gestational age (mid pregnancy)

- Visit 3: 28 gestational weeks or any time in 3rd trimester from 27 weeks and 0 days gestational age onwards (late in pregnancy)
- Visit 4: from the time of birth to 1 week postnatal age (postpartum period)

The timing for each visit was chosen following a discussion with the obstetric member of the trial management team (Professor Asma Khalil) and our PPI group (the South London Maternal and Paediatric Infection & Immunity Patient and Public Involvement group) to strike a balance of coinciding with routine antenatal appointments for the convenience of the participants to promote on-going participation, and capturing samples throughout pregnancy at varying gestational stages. Where a visit is not possible to coincide with routine antenatal (or postnatal) appointments (in particular Visit 1), this will then take place in a clinical setting, and if still not possible, in the participant's home.

9.3.1 Visit 1

At Visit 1, after the participant has signed the written informed consent, the relevant study procedures can be performed in any order, which are:

- Background questionnaire
- Contact questionnaire
- Self-sampling of saliva, urine and vaginal secretions
- Venous blood sampling where consent is obtained

10.3.1.1 Questionnaires

The participant will be asked to complete two questionnaires:

- Background questionnaire: This will collect information on sociodemographic details such as ethnicity, age, living arrangements, number of children, occupation, age of child(ren), as well as existing medical conditions, previous child(ren) with cCMV and their awareness of CMV.
- Contact questionnaire: This will collect information on the participant's amount of handling and contact with child(ren)'s urine and saliva over the preceding two weeks. This will be collected at each visit.

Both questionnaires will be completed electronically on an electronic device (and if not possible on a paper copy) and will be completed during Visit 1.

10.3.1.2 Self-sampling of Saliva, Urine and Vaginal Secretions

At the Visit 1, the study team will explain and provide the participant with the ethically approved, study specific, written instructions on how to perform self-sampling of saliva, urine and vaginal secretions. The study team will collect these samples on the same day of the sampling. Self-sampling of saliva, urine and vaginal secretions will be performed on the day of each visit by the participant, using the written instruction provided without the need for any supervision by the study team, however the participant will be encouraged to seek advice and ask the study team any questions they may have regarding the self-sampling techniques. The participant will be provided with enough self-sampling kits to last them for the whole study period.

10.3.1.3 Venous Blood Sampling

Where consent is obtained, a venous sample will be collected at Visit 1, and at every visit. Any person that has received the appropriate training to perform venepuncture can collect the study blood samples. The participant's antenatal (or postnatal) clinical care team can also collect the study blood samples, for example, the participant may be due for a routine blood sampling as part of her antenatal (or postnatal) care and therefore the additional venous blood sample could be collected by her obstetrician or midwife at the same time for the study. However a member of the study team must perform any processing, packaging and transporting of the study sample to the laboratory.

9.3.2 Visit 2

Pregnant women booked for their antenatal care in St George's Hospital will attend a routine antenatal appointment at 20 gestational weeks. This is the timing chosen for Visit 2. Participants will be asked to inform the study team when their 20 gestational week antenatal appointment will be, and if any changes to the appointment date, the participant will be asked to make contact with the study team to inform them. If this timing is not suitable, the Visit 2 can be performed at a different time within the participant's second trimester from 17 weeks and 0 days gestational age to 26 weeks and 6 days gestational age.

At Visit 2, the relevant study procedures are:

- Contact questionnaire
- Self-sampling of saliva, urine and vaginal secretions
- Venous blood sampling where consent is obtained

The study team will send the participant a text reminder a day in advance of the Visit 2 to:

- Remind them of the visit
- Remind them to perform the self-sampling of saliva, urine and vaginal secretions on the date of the Visit 2 and to bring it with them to Visit 2
- Inform them that an email has been sent to them with a link to complete the contact questionnaire for Visit 2.

Although a face-to-face contact with the participant may not be necessary, a member of the study team will need to be available on the day of Visit 2 to:

- Collect the self-samples from the participant (or the participant can leave them with their direct antenatal team if the Visit 2 is coincided with their 20 gestational week routine antenatal appointment and the study team can collect it from the direct antenatal team)
- Perform venepuncture to collect a venous blood sample (or to collect the venous blood sample already taken in the event that the venepuncture and blood sampling is performed by the participant's direct antenatal team)
- If the participant does not have access to an email address, a paper copy of the contact questionnaire can be posted to the participant's home address.

9.3.3 Visit 3

- Pregnant women booked for their antenatal care in St George's Hospital will attend a routine antenatal appointment at 28 gestational weeks, which also includes a routine antenatal blood test. This is the timing chosen for Visit 3. Participants will be asked to inform the study team when their 28 gestational week antenatal appointment will be, and if any changes to the appointment date, the participant will be asked to make contact with the study team to inform them. If this timing is not suitable, Visit 3 can be performed at a different time within the participant's third trimester from 27 weeks and 0 days gestational age onwards.

At Visit 3, the relevant study procedures are the same as Visit 2, which are:

- Contact questionnaire
- Self-sampling of saliva, urine and vaginal secretions
- Venous blood sampling where consent is obtained

Just like Visit 2, the study team will send the participant a text reminder a day in advance of Visit 3 to:

- Remind them of the visit
- Remind them to perform the self-sampling of saliva, urine and vaginal secretions on the date of the Visit 3 and to bring it with them to Visit 3
- Inform them that an email has been sent to them with a link to complete the contact questionnaire for Visit 3.

Just like Visit 2 where a face-to-face contact with the participant may not be necessary, a member of the study team will need to be available on the day of the participant's Visit 3 to:

- Collect the self-samples from the participant (or the participant can leave them with their direct antenatal team if the Visit 3 coincided with their 28 gestational week routine antenatal appointment and the study team can collect it from the direct antenatal team)
- Perform venepuncture to collect venous blood sample (or to collect the venous blood sample already taken in the event that the venepuncture and blood sampling is performed by the participant's direct antenatal team)
- If the participant does not have access to an email address, a paper copy of the contact questionnaire can be posted to the participant's home address.

9.3.4 Visit 4

The participant will be reminded to let the study team know when they are in labour/have delivered. A reminder text will also be sent to all participants at 37 gestational weeks to remind them to let the study team know when they are in labour/have delivered. Effort will be made to perform Visit 4 when the participant is still on the postnatal ward provided this also suits the participant. If this timing is not suitable, Visit 4 can be performed at a different time within the first postnatal week, either at home or at a clinical setting, provided the participant is happy to bring her newborn with her to perform the newborn saliva sample. A face-to-face contact with the participant will be necessary to collect the saliva sample from the newborn for cCMV screening.

In the event that the participant delivers prematurely, provided the participant is still happy to remain in the study and the investigator has no concern with the participant remaining in the study, the specified visit number for this will be the subsequent visit of the previous visit and no further visits will be required (for example if the participant delivers her newborn at 32 weeks and the last visit she had was Visit 2, this will be Visit 3 labelled study visit equivalent to Visit 4).

Study Procedures	Screening	Visit 1	Visit 2	Visit 3	Visit 4
Eligibility criteria	X				
Informed consent		X			
Background questionnaire		X			
Contact questionnaire		X	X	X	X
Self-sampling of saliva, urine & vaginal secretions		X	X	X	X
Venous blood sampling*		X	X	X	X
Feedback questionnaire					X
Salivary swab of newborn*					X

At Visit 4, the relevant study procedures are:

- Self-sampling of saliva, urine and vaginal secretions
- Venous blood sampling where consent is obtained
- Screening newborn for cCMV
- Contact questionnaire
- Feedback questionnaire

10.3.4.1 Study Feedback Questionnaire

At Visit 4, a study feedback questionnaire will be completed. This will collect participants' feedback on the feasibility and acceptability of the study procedures. An email containing the link to the study feedback questionnaire (as well as the contact questionnaire) will be sent, which will be notified to the participant via a text reminder. This could also be completed in person with the study team using the hand held device provided, or a paper copy of the feedback questionnaire can be posted to the participant's home address.

10.3.4.2. Screening Newborn for cCMV

Although the vertical transmission rate for non-primary maternal CMV infection (as all participants are CMV seropositive) is very low at around 2% [Liesnard 2000; Revello 2002], all participants entering into the cCHIPS study will be offered the opportunity for their newborn to have cCMV screening where a saliva swab of the newborn will be collected by the study team. This will be done within the first week of the newborn's life, to ensure that if the newborn was diagnosed with cCMV and meets the criteria for treatment, this can be done in a timely way, as the current guidelines recommend treatment to begin within the first month of life. The decision to offer to screen the participants' newborns for cCMV was made following a discussion with our PPI group.

A positive CMV salivary swab result will be communicated to the participants by phone by a clinically qualified member of the research team and arrangements will be made for appropriate clinical follow up. This will involve referral to the paediatric infectious diseases team, according to established clinical pathways. Responsibility for the clinical care of the newborn, whether established as having cCMV infection or not, will remain with the clinical team

9.4 Summary flow chart of study assessment

*If consent obtained

9.5 Process Evaluation

Up to 20 participating mothers will be asked to take part in a process evaluation at the end of the study. Consent for this will be included in the consent form at the start of the study. This will be done by phone, skype or face to face interviews at the convenience of the participants. Interviews will be recorded. The recording will start after introductions have been done and will only contain first names. These recordings will be stored securely on NHS or University computers before being transcribed. After transcription the audio files will be destroyed. Interviews will be a maximum of 40 minutes long. Interviews will be performed until data saturation is reached, that is until additional interviews will not provide new information over and above that already obtained. The interviews will explore in depth their experiences during the study participation. They will explore optimal ways for information to be shared in future studies.

9.6 Methods

10.6.1 Blood sampling

Blood samples will be taken according to the normal procedures for venepuncture in an adult by experienced staff; any suitably trained staff that could either be a member of the study team or part of the participant's routine antenatal care team will perform the blood sampling. Approximately 12mls of venous blood will be collected in 4 tubes (approximately 8ml, 1.3ml, 1.3ml and 1.3ml in respective tubes).

10.6.2 Sampling of saliva, urine and vaginal secretion

Sampling of saliva, urine and vaginal secretions will be performed by the participants themselves using the ethically approved, study specific, participant friendly written instructions on how to perform self-sampling of saliva, urine and vaginal secretions with the self-sampling kit provided.

1. Self-sampling of urine: Approximately 10ml of mid-stream urine is directly collected into a universal specimen container
2. Self-sampling of saliva: Saliva is swabbed from the oral cavity using a salivary swab in PCR transport medium
3. Self-sampling of vaginal secretions: Vaginal secretions are obtained from the low vaginal wall using a vaginal swab

10.6.2 Sample labelling and transport

For all of the study samples, the participant study identification number, date of sampling and the participant's initials will be written on the sample bottle (or tube), and time of sampling for blood samples. A pre-filled sticker will be attached to the bag containing the samples to indicate study involvement in this specific study.

All study samples will be provided to the St George's research laboratory team by the study team where the samples will be stored under the relevant St George's laboratory Standard Operating Procedures.

10.6.3 Laboratory procedures

All saliva, urine and vaginal secretion samples collected from the participants will be tested for CMV DNA via polymerase chain reaction (PCR). These tests will be performed in multiple batches and not in 'real-time' within the limitations of sample integrity. All saliva samples collected from the newborns will be tested for CMV DNA PCR in 'real-time'.

For the blood samples, CMV-specific T cell immune responses will be analysed using two commercially available IGRAs; the QuantiFERON-CMV assay and CMV-ELISPOT assay.

The blood samples that will be analysed will take into account several factors; for all participants where CMV shedding is detected, the corresponding blood samples (same participant and same visit number as when shedding was detected) and other blood samples (same participant but at different visit numbers even when shedding does not occur) will be tested. A proportion of participants where CMV shedding is not detected at all will have their blood samples tested, and will take into account the prevalence, sociodemographic details, gestational stages and the source of shedding (saliva, urine and/or vaginal secretions) of the participants that shed.

10.6.3.1 QuantiFERON-CMV

Quantiferon-CMV (manufactured by QIAGEN), is an in-vitro diagnostic test using a cocktail of human leukocyte antigen class 1-restricted CMV peptide antigens that are associated with CMV infection (pp28, pp50, pp65, IE-1, IE-2, and gB) to stimulate cells in heparinised whole blood via the detection and measurement of IFN-γ production by Enzyme-Linked Immunosorbent Assay (ELISA).

Loss of this immune function (CMV-specific CD8+ responses) may be associated with the development of CMV disease. The intended use of QuantiFERON-CMV is to monitor the level of anti-CMV cell mediated immunity in persons at risk of developing CMV disease. QuantiFERON-CMV is not a test for determining CMV infection and should not be used to exclude CMV infection.

The QuantiFERON-CMV test system uses specialised blood collection tubes, which are used to collect whole blood. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens.

The QuantiFERON-CMV test is performed in two stages.

First, whole blood is collected into each of the three QuantiFERON-CMV blood collection tubes, which include a Nil Control tube, CMV Antigen tube and a Mitogen tube. The CMV Antigen tube is coated with peptides simulating CD8+ specific epitopes of CMV proteins where stimulation of CD8+ T cells in whole blood with the CMV peptides results in the production of IFN-γ in infected individuals. The Nil Control tube is used in the QuantiFERON-CMV test as a negative control. The Mitogen tube is used in the QuantiFERON-CMV test as a positive control, such as when there is doubt as to the individual's immune status and can also serve as a control for correct blood handling and incubation.

Second, the tubes are incubated at 37°C as soon as possible, and within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/ml) in the plasma is measured by ELISA. A robust IFN-γ response in the CMV Antigen tube is indicative of immunity to CMV (11).

10.6.3.2 CMV-ELISPOT

The CMV-ELISPOT test (manufactured by Oxford Immunotec) called T-SPOT®.CMV, is an in-vitro diagnostic test using CMV antigens pp65 and IE-1 to stimulate cells in peripheral blood mononuclear cells (PBMCs) isolated from whole blood via the detection and measurement of IFN-γ production by enzyme-linked immunospot (ELISPOT) assay.

Loss of this immune function (CMV-specific CD4+ plus CD8+ responses) may be associated with the development of CMV disease. The intended use of CMV-ELISPOT is to monitor the level of anti-CMV cell mediated immunity in persons at risk of developing CMV disease. CMV-ELISPOT is not a test for determining CMV infection and should not be used to exclude CMV infection.

The CMV-ELISPOT test system uses a heparinised blood collection tube to collect whole blood that is then washed to remove any sources of background interfering signal and isolated into PBMCs and tested for the presence of IFN-γ produced in response to the peptide antigens by capturing them directly around the secreting cell, before it is diluted in the supernatant, bound by receptors of adjacent cells or degraded.

Four wells are required for each sample:

1. Nil Control to identify non-specific cell activation
2. Panel CMV-A: CMV-specific antigen, IE-1

3. Panel CMV-B: CMV-specific antigen, pp65
4. Positive Control: Mitogen solution containing phytohaemagglutinin (PHA, a known polyclonal activator2) to confirm PBMC functionality.

The PBMCs are incubated with the antigens to allow stimulation of any sensitised T cells present. IFN-γ is captured by specific antibodies on the membrane, which forms the base of the well, and the PBMCs and other unwanted materials are removed by washing. A second antibody, conjugated to alkaline phosphatase and directed to a different epitope on the cytokine molecule, is added and binds to the cytokine captured on the membrane surface. Any unbound conjugate is removed by washing. A soluble substrate is added to each well; this is cleaved by bound enzyme to form a spot of insoluble precipitate at the site of the reaction. Each spot represents the footprint of an individual IFN-γ secreting T cell, and evaluating the number of spots obtained provides a measurement of the abundance of CMV-sensitive T cells in the peripheral blood.

10.6.3.3 CMV PCR DNA

All saliva, urine and vaginal secretion samples collected from the participants, and all saliva samples collected from the participants' newborns, will be tested for CMV DNA via real-time polymerase chain reaction (rtPCR) with internal controls using automative molecular processing in the St George's laboratory under the relevant St George's laboratory Standard Operating Procedures. Quantification standards will be used to evaluate the quantitative detection to copies/ml and where necessary the semi-quantitative detection (CT value -cycle threshold) of CMV DNA in clinical samples.

10.6.4 Sample storage

Samples are stored in the St George's research laboratory under relevant Standard Operating Procedures. Samples will be stored in the lab for two years in case they need to be referred to for a patient's clinical care. Any further storage for further research purposes will be anonymous and subject to prior consent. After this point they will be destroyed according to routine practice.

10.6.5. Sample collection and sample storage due to disruption caused by COVID-19 Pandemic

During the COVID-19 outbreak, face-to-face study visits, new recruitment and screening are suspended. Remaining participants will be offered the opportunity to continue with their study participation through the storing of their samples according to their usual study visit time-points at home until it is safe for the study team to collect the samples from the participants. This will involve the participants continuing to perform self-sampling of saliva, urine and vaginal secretions but instead of providing the samples to the research team on the day of the sampling, they will place the samples inside a securely sealed bag and store the sealed bag in the freezer at home. Participants will be provided with written instructions on how to do this and any additional sampling consumables via post. During this period of time it will not be possible to take blood samples or saliva swabs of newborns and so these will be seen as missing data points. For participants who do not agree to the storage of their samples in the freezer, or who lack access to a freezer, they will be withdrawn from the study or resume their study visits normally when it is safe to do so if suitable. Collection of home frozen samples will take place once the situation

has improved and we are able to see participants face-to-face. Participants will be contacted to update them on the changes required to the study due to the COVID-19 outbreak.

10 Safety Events

10.1 Definitions

Adverse Event (AE)—any untoward medical occurrence in a participant whether it is considered to be related to the intervention or not, that includes a clinical sign, symptom, or condition and /or an observation of a near incident. (This does not include pre-existing conditions recorded as such at baseline)

Serious Adverse Event (SAE)—any Adverse Event or untoward medical occurrence in a trial participant that can be wholly or partly to the intervention, which resulted in any of the following:

- Results in death; or
- Is life-threatening (places the participant, in the view of the Investigator, at immediate risk of death)
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay; even if it is a precautionary measure for observation; including hospitalisation for an elective procedure, for a pre-existing condition)
- 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 participants regardless of time of diagnosis).
- Or is another important medical condition

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the outcomes listed in the definition of serious will also be considered serious.

10.2 Recording Adverse Events (AEs)

A record of all AEs, whether related or unrelated to study participation will be kept in the CRF and the Sponsor's AE Log JRESLOG0007. We will not record AEs which can reasonably be considered to be part of pregnancy including nausea, vomiting, back, hip or pelvic pain, abdominal discomfort, reduced sleep, constipation and lower limb swelling.

If the investigator suspects that the disease or condition has progressed faster due to the intervention, then they will report this as an unexpected adverse event to the sponsor.

10.3 Investigator Responsibilities relating to Safety Reporting

Collection, recording and reporting of AEs (including serious and non-serious events and reactions) to the Sponsor will be done according to the Sponsor's Safety reporting for non-CTIMP studies SOP JRESSOP0033.

All SAEs will be recorded in the CRF, and the Sponsor's AE Recording Log JRESLOG0007. The AE Log will be sent to the Sponsor on request and every 2 months.

All SAEs will be reported both to the Sponsor via the JRES & REC using the SAE report form for research other than CTIMPs (non-CTIMPs) published on the HRA website. The Chief or Principal Investigator at any participating site will complete the SAE form which will be faxed to the JRES on 020 8725 0794 or e-mailed to adverseevents@sgul.ac.uk, within 48hrs of the Investigator becoming aware of the event, and via email to the relevant REC.

The Chief or Principal Investigator will respond to any SAE queries raised by the Sponsor as soon as possible. Follow up reports must continually be completed within acceptable time-frames and sent as detailed above until the reportable event is considered resolved

Events will be followed up until resolution; any appropriate follow-up information will be clearly marked as such and reported to the sponsor via the JRES as above in a timely manner.

Full reports should be completed and submitted to REC within 15 days of the event

10.4 Notification of deaths

All deaths will be reported to the Sponsor irrespective of whether the death is related to disease progression, the intervention, or an unrelated event.

11 Data management and quality assurance

11.1 Confidentiality

St George's, University of London is the sponsor for this study and is based in the United Kingdom. They will be using information from participants (directly and/or medical records) in order to undertake this study and will act as the data controller for this study. All data will be handled in accordance with the Data Protection Act 2018.

The Case Report Forms (CRFs) will not bear the participant's name or other directly identifiable data. The participant's trial Identification Number (ID) only, will be used for identification. The sponsor Subject ID log JRESLOG0002 can be used to cross reference participant's identifiable information.

11.2 Data collection tool

Case Report Forms will be designed by the CI or a delegated member of the study team. All data will be entered legibly in black ink with a ball-point pen. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log JRESLOG0004 will identify all trial personnel responsible for data collection, entry, handling and managing the database.

Data will be recorded directly onto the CRF.

An initial entry will be made in the participant's handheld notes describing the participant's involvement in a research study. The front page of the handheld notes will have a sticker placed on it to alert clinical staff to study participation. Any further correspondence between the study team and clinical colleagues will take place by letter.

There are three types of study-specific questionnaires to be completed throughout the study period (background and contact questionnaires at Visit 1, contact questionnaire at Visit 2 and 3, contact and feedback questionnaires at Visit 4). At Visit 1 and 4 which require face to face contact, the questionnaires will be completed electronically on an electronic device (and if not possible on hard copy). For Visit 2 and 3, a link to the contact questionnaire will be emailed out to participants along with a text. If participating women do not have access to a computer, the questionnaire may be mailed to them and a pre-paid envelope provided.

11.3 Incidental Findings

In this study the participants' newborns are offered screening for evidence of cCMV. A positive CMV salivary swab result will be communicated to the participants by phone by a clinically qualified member of the research team and arrangements will be made for appropriate clinical follow up. This will involve referral to the paediatric infectious diseases team, according to established clinical pathways. Whilst a discovery of cCMV in their newborns may cause anxiety and distress to an individual and her family, this will provide the opportunity for appropriate treatment to be provided for her infant and therefore represents an opportunity for early diagnosis and potentially faster treatment for infants. The decision to offer to screen the participants' newborns for cCMV was made following a discussion with our PPI group (the South London Maternal and Paediatric Immunity & Infection Patient and Public Involvement group).

A very small minority of women may be found to have recent infection and as receiving these results may generate anxiety in the women and their families these will be communicated by a clinical member of the team by phone. A letter may subsequently be sent outlining what was said for reference. This group of women will be referred to the Fetal Medicine Unit (FMU) for further investigation and counselling. To expedite this process all such results will be discussed with Dr Asma Khalil (or a designated specialist in her absence) to arrange a timely clinic appointment at the FMU. A letter will also be sent to the GP to inform them of the result and the referral to FMU.

11.4 Data handling and analysis

Paper

Paper CRFs will not contain identifiable information. Contact details will be stored in the same folder as the CRF, but will not be attached to it allowing easy separation if necessary. CRFs will be stored in lockable filing cabinets in a secure office. Informed consent forms completed will be collected by a

member of the study team and filed and the file will be stored in a lockable cupboard in a secure office.

Digital

Identifiable information about participants (contact details) will be stored alongside their unique identification number in a password-protected database on a password-protected NHS or University computer. This information is important to allow contact to be made by the study team. Details about investigation results will be stored in a second password-protected database in which participants are only identified by their unique identification number. Information for the study will be entered onto a REDCap database designed by the study team. No identifiable information will be stored in this database. Data will be entered and checked by two separate members of the study team.

12 Archiving arrangements

The trial essential documents along with the trial database will be archived in accordance with the sponsor SOP JRESSOP0016. The agreed archiving period for this trial will be 5 years.

13 Statistical design

13.1 Statistical input in trial design

The statistical aspects of this study have been reviewed by epidemiologist Dr Charlotte Jackson.

13.2 Endpoints

The overall aim of the study is to determine the feasibility of future large studies to evaluate the relationship between CMV shedding in pregnancy and congenital CMV. This feasibility study will generate the essential data upon which to appropriately power this study. A key element of determining the sample size for future studies is to estimate the prevalence of CMV shedding in CMV seropositive women during pregnancy.

14.2.1 Primary endpoints

The primary end point (on which this study is powered) is the percentage of subjects with detectable CMV virus (measured via quantitative PCR) in any bodily fluid (saliva, urine or vaginal secretions) at any point in pregnancy

14.2.2 Secondary endpoints

- The percentage of subjects with detectable CMV virus in saliva (measured via quantitative PCR) at individual study visits and cumulatively
- The percentage of subjects with detectable CMV virus in urine (measured via quantitative PCR) at individual study visits and cumulatively

- The percentage of subjects with detectable CMV virus in the vaginal secretions (measured via quantitative PCR) at individual study visits and cumulatively
- The quantity of CMV virus in saliva (measured via quantitative PCR) in the subjects at individual study visits cumulatively
- The quantity of CMV virus in urine (measured via quantitative PCR) in the subjects at individual study visits and cumulatively
- The quantity of CMV virus in vaginal secretions (measured via quantitative PCR) in the subjects at individual study visits and cumulatively
- Risk factors associated with CMV shedding in the subjects
- Assessment of the acceptability of the study procedures to participating pregnant women
- Proportion of subjects approached who are willing to participate in the study
- Proportion of subjects completing the study

14.2.3 Tertiary endpoints

- Detection and quantity of IFN-γ (IU/ml) in the plasma measured by CMV-QuantiFERON ELISA at a study visit when CMV shedding occurs in the subject
- Detection and quantity of IFN-γ (IU/ml) in the plasma measured by CMV-QuantiFERON ELISA at a study visit when CMV shedding does not occur in the subject
- Detection and quantity of IFN-γ (IU/ml) in the plasma measured by ELISPOT-CMV assay at a study visit when CMV shedding occurs in the subject
- Detection and quantity of IFN-γ (IU/ml) in the plasma measured by ELISPOT-CMV assay at a study visit when CMV shedding does not occur in the subject

13.3 Sample size and recruitment

13.3.1 Sample size calculation

This is a feasibility study and the sample size has been calculated to allow an acceptably precise estimate of the percentage of seropositive women who shed CMV during pregnancy.

There is a recently published longitudinal study [Barbosa 2018] looking at the prevalence of CMV shedding throughout pregnancy of CMV seropositive women in Brazil, which found that shedding was detected at least once in 35% of the pregnant women, with saliva being the most common (20%), followed by urine (13.3%), vaginal secretions (12.5%) and blood (0.8%). This study also found that mothers living with or providing daily care to young children were twice as likely to shed CMV at least once compared to women with less exposure to young children (58% vs 26%).

Based on this study, the sample size was calculated using an estimated proportion (prevalence of CMV shedding at least once in any bodily fluid in CMV seropositive women throughout pregnancy) of 30%. Thus a sample size of 200 participants, which is feasible in practice, will provide tight 95% confidence levels for this estimate of +/-6% (i.e. 24-36%).

13.3.2 Planned recruitment rate

For the parallel-run RACEFIT study, approximately 4800 women is estimated to be approached over a one-year recruitment period (approximately 400 per month). The RACE-Fit study team have estimated from their experience that 70% of women approached will consent to serological screening of a blood sample taken with their routine antenatal bloods and 50% of those to consent to taking part in the full study. Approximately 23% of women approached are eligible for the screening process. Assuming that 55% [Tookey 1992] of the population will be CMV seropositive, it means that 212 (4800 x 23% x 70% x 50% x 55%) women from those approached are expected to take part in the study over 12 months. Therefore, a sample size of 200 participants over 12-month recruitment period should be achievable.

13.4 Statistical analysis plan

13.4.1 Summary of baseline data and flow of patients

Baseline data will be summarised descriptively. The percentage of eligible women consenting to participate will be calculated as (number consenting) / (number of eligible women identified to be CMV seropositive from the screening). To assess the representativeness of women participating in the study, characteristics of those consenting (ethnicity and age) will be compared to those who do not consent in univariate analyses (e.g. using chi squared and t tests).

13.4.2 Primary endpoint analysis

The percentage of participating women with at least one study sample with CMV DNA detected (saliva, urine or vaginal secretions taken at any study visit during pregnancy) as measured by PCR, will be calculated with 95% confidence intervals (CIs).

13.4.3 Secondary endpoint analysis

For each sample type (saliva, urine, vaginal secretions), the percentage of participating women with detectable CMV DNA as measured by quantitative PCR, will be calculated for each study visit (Visit 1, Visit 2, Visit 3, Visit 4), and cumulatively, with 95% CIs.

For each study visit (Visit 1, Visit 2, Visit 3, Visit 4), the percentage of participating women with detectable CMV DNA as measured by PCR, will be calculated for each sample type (saliva, urine, vaginal secretions), and cumulatively, with 95% CIs

CMV shedding will also be assessed quantitatively, as measured by quantitative PCR on the amount of CMV DNA detected, in each sample type and at each study visit, separately and cumulatively, with 95% CIs.

The distributions of sample type, study visit number, detectable shedding and quantity of shedding will be summarised graphically and described using means and medians, as appropriate.

Factors associated with CMV shedding as a binary outcome (detectable shedding versus no detectable shedding) will be investigated using multivariable mixed effects models. Potential risk factors will be those assessed using the study sociodemographic questionnaire at Visit 1 and risk factor questionnaires at all visits (these variables may be time-varying) – these will include ethnicity, age, number of children, parental occupation, age of child(ren), and type of handling with child(ren)'s urine and saliva.

Acceptability of the study design and procedures will be analysed descriptively, for example by calculating mean scores derived from satisfaction scales. We will also investigate factors associated with satisfaction, defined as a binary variable, using logistic regression. We will summarise free text comments made through the feedback questionnaire.

The percentage of eligible women who consent to participate, and the percentage of participants who complete the study (provide urine, saliva and vaginal secretion samples and questionnaire data at all four study visits, with or without blood and newborn saliva samples), will be calculated.

13.4.4 Tertiary endpoint analysis

For each visit, the percentage of women with a positive result from each IGRA will be estimated with 95% CIs, separately amongst participating women with and without CMV shedding. The distribution of CMV-specific T cell immune responses as measured by each IGRA will also be plotted for each of these groups.

Agreement between the categorical results from the two IGRAs will be assessed using the kappa statistic.

13.4.5 Sensitivity and other planned analyses (if applicable)

A subgroup analysis will be performed using data only from women with samples at all three time points.

13.5 Interim analysis

There will be no need for interim analyses to be performed.

14 Committees involved in the trial

This study will have a Trial Management Group. This group will include those individuals responsible for the day-to-day management of the study, including the CI, the PI, senior investigators at the site, the study midwife involved in the study, the virologist coordinating the laboratory analysis of the samples, the project manager and the statistician. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and ensure the quality of the trial.

15 Direct access to source data

The Investigator(s)/institution(s) will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

16 Ethics and Research Governance requirements

Before the site can enrol patients into the trial, the Chief Investigator must ensure written permission to proceed has been granted by their Research & Development (R&D) department. At St George's the governance team within the JRES can provide assistance.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and which was given favourable opinion by the Research Ethics Committee (REC).

The Chief Investigator will be provided (via the Sponsor) with file indexes E.G. JRESDOC0003 TMF index and JRESDOC0004 ISF index for use with SOP JRESSOP0019 'Preparation and Maintenance of the TMF' The CI will be responsible for the maintenance of the TMF and may delegate the responsibility of ISF file maintenance.

It is the responsibility of the Chief Investigator to ensure that all subsequent amendments gain the necessary approval. Refer to JRESSOP0011 'Management of Amendments'.

Within 90 days after the end of the trial, the CI and Sponsor will ensure that the REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial. Refer to JRESSOP0015 'End of study declaration'

The CI will supply an End of Study report of the clinical trial to the REC within one year after the end of the trial. The sponsor can provide JRESDOC0059 End of study Report template

17.1 Annual Progress Reports (APRs)

The Chief Investigator will prepare the APR in accordance with JRESSOP0043. Following review by the sponsor the report will be sent to the REC. The APR is due for submission annually within 30 days of the anniversary date on which the Ethics committee gave the favourable opinion, until the trial is declared ended.

17.2 Notification of Serious Breaches of GCP and/or the protocol

Any Protocol Deviations or violations will be documented using JRESDOC0061, and entered onto the Sponsor's log JRESLOG0005. Potential Serious Breaches and Urgent Safety Measures will be recorded both on the Sponsor's Log JRESLOG0005 and processed according to JRESSOP0012 and where necessary JRESSOP0032

A "serious breach" is a breach that is likely to effect to a significant degree:

(a) The safety or physical or mental integrity of the participants of the trial; or
(b) The scientific value of the trial.

The CI will notify the Sponsor immediately of any case where there exists a possible occurrence of a serious breach.

17 Finance

This study is funded by the Merck Investigator Studies Program (MISP No. 58414).

18 Insurance and indemnity

St George's, University of London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that St George's, University of London has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the Trial is conducted in a hospital, the hospital has a duty of care to participants. St George's, University of London will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to St George's, University of London, upon request.

Participants may be able to claim compensation for injury caused by participation in this Trial without the need to prove negligence on the part of St George's, University of London or another party.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of St George's, University of London immediately.

Failure to alert St George's, University of London without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

19 IP and development policy

Unless otherwise specified in agreements, the following guidelines shall apply: All Intellectual Property Rights and Know How (IP) related to the Protocol and the Trial are and shall remain the property of the Sponsor excluding

1) Pre-existing IP related to clinical procedures of any Hospital.

2) Pre-existing IP related to analytical procedures of any external laboratory.

All contributors shall assign their rights in relation to all Intellectual Property Rights and in all Know How, not excluded above to the Sponsor and at the request and expense of the Sponsor, shall execute all such documents and do all such other acts as the Sponsor may reasonably require in order to vest fully and effectively all such Intellectual Property Rights and Know How in the Sponsor or its nominee. They shall promptly disclose to the Sponsor any Know How generated pursuant to this Protocol and not excluded above and undertake to treat such Know How as confidential information jointly owned between it and the Sponsor.

Nothing in this section shall be construed so as to prevent or hinder any medical professional from using Know How gained during the performance of the Trial in the furtherance of its normal business

activities, to the extent such use does not result in the disclosure or misuse of Confidential Information or the infringement of any Intellectual Property Right of the Sponsor.

20 Publication policy

Publication: "Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations."

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance.

20.1 Before the official completion of the Trial

If an investigator wishes to publish a sub-set of data before completion of the trial, the Chief Investigator must be consulted.

20.2 Up to 180 days after the official completion of the Trial

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.
- Other investigators shall be listed as compatible with the policies of the publication outlet and good academic practice.
- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.

20.3 Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

21 Statement of Compliance

The trial will be conducted in compliance with the protocol, Sponsor's Standard Operating Procedures (SOPs), GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the Data Protection Act 2018, the Human Medicines Regulations 2012, ICH GCP**,** the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the NHS Research Governance Framework for Health and Social Care (Version 2, April 2005).

This study will be conducted in compliance with the protocol approved by the REC and according to GCP standards. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor and REC except where it may be necessary to eliminate an immediate hazard to a research participant. In such case, the deviation will be reported to the Sponsor and REC as soon as possible.

22 References

Peckham CS, Chin KS, Coleman JC, Jenderson K, Jurley R, Preece PM Cytomegalovirus infection in pregnancy: preliminary findings from a prospective study. Lancet 1983; 1(8338): 1352-1355.

Griffiths PD, Baboonian C, Rutter D, Peckham C. Congenital and maternal cytomegalovirus infections in a London population. Br J Obstet Gynaecol 1991; 98: 135-140.

Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007; 17: 355-363.

Barbosa NG, Yamamoto AY, Duarte G, Aragon DC, Fowler KB, Boppana S, Britt WJ, Mussi-Pinhata MM. Cytomegalovirus (CMV) shedding in seropositive pregnant women from a high seroprevalence population: "The Brazilian Cytomegalovirus Hearing and Maternal Secondary Infection Study" (BraCHS). Clin Infect Dis 2018; 67: 743.

de Vries JJ, van Zwet EW, Dekker FW, Kroes AC, Verkerk PH, Vossen AC. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: A population-based prediction model. Rev Med Virol 2013; 23: 241-249.

Mussi-Pinhata MM, Yamamoto AY, Aragon DC, Duarte G, Fowler KB, Boppana S, Britt WJ. Seroconversion for Cytomegalovirus Infection During Pregnancy and Fetal Infection in a Highly Seropositive Population: "The BRAHCS Study". J Infect Dis 2018; 218(8).

Atkinson C, Emery VC, Griffiths PD. Development of a novel single tube nested PCR for enhanced detection of cytomegalovirus DNA from dried blood spots. J Virol Methods 2014; 196: 40-44.

Kimberlin DW, Lin CY, Sánchez PJ, Demmler GJ, Dankner W, Shelton M, Jacobs RF, Vaudry W, Pass RF, Kiell JM, Soong SJ, Whitley RJ. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. J Pediatr 2003; 143(1): 16-25.

Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol 2011; 21: 240-255.

Williams EJ, Gray J, Luck S, Atkinson C, Embleton ND, Kadambari S, Davis A, Griffiths P, Sharland M, Berrington JE, Clark JE. First estimates of the potential cost and cost saving of protecting childhood hearing from damage caused by congenital CMV infection. Arch Dis Child Fetal Neonatal Ed 2015; 100(6): F501-F506.

Marty FM, Ljungman P, Chemaly RF. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med 2017; 377: 2433-2444.

Pass RF, Zhang C, Evans, Simpson T, Andrews W, Huang M, Corey L, Hill J, Davis E, Flanigan C, Cloud G. N Engl J Med 2009; 360(12): 1191-1199.

Sabbaj S, Pass RF, Pichon S, Goepfert PA. CMV Glycoprotein B vaccine is capable of boosting both antibody and CD4 T cell responses to CMV in chronically infected women. J Infect Dis 2011; 203(11):1534-1541.

Griffiths PD, Stanton A, McCarrell E, Smith C, Osman M, Harber M, Davenport A, Jones G, Wheeler DC, O'Beirne J, Thorburn D, Patch D, Atkinson CE, Pichon S, Sweny P, Lanzman M, Woodford E, Rothwell E, Old N, Kinyanjui R, Haque T, Atabani S, Luck S, Prideaux S, Milne RS, Emery VC, Burroughs AK. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. Lancet 2011; 377(9773): 1256-1263.

Adachi K, Xu J, Ank B, Watts DH, Mofenson LM, Pilotto JH, Joao E, Santos B, Fonseca R, Kreitchmann R, Pinto J, Mussi-Pinhata MM, Gray G, Theron G, Morgado MG, Bryson YJ, Veloso VG, Klausner JD, Moye J, Nielsen-Saines K. Cytomegalovirus Urinary Shedding in HIV-infected Pregnant Women and Congenital Cytomegalovirus Infection. Clin Infect Dis 2017; 65(3): 405-413.

Lee H, Park KH, Ryu JH, Choi AR, Yu JH. Cytomegalovirus (CMV) immune monitoring with ELISPOT and QuantiFERON-CMV assay in seropositive kidney transplant recipients. PLoS One 2017; 12(12): e0189488.

Revello MG, Lilleri D, Zavattoni M, Furione M, Genini E, Comolli G, Gerna G. Lymphoproliferative response in primary human cytomegalovirus (HCMV) infection is delayed in HCMV transmitter mothers. J Infect Dis 2006; 193: 269-276.

Bialas KM, Tanaka T, Tran D, Varner V, Cisneros De La Rosa E, Chiuppesi F, Wussow F, Kattenhorn L, Macri S, Kunz EL, Estroff JA, Kirchherr J, Yue Y, Fan Q, Lauck M, O'Connor DH, Hall AH, Xavier A, Diamond DJ, Barry PA, Kaur A, Permar SR. Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of placental cytomegalovirus transmission. Proc Natl Acad Sci USA 2015; 112: 13645-13650.

Saldan A, Forner G, Mengoli C, Gussetti N, Palu G,Abate D. Strong Cell0Mediated Immune Response to Human Cytomegalovirus Is Associated With Increased Risk of Fetal Infection inPrimarily Infected Pregnant Women. Clin Infect Dis 2015; 61: 1228-1234.

Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet Gynecol 2000; 95: 881 -888.

Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev 2002; 15: 680-715.

Tookey PA, Ades AE, Peckham CS. Cytomegalovirus prevalence in pregnant women: the influence of parity. Arch Dis Child 1992; 67: 779-783.

23 List of Protocol appendices

Appendix 1 Summary of Protocol Revision History

Appendix 1

There are currently no amendments to the protocol.

Protocol amendment /Revision History

Protocol Version and Date	New text
2.0 08/08/19	Details on screening process has been updated throughout the study protocol to reflect changes required for the study screening period to extend to 12 months. The range of gestational ages to reflect the appropriate corresponding trimesters were also corrected.
3.0, 25/03/20	Addition of section 10.6.5. Sample collection and sample storage due to disruption caused by COVID-19 Pandemic