Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut, 89
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eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Date of protocol	Final: 06 April 2012
Date of protocol amendment	Protocol Amendment 1 Final: 31 July 2013 Protocol Amendment 2 Final: 19 February 2015
Title	A study in adolescent females to explore cytomegalovirus infection.
Detailed Title	A study to explore cytomegalovirus primary infection, re-activation and re-infection in an adolescent female population.
Co-ordinating author	PPD (Keyrus Biopharma contractor for GSK Biologicals), PPD, Scientific Writers
Contributing authors **(Amended 19 February 2015)**	• PPD, PPD, Clinical ***Research and*** Development ***Leads*** • PPD, PPD, Study Delivery Lead • PPD ***, Study Delivery Manager*** • PPD, PPD, Project Statistician • PPD, PPD, PPD ***(Business & Decision Life Sciences for GSK Biologcals), GVCL*** Representatives • PPD, PPD, PPD (Keyrus Biopharma contractor for GSK Biologicals), PPD ***(TCS Consultant for GSK Biologicals),*** PPD **,** Study Data Manager • PPD ***, Project Data Manager*** • PPD, PPD **,** PPD **,** Safety Representative**s** • PPD, Epidemiologist

GSK Biologicals' protocol template for observational studies and interventional studies without administration of medicinal products as described in a research protocol based on the Protocol Document Standard version 13.2

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Date of protocol amendment	Protocol Amendment 2 Final: 19 February 2015
Detailed Title	A study to explore cytomegalovirus primary infection, re-activation and re-infection in an adolescent female population.
Sponsor signatory	Jeanne-Marie Devaster
(Amended 19 February 2015)	***Director*** Clinical ***Research and Translational Science***

Signature _____

Date _____

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Protocol Amendment 2 Rationale

Amendment number: Amendment 2 Final
Rationale/background for changes:
Protocol amendment 2 was prepared: • To cancel the planned interim analyses and to keep the final analysis only: The need to validate and develop some assays has led to a delay in the release of data on time. Since no vaccine is administered in the CMV-014 EXPLO study and as the interim analyses were not linked to subjects' safety or efficacy, but rather to exploring the natural history of CMV disease, it was decided to group the planned interim analysis after Year 1 and Year 2 and to have a single final analysis of the total follow-up at the end of the study. • To clarify that the tertiary endpoints (i.e. exploratory tests) and the subcohort selected for testing will be based on the evaluations of primary and secondary endpoints. • To stop collection of blood samples for gene expression signature (qPCR or RNA microarray) as of protocol amendment 2 approval: A Year 1 dataset will be used for analysis of gene expression in a subset of subjects. In addition, the list of contributing authors together with the Sponsor Signatory Approval page have been updated following changes in the team members/ function names. The notation of the terms "ATP cohort" and "Total cohort" were adapted to maintain consistency throughout the protocol.

The summary of the amendment will be provided in APPENDIX C.

Protocol Amendment 2 Investigator Agreement

I agree:

- **To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline Biologicals (GSK Biologicals).**
- **To assume responsibility for the proper conduct of the study at this site.**
- **That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.**
- **To ensure that all persons assisting me with the study are adequately informed about study-related duties and functions as described in the protocol.**
- **To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.**
- **To ensure that no samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally authorised representative.**
- **To perform no other biological assays on the samples except those described in the protocol or its amendment(s).**
- **To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.**
- **That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.**

Hence I:

- **Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).**
- **Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.**
- **Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.**
- **Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.**

eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Date of protocol amendment	Protocol Amendment 2 Final: 19 February 2015
Detailed Title	A study to explore cytomegalovirus primary infection, re-activation and re-infection in an adolescent female population.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut, 89
1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 6.3.2.

SYNOPSIS

Detailed Title

A study to explore cytomegalovirus primary infection, re-activation and re-infection in an adolescent female population.

Rationale for the study

GSK Biologicals anticipates developing a prophylactic cytomegalovirus (CMV) vaccine indicated for the use in women and adolescents to protect their offspring against congenital cytomegalovirus (cCMV) infection and disease. This study is therefore designed:

- To estimate the incidence of CMV secondary infections (re-infections / re-activations) in adolescent females.
- To estimate the incidence of CMV primary infection in adolescent females.
- To develop assays able to distinguish and detect re-infections versus re-activations.

Objectives

Primary

- To estimate the incidence of CMV secondary infections in seropositive adolescent females.

Secondary

- To estimate the incidence of CMV primary infections in seronegative adolescent females.

Tertiary

- To develop assays that allow the differentiation of CMV secondary infections caused either by re-infection or by re-activation, in CMV seropositive adolescent females.
- To assess the diagnostic value of different assays to evaluate seroconversion in blood and the detection of CMV DNA in different body fluids.
- To describe the proportion of secondary infection caused either by re-infection or re-activation.
- To collect samples for immunological and biological disease-related exploratory assays.
- To explore socio-demographic or behavioral factors associated with CMV infection.
- In case of pregnancies, to document the birth prevalence of CMV congenital infections.

- To evaluate the concordance between screening testing data at local laboratories (or alternatively, central laboratories) and the GSK Biologicals or designated laboratory testing data.

Study design

- Prospective, multi-center, multi-country study.
- **Visit schedule**:
  - 10 scheduled **Site Visits** approximately every 4 months.
  - 9 **Sample Collection Visits** approximately at mid-term between 2 site visits (i.e. approximately 2 months after a Site Visit) where samples will be collected either through self-collection by the subjects, through collection by a home-visiting nurse or through a new visit at the study center.
- **Study population:** Adolescent females aged between 10 and 17 years regardless of CMV status.
- **Type of study**: Self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Duration of the study**:

  Participant: Approximately 36 months.

  Newborn (in case of pregnancy): Approximately 10 days.
  - Epoch 001: Prospective data collection starting at Visit 1 (Month 0) and ending at Visit 10 (Month 36).

Synopsis Table 1 Study groups foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)
S+	+/- 240 seropositive subjects	10 – 17 years
S-	Estimated approximate number of seronegative subjects[1]: 160	10 – 17 years

[1] As subjects will be enrolled consecutively without previous CMV screening with a target number of 240 seropositive subjects, the number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries.

Discussion of study design

The study sites will be selected in multiple countries, including countries where the CMV prevalence is assumed to be high in order to enroll primarily CMV seropositive adolescent females to allow the evaluation of CMV secondary infections. Since enrolment will proceed without previous CMV screening, some of the enrolled subjects will be seronegative. The enrolled seronegative subjects will allow the estimation of the incidence of CMV primary infections in the adolescent population.

To increase the probability to capture all CMV infections (primary and secondary infections), regular sample collection time-points have been arranged:

- **Site Visits**: Subjects will be asked to perform a Site Visit approximately every 4 months for 3 years (10 Site Visits in total). At Site Visits, blood, urine and saliva, will be collected.
- **Sample Collection Visits**: Subjects will be contacted to perform urine and saliva sample collections at home either by self-collection or by a home-visiting nurse. Alternatively subjects may be asked to come back to the study center where the investigator or designate will collect the samples. These Sample Collection Visits will occur approximately every 4 months for 3 years between 2 Site Visits (9 Sample Collection Visits in total).

Number of subjects

Subjects will be enrolled consecutively without previous CMV screening. Enrolment will be terminated once approximately 240 seropositive subjects are included in the trial. The number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries. It is estimated that approximately 400 subjects will be enrolled in total.

Endpoints

Primary

- Occurrence of CMV secondary infections determined in all seropositive subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*

Secondary

- Occurrence of CMV primary infection determined in all seronegative subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*

Tertiary (amended 19 February 2015)

- Further characterization of primary infection in initially seronegative subjects during the 4-month Site Visits until study conclusion (testing done upon seroconversion) ***might be done***:
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
- Further characterization of primary and /or secondary CMV infections in a subset of subjects on selected samples of the Sample Collection Visits* ***might be done***.
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR).*

** In case of a positive sample at a 4-month Site Visit time point, testing **might** be done on selected samples collected during the Sample Collection Visit. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.*

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits ***might be done***:
  - *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody concentration in serum (ELISA).*
  - *Anti-CMV IgM antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva and blood (qPCR).*
  - *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*

- Development of assays that will allow differentiating re-infection from re-activation from primary infection in a subset of subjects ***might be done***.
  - *Characterization of CMV strains by genotyping/sequencing.*
  - *Exploring the CMV strain specific antibody profile using peptide microarrays.*
- Assessment of the expression of host genes* using techniques such as qPCR or mRNA microarray in a subset of subjects ***might be performed***.

* excluding genes related to hereditary characteristics of the subject.

- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection ***might be performed***.
- Occurrence of congenital CMV infection in newborns of subjects who become pregnant during the study ***might be evaluated***:
  - *Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes)* ***might be tested****.*

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LIST OF ABBREVIATIONS (amended 19 February 2015)

AE	Adverse Event
Ag	Antigen
ATP	***a***ccording-***t***o-***p***rotocol
CI	Confidence Interval
cCMV	congenital Cytomegalovirus
CIOMS	Council for International Organizations of Medical Sciences
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
eCRF	electronic Case Report Form
ED_{50}	Effective dose 50
ELISA	Enzyme-linked immunosorbant assay
EU	ELISA unit
gB	Glycoprotein B
GCP	Good Clinical Practice
gH	Glycoprotein H
GSK	GlaxoSmithKline
GMT	Geometric mean titer
HIV	Human Immunodeficiency Virus
HRP	Horseradish peroxidase
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IgG	Immunoglobulin class G

IgM	Immunoglobulin class M
IRB	Institutional Review Board
LAR	Legally Acceptable Representative
NA	Not applicable
mRNA	Messenger Ribonucleic acid
OD	Optical density
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
pp65	Phosphoprotein 65
RDE	Remote Data Entry
RNA	Ribonucleic acid
S-	CMV Seronegative group
S+	CMV Seropositive group
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDV	Source Document Verification
SPM	Study Procedures Manual
TBD	To be determined

GLOSSARY OF TERMS

Adverse event:	Any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.
Anonymisation:	Information that identifies a specific individual (including, but not limited to name, address and national identification number such as social security number, date of birth) has been removed and no link to the donor, through a code number for example, is maintained.
Child in care:	A child who has been placed under the control or protection of an agency, organization, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
CMV secondary infection:	Recurrent CMV infection, defined as either a viral re-activation or a re-infection with a new strain of CMV.
Coded:	Information is associated with a subject number i.e. a code number. Coded information can only be linked back to the individual via a key code i.e. a listing of the research participants and their code. Within the pharmaceutical industry coding data is the usual mechanism used for protecting an individual's research data. The key code is kept secure, usually by the investigator, and GSK researchers cannot identify the research individual other than in exceptional and controlled circumstances.

Cohort study:	A form of epidemiology study where subjects in a study population are classified according to their exposure status/disease and followed over time (prospective / retrospective) to ascertain the outcome(s).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epidemiology study:	An observational study or an interventional study without administration of medicinal product(s) as described in a research protocol.
Epoch:	An epoch is a well defined part of a protocol that covers a set of consecutive time points. Generally, an epoch is self-contained and allows to perform a data analysis to address some of the trial objectives (e.g. primary, booster, yearly follow-ups, retrospective data collection, prospective data collection).
eTrack:	GSK's tracking tool for clinical/epidemiology trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Section 8.4 for details on criteria for evaluability).
Interventional Human Subject Research:	Studies in which participants are administered medical care, medicinal products and/or medical/scientific procedures as described in a research protocol.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study endpoints.
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents.
Seronegative subject:	Subject for whom anti-CMV immunoglobulin G (IgG) antibodies are not detected in serum sample collected at Visit 1.
Seropositive subject:	Subject for whom anti-CMV IgG antibodies are detected in serum sample collected at Visit 1.

Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical/ epidemiology studies at one or more investigational sites.
Study population:	Sample of population of interest.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical/ epidemiology study, or a person about whom some medical information has been recorded in a database.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.

1. INTRODUCTION

1.1. Background

Cytomegalovirus (CMV), a member of the herpes virus family, is a double-stranded deoxyribonucleic acid (DNA) virus. CMV infects a large proportion of the world's population, with a seropositivity ranging from 30% to 100% in adults, depending on the countries, populations, density and socioeconomic status [Cannon, 2010].

CMV is generally passed from infected people to others through direct contact with body fluids (such as saliva, tears, blood, breast milk, semen and urine). It can also be transmitted from a pregnant woman to her fetus during pregnancy. The virus in the mother's blood crosses over the placenta and infects the fetus's blood. A primary infection with CMV is likely to be asymptomatic in healthy individuals, although it may cause a mild mononucleosis-like syndrome. However, CMV infection can be serious or even life-threatening in subjects with deficient or immature immune systems, such as congenitally infected fetuses, organ-transplant patients and human immunodefiency virus (HIV)-infected patients. The most frequent complications from congenital CMV (cCMV) infection are neonatal neurological sequelae (mainly sensorineural hearing loss) following infection of the mother during pregnancy [Mussi-Pinhata, 2009; Fowler, 2006].

Congenital CMV infection results from *in utero* transmission from the mother to her fetus, either because she undergoes a primary (i.e. first) CMV infection during pregnancy or because she experiences a recurrent CMV infection, defined as either viral re-activation or viral re-infection with a different strain of CMV [Hyde, 2010]. Recent studies have shown that maternal re-infection by new strains of CMV is a major source of congenital infection in seropositive population [Yamamoto, 2010; Ross, 2006].

The overall prevalence of cCMV infection in industrialized countries, regardless of the serostatus of the mother, is estimated to be 0.6-0.7% [Dollard, 2007]. From these, it is estimated that 12.7% of the CMV infected children present CMV-specific symptoms (such as mental retardation, deafness) at birth. From the remaining 87.3% of children without symptoms at birth an additional 13.5% may develop permanent sequelae (such as primarily deafness) [Dollard, 2007].

Upon primary infection of the mother during pregnancy (between 1.38% and 3.85% per year [Colugnati, 2007]), the transmission rate of the virus to the fetus is approximately 30% in contrast to seropositive women in whom vertical transmission has been estimated to be 1%. In countries or populations where high seropositivity rates are observed actually more cCMV infected babies will be born from secondary infections (re-infection/ re-activation) than from primary infections.

As pre-existing maternal immunity to CMV provide some protection against vertical transmission of the virus [Nyholm, 2010], primary maternal infection may place the child at higher risk of permanent and serious disabilities [Hyde, 2010]. It is generally believed that primary infections cause more severe disease than re-infections or re-activations [Fowler, 2003]. However, several investigators have also reported severe cCMV disease in babies born from seropositive mothers resulting in lifelong disabilities, mainly leading

to hearing loss [Boppana, 1999; Ross, 2006; Nyholm, 2010; Yamamoto, 2011; Mussi-i-Pinhata, 2009].

Some attempts have been made to evaluate the relative contribution of re-activation of latent virus or re-infection with a different CMV strain to intrauterine transmission of the virus to the infants born to women with pre-existing immunity. An enzyme-linked immunosorbant assay (ELISA) was developed to distinguish serological responses against infection with different CMV strains [Novak, 2008; Boppana, 2001]. The principle of this assay was based on the defined heterogeneity of antibody binding epitopes of glycoprotein H (gH) and/or glycoprotein B (gB) from two laboratory strains of CMV (AD169 and Towne). The detection of new antibody reactivity to either epitope on gH or gB in serum samples of seropositive women was considered as seroconversion and infection with a new virus strain (re-infection). One limitation of this assay is that not all CMV-specific IgG-positive individuals can be identified using these antigens. Specimens from more than a third of the seropositive individuals did not contain antibodies against any of the four antigenic determinants tested. This suggests the presence of additional polymorphic epitopes on gH and gB as well as on other envelope glycoproteins of CMV, such as glycoprotein N.

Providing protection against cCMV infection in babies born to mothers with pre-existing immunity will be a major challenge for any vaccine in development. The feasibility of running vaccine trials to demonstrate vaccine efficacy in this seropositive population needs to be carefully explored.

1.2. Rationale for the study

GSK Biologicals anticipates developing a prophylactic CMV vaccine indicated for the use in women and adolescents to protect their offspring against cCMV infection and disease. This study is therefore designed:

- To estimate the incidence of CMV secondary infections (re-infections / re-activations) in adolescent females.
- To estimate the incidence of CMV primary infection in adolescent females.
- To develop assays able to distinguish and detect re-infections versus re-activations.

2. OBJECTIVES

2.1. Primary objective

- To estimate the incidence of CMV secondary infections in seropositive adolescent females.

Refer to Section 8.1 for the definition of the primary endpoint.

2.2. Secondary objective

- To estimate the incidence of CMV primary infections in seronegative adolescent females.

Refer to Section 8.1.2 for the definition of the secondary endpoint.

2.3. Tertiary objectives

- To develop assays that allow the differentiation of CMV secondary infections caused either by re-infection or by re-activation, in CMV seropositive adolescent females.
- To assess the diagnostic value of different assays to evaluate seroconversion in blood and the detection of CMV DNA in different body fluids.
- To describe the proportion of secondary infection caused either by re-infection or re-activation.
- To collect samples for immunological and biological disease-related exploratory assays.
- To explore socio-demographic or behavioral factors associated with CMV infection.
- In case of pregnancies, to document the birth prevalence of CMV congenital infections.
- To evaluate the concordance between screening testing data at local laboratories (or alternatively, central laboratories) and the GSK Biologicals or designated laboratory testing data.

Refer to 8.1.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

Time point							
	Year 1						
Month	M0	M2	M4	M6	M8	M10	M12
Site Visits	V1		V2		V3		V4
Sample Collection Visits		SCV1		SCV2		SCV3	
	Year 2						
Month		M14	M16	M18	M20	M22	M24
Site Visits			V5		V6		V7
Sample Collection Visits		SCV4		SCV5		SCV6	
	Year 3						
Month		M26	M28	M30	M32	M34	M36
Site Visits			V8		V9		V10
Sample Collection Visits		SCV7		SCV8		SCV9	
Samples:							
Blood	✔		✔		✔		✔
Saliva	✔	✔	✔	✔	✔	✔	✔
Urine	✔	✔	✔	✔	✔	✔	✔

In case of pregnancy resulting in live birth: Urine and or saliva from the newborn (please refer to Table 3) will be collected (if possible) as per standard of care in the center where the subject has delivered or through a home visiting nurse or alternatively, the subject may be asked to come back to the study center within 10 days of delivery.

- **Type of design:** Prospective, multi-center, multi-country study.
- **Study Design**:
  - 10 scheduled **Site Visits** approximately every 4 months.
  - 9 **Sample Collection Visits**, approximately at mid-term between 2 Site Visits (i.e. approximately 2 months after a Site Visit) where samples will be collected either through self-collection by the subjects, by a home-visiting nurse or through a new visit at the study center.
- **Study population:** Adolescent females aged between 10 and 17 years regardless of CMV status.
- **Number of subjects and study groups:**

  At Visit 1, subjects will be enrolled regardless of their seropositivity status. Subjects will be allocated to the CMV seropositive (S+) or the CMV seronegative (S-) groups based on the local laboratory results (alternatively, a GSK designated and validated central laboratory can perform the analyses). Enrolment will be terminated when approximately 240 CMV seropositive females have been enrolled.

  - CMV Seropositive Group (S+): Subjects defined as CMV seropositive based on the local laboratory results (or alternatively based on central laboratory results) obtained from the sample collected at Visit 1.
  - CMV Seronegative Group (S-): Subjects defined as CMV seronegative based on the local laboratory results (or alternatively based on central laboratory results) obtained from the sample collected at Visit 1.

  For statistical analysis, the serostatus will be based on the laboratory results obtained at the GSK Biologicals or designated laboratory.

- **Controls**: Uncontrolled.
- **Type of study:** Self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).
- **Duration of the study**:
  - Participant: Approximately 36 months.
  - Newborn (in case of pregnancy): Approximately 10 days.

  Epoch 001: Prospective data collection starting at Visit 1 (Month 0) and ending at Visit 10 (Month 36).

Table 1 Study groups foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)
S+	+/- 240 seropositive subjects	10 – 17 years
S-	Estimated approximate number of seronegative subjects(1): 160	10 – 17 years

(1) As subjects will be enrolled consecutively without previous CMV screening with a target number of 240 seropositive subjects, the number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries.

- **Time points and biological samples**
  - Blood: approximately every 4 months;
  - Saliva and urine: approximately every 2 months;
  - Urine and /or saliva of newborn (in case of pregnancy): within 10 days post-delivery.

3.1. Discussion of study design

The study sites will be selected in multiple countries, including countries where the CMV prevalence is assumed to be high in order to enroll primarily CMV seropositive adolescent females to allow the evaluation of CMV secondary infections. Since enrolment will proceed without previous CMV screening, some of the enrolled subjects will be seronegative. The enrolled seronegative subjects will allow the estimation of the incidence of CMV primary infections in the adolescent population.

To increase the probability to capture all CMV infections (primary and secondary infections), regular sample collection time-points have been arranged:

- **Site Visits**: Subjects will be asked to perform a Site Visit approximately every 4 months for 3 years (10 Site Visits in total). At Site Visits, blood, urine and saliva will be collected.
- **Sample Collection Visits**: Subjects will be contacted to perform urine and saliva sample collections at home either by self-collection or by a home visiting nurse. Alternatively subjects may be asked to come back to the study center where the investigator or designate will collect the samples. These Sample

Collection Visits will occur approximately every 4 months for 3 years between 2 Site Visits (9 Sample Collection Visits in total).

4. STUDY POPULATION

4.1. Number of subjects/ centers

The study will be conducted world-wide in countries with a high seroprevalence. Considering a 15-20% drop-out rate over the study period it is estimated that approximately 240 seropositive subjects should be enrolled to obtain approximately 200 evaluable seropositive subjects.

Overview of the recruitment plan

Subjects will be enrolled consecutively without previous CMV screening. Enrolment will be terminated once approximately 240 seropositive subjects are included in the trial, based on the local laboratory results (or alternatively, based on the GSK designated and validated central laboratory results) obtained from the sample collected at Visit 1. The number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries. It is estimated that approximately 400 subjects will be enrolled in total.

4.2. Inclusion criteria for enrolment

All subjects must satisfy ALL the following criteria at study entry:

- A female adolescent between, and including 10 and 17 years at the time of enrolment (subjects become ineligible for enrolment on their 18th birthday) regardless of pregnancy status and contraception method used or not used.
- Subjects who the investigator believes that the subject and/or the subject's parent(s)/Legally Acceptable Representative(s) (LAR[s]) can and will comply with the requirements of the protocol (e.g.: sample collection either through self – collection or through a home-visiting nurse, return for follow-up visits).
- Written informed assent and/or consent obtained from the subject and/or the parent(s)/LAR(s) of the subject.
- Subject is likely to remain in the area and/or return for required study Site Visits and complete Sample Collection Visits.

4.3. Exclusion criteria for enrolment

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Child in care

  Please refer to the glossary of terms for the definition of child in care.
- Use or planned use of any investigational or non-registered antiviral drug or vaccine during the study period.
- Known medical history of any recurrent clinical herpes episodes requiring episodic or chronic suppressive treatment with oral or parenteral antiviral treatment such as acyclovir, famciclovir, valacyclovir or any other anti-herpes virus anti-viral during the year preceding enrolment (no laboratory testing required). Topical anti-viral are allowed.
- Subjects with history of previous vaccination against CMV.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs within 6 months prior to Visit 1 or planned administration during the study (for corticosteroids, this will mean prednisone, 0.5 mg/kg/day, or equivalent). Inhaled and topical steroids are allowed.
- Administration of immunoglobulins and/or any blood products within 3 months prior to Visit 1 or planned administration during the study.
- Any confirmed or suspected immunosuppressive or immunodeficient condition including HIV-infection, based on medical history and physical examination (no laboratory testing required).
- Any major congenital defects, serious chronic illness or organ transplantation.

The criterion that may eliminate subjects from ATP analyses can be found in Section 5.5.2.9.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study has been designed and will be conducted in accordance with the ICH Harmonized Tripartite Guideline for clinical investigation of medicinal products in the pediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country or will document that neither a favorable opinion nor an approval to conduct the study is needed.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Subject/ subject's parent(s)/LAR(s) informed consent and subject informed assent, as appropriate.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each subject and/or each subject's parent(s)/LAR(s) and subject informed assent, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the applicable ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgment, local regulations and requirements should guide the final structure and content of the local version of the ICF.

In accordance with the ICH Harmonized Tripartite Guidelines for GCP, those subjects who can only be enrolled in the study with the consent of the subject's parent(s) or legally acceptable representative (e.g. minors), should be informed about the study to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date a written informed assent. It is required that the assent be signed

by each subject, if capable, in addition to the informed consent that is to be signed by his/her parent or legal representative. It should be assessed whether an assent is required depending on the age of the study population and the local requirements.

GSK Biologicals strongly recommends that if the subject reaches the age of consent during the study they will be asked to provide consent at the next study visit (if applicable). This procedure should be applied according to local laws and regulations.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

5.2. Subject identification

Subject numbers will be assigned sequentially to subjects consenting to participate in the study, according to the range of subject numbers allocated to each study center.

5.3. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM). The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.4. Outline of study procedures

Table 2 List of study procedures (amended 19 February 2015)

Age	10-17 years							
Epoch	001							
	Year 1							
Time-point (Months)	M0	M2	M4	M6	M8	M10	M12	
Visit/Sample Collection Visit	V1	SCV1	V2	SCV2	V3	SCV3	V4	
	Year 2							
Time-point (Months)		M14	M16	M18	M20	M22	M24	
Visit/Sample Collection Visit		SCV4	V5	SCV5	V6	SCV6	V7	
	Year 3							
Time-point (Months)		M26	M28	M30	M32	M34		M36
Visit/Sample Collection Visit		SCV7	V8	SCV8	V9	SCV9		V10
Informed consent	●							
Check inclusion/exclusion criteria	●							
Record demographic data	●							
Record social and behavioral data(1)	●						●	●
Medical history	●							
CMV serostatus(2) (~3.5 to 5 mL)	●							
Blood sample(3) - for humoral immunity (10 mL) - for molecular biology (4 mL)	●		●		●		●	●
Blood sample for gene expression signature (qPCR or RNA microarray; 2.5 mL)(4)	●						●	
Urine sample(5) (~10 mL)	●	●	●	●	●	●	●	●
Saliva	●	●	●	●	●	●	●	●
Subject sample collection booklet distribution(6)	○							
Check elimination criteria	●		●		●		●	●
Record any concomitant medication/vaccination(7)	●		●		●		●	●
Reporting of SAEs related to study participation	●		●		●		●	●
Reporting of pregnancy and outcome	●		●		●		●	●
Study conclusion								●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF
(1) Social and behavioral data will be recorded at inclusion (Visit 1 [Month 0]), at Visit 4 (Month 12), Visit 7 (Month 24) and Visit 10 (Month 36).
(2) CMV serostatus, for allocation to a study group (S+ or S-), will be determined by the local laboratory as per local practices or by a GSK designated and validated central laboratory.
(3) CMV serostatus for endpoints analysis will be determined by GSK Biologicals or designated laboratory at Visit 1 (Month 0).
(4) These blood samples will be collected until approval of protocol amendment 2.
(5) Prepared from the 20 mL urine samples collected from the subject.
(6) The sample collection booklet will instruct the subjects of the study procedures to be performed at the Sample Collection Visits. The sampling can be done through self-collection, through a home-visiting nurse or the subject may be asked to come back to the study center.
(7) Only concomitant medication/vaccination related to inclusion/exclusion and elimination criteria will be recorded.

Table 3 List of study procedures for newborns/mothers (in case of pregnancy)

Age	0-10 days old
Visit/ time-point	Visit 1/Day 0-10
Informed consent(1)	●
Collect urine (5 mL) and/or saliva from newborn	●
Record CMV infection status, if available	●
Assessment of CMV disease from newborn, if applicable(2)	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
(1) For practical reasons the informed consent may be obtained during pregnancy.
(2) Symptomatic or asymptomatic.

Table 4 Intervals between study Site Visits/Sample Collection Visits

Interval	Optimal length of interval(1) (Days)	Allowed interval(2) (Days)
Visit 1 (M0) → Visit 2 (M4)	122	112 - 132
Visit 1 (M0) → Visit 3 (M8)	244	234 - 254
Visit 1 (M0) → Visit 4 (M12)	365	355 - 375
Visit 1 (M0) → Visit 5 (M16)	488	478 - 498
Visit 1 (M0) → Visit 6 (M20)	610	600 - 620
Visit 1 (M0) → Visit 7 (M24)	730	720 - 740
Visit 1 (M0) → Visit 8 (M28)	854	844 - 864
Visit 1 (M0) → Visit 9 (M32)	976	966 - 986
Visit 1 (M0) → Visit 10 (M36)	1095	1085 - 1105

(1) Whenever possible the investigator should arrange study visits within this interval.
(2) Subjects will not be eligible for inclusion in the according-to-protocol (ATP) cohort if they make the study visit outside this interval (See Section 8.4.2 for definition of the ATP cohort).

(Amended 19 February 2015)

Between study visits, Sample Collection Visits should occur at approximately 2 months interval of the Site Visits (i.e. ± mid-term between 2 Site Visits). Every effort should be made to collect samples at 61-day (2-month) intervals with approximately a ± 10-day range.

5.5. Detailed description of study procedures

5.5.1. Procedures prior to study participation

5.5.1.1. Informed consent

Before performing any other study procedure, the signed informed consent of the subject or subject's parent(s)/LAR(s) needs to be obtained. If capable, before performing any other study procedure, the signed informed assent of the subject below the age of consent should be obtained in addition to the signed informed consent by her parent(s)/LAR(s) according to local rules and regulations.

In case of pregnancy and before performing any study procedure for the newborns, the subject /subject's parent(s)/LAR(s) informed consent must be obtained. If capable, before performing any other study procedure, the signed informed assent of the subject below

the age of consent should be obtained in addition to the signed informed consent by her parent(s)/LAR(s) according to local rules and regulations.

Refer to Section 5.1 for the requirements on how to obtain informed consent and assent, as appropriate.

5.5.1.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.5.2. Procedures during study participation

5.5.2.1. Collect demographic data

Record demographic data such as age and race in the subject's eCRF.

5.5.2.2. Collect social and behavioral data

Record the social and behavioral data in the subject's eCRF, based on the questionnaire completed by the subject (a blank questionnaire is provided in the SPM).

5.5.2.3. Medical history

Perform a history-directed medical examination and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study in the eCRF. Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

5.5.2.4. CMV serostatus

Collect a blood sample (approximately 3.5 to 5 mL) at Site Visit 1 to determine the subject's serostatus by the local laboratory (or alternatively, by a GSK designated and validated central laboratory). Record results of the test in the eCRF.

5.5.2.5. Blood sampling

As specified in the List of Study Procedures in Section 5.4 (Table 2), blood samples will be collected at each Site Visit (approximately 14 to 16.5 mL). Refer to the module on Biospecimen Management in the SPM for general handling and storage condition of blood samples.

- A volume of approximately 10 mL of whole blood should be drawn from all subjects for analysis of humoral immune response ***at each Site Visit. This blood sample might also be used for exploratory testing.***
- A volume of approximately 4 mL of whole blood should be drawn from all subjects for molecular biology tests ***at each Site Visit. This blood sample might also be used for exploratory testing.***

- A volume of approximately 2.5 mL of whole blood should be drawn from all subjects for ribonucleic acid (RNA) assays using qPCR or microarray at Site Visits 1 (Month 0) ***and*** 4 (Month 12). ***This blood sample might also be used for exploratory testing.***

(Amended 19 February 2015)

5.5.2.6. Urine sampling

A volume of approximately 10 mL of urine will be collected from all subjects at each Site Visit by the investigator or designate.

A volume of approximately 10 mL of urine will also be collected at each Sample Collection Visit either by the subject herself or a home-visiting nurse. Alternatively, the subject can be asked to come back to the study center and the sample will be collected by the investigator or designate.

If samples are self-collected by the subject, the investigator or designated is responsible for instructing the subjects on how to take the sample appropriately.

Refer to the module on Biospecimen Management in the SPM and the sample collection booklet for the general handling of urine samples.

5.5.2.7. Saliva sampling

Saliva will be collected from all subjects using a specific device at each Site Visit by the investigator or designate.

Saliva will also be collected at each Sample Collection Visit either by the subject herself or a home visiting nurse. Alternatively, the subject can be asked to come back to the study center and the sample will be collected by the investigator or designate.

If samples are self-collected by the subject, the investigator or designate is responsible for instructing the subjects on how to take the sample appropriately.

Refer to the module on Biospecimen Management in the SPM and the sample collection booklet for general handling of saliva samples.

5.5.2.8. Distribution of subject sample collection booklet

If it's foreseen that the subject herself or a home-visiting study nurse will collected urine and saliva at the Sample Collection Visit, a sample collection booklet will be given to the subject at the first Study Visit (SV1, Month 0). This sample collection booklet will detail the study procedures to be performed during Sample Collection Visit.

5.5.2.9. Check Elimination criteria

Check the following elimination criteria:

- Known medical history of any recurrent clinical herpes episodes requiring episodic or chronic suppressive treatment with oral or parenteral antiviral treatment such as acyclovir, famciclovir, valacyclovir or any other anti-herpes virus anti-viral since previous visit (no laboratory testing required). Topical anti-viral are allowed.

5.5.2.10. Record any concomitant medication/vaccination

Concomitant medication/vaccination related to inclusion/exclusion and elimination criteria (i.e. administration of immunosuppressants, other immune-modifying drugs, immunoglobulins, any blood products and/or investigational/non-registered vaccine, any antiviral drug including anti-herpes) must be recorded in the eCRF.

5.5.2.11. Recording of Serious Adverse Events related to study participation

The subject/subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should they/the subjects manifest any signs or symptoms she/they perceive as serious.

Refer to Section 6.2 for procedures for the Investigator to record serious adverse events (SAEs) that are related to study participation or GSK concomitant medication/vaccination and to Section 6.3 for guidelines on how to report these SAEs to GSK Biologicals.

5.5.2.12. Reporting of pregnancy and pregnancy outcome

If the investigator becomes aware that a subject is pregnant, the pregnancy should be reported in the eCRF of the subject.

Pregnancy will be followed-up only during the study period. If the delivery occurs during the study period, the pregnancy outcome should be recorded.

5.5.2.13. Study conclusion

The investigator will review safety data collected to ensure accuracy and completeness and will complete the Study Conclusion screen in the eCRF.

5.5.3. Procedure during study participation for the newborns (in case of pregnancy and delivery during the study period)

If the delivery occurs during the study period, the pregnancy and, if the subject and/or subject's parent(s)/LAR(s) has given her/his/their consent, information regarding CMV infection of the newborn will be documented (see Section 5.5.3) in the newborn's eCRF.

5.5.3.1. Urine/saliva collection

A urine (approximately 5 mL) and/or saliva sample will be collected from newborns for virology analysis. Samples will be collected (if possible):

- As per standard of care in the center where the subject has delivered.
- OR through a home visiting nurse within 10 days of delivery.
- OR the subject may be asked to come back to the study center within 10 days of delivery.

Refer to the module on Biospecimen Management in the SPM for general handling of urine and/or saliva samples.

5.5.3.2. Record CMV infection status

The CMV infection status (positive or negative) of the newborns will be recorded in the newborn's eCRF.

5.5.3.3. Assessment of CMV disease at birth

Assessment of CMV disease (if applicable, symptomatic or asymptomatic) will be performed for newborns diagnosed as CMV-positive and record in the newborn's eCRF. The investigator or designate (such as a pediatrician) will perform the assessment him/herself during the Site Visit which should occur within 10 days of delivery or will contact the center where the subject has delivered to obtain a copy of the medical dossier.

5.6. Biological Sample handling and analysis

Please refer to the SPM and sample collection booklet for details of biospecimen management (handling, storage and shipment).

Samples will not be labeled with information that directly identifies the subjects but will be coded with the identification number for the subject (subject number).

Collected samples may be used in other assays, for test improvement or test development of analytical methods related to a study vaccine(s)/product(s) and its constituents or the disease under study to allow to achieve a more reliable measurement of the vaccine response. Under these circumstances, additional testing on the samples may be performed by GSK Biologicals outside the scope of this protocol.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject/subject's parent(s)/LAR(s).

Any human pharmacogenetic testing will require specific consent from the individual subjects/subject's parent(s)/LAR(s) and the ethics committee approval. Any HIV testing will also require specific consent and ethics committee approval.

Refer also to the Investigator Agreement, where it is noted that the Investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for up to 15 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subjects/subject's parent(s)/LAR(s) consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.6.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 8.4 for the definition of study cohorts to be evaluated). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.6.2. Biological samples

Each sample will be collected from all subjects.

Table 5 Biological samples (amended 19 February 2015)

Sample type	Quantity	Time point	Priority rank
Blood for humoral immunity determination	Approximately 10 mL	Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36	1
Blood for molecular biology	Approximately 4 mL	Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36	1
Blood for gene signature expression (RNA transcript)	Approximately 2.5 mL	Months 0, ***and*** 12*	2
Urine	Approximately 10 mL	Months 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36	1
Saliva	NA	Months 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36	1

**** Blood samples will be collected until approval of protocol amendment 2.***

5.6.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study and to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Table 6 Humoral Immunity (Antibody determination) (amended 19 February 2015)

System	Component	Method	Kit / Manufacturer	Unit	Cut-off	Number of subject	Laboratory (3)
Serum(1)	Anti-CMV tegument protein IgG	ELISA	Biotest like assay	EU/mL	TBD	All subjects	TBD
Serum(1)	Anti-gB IgG	ELISA	In house	EU/mL	54	Subset(2)	TBD
Serum(1)	Anti-CMV tegument protein avidity index	ELISA	Biotest like assay	NA	NA	Subset(2)	TBD
Serum(1)	Anti-gB IgG avidity index	ELISA	In house	NA	NA	Subset(2)	TBD
Serum(1)	Anti-CMV IgM	ELISA	Diasorin or equivalent	NA	NA	Subset(2)	TBD
Serum	Anti-CMV neutralizing antibodies (multiple cell lines) and /or other virus strains	Seroneutralisation	In house	ED_{50}	TBD	Subset(2)	TBD
Serum(1)	Antibody recognition profile	Peptide microarray	TBD	NA	NA	Subset(2)	TBD

Note: Most of the read-outs described in this table might be done if deemed necessary.

(1) Coating Ag will be defined based on available commercial kits or in house developed assays (such as whole virus proteins or tegument proteins or others to be defined).

(2) Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.

(3) Refer to APPENDIX B for the laboratory addresses.

TBD = To be determined, gB = glycoprotein B; Ig = immunoglobulin, ELISA = Enzyme-linked immunosorbant assay, EU = ELISA unit, NA = not applicable; ED_{50} = effective dose 50, Ag = antigen.

The laboratories that will perform antibody determination are not yet identified and will be defined as soon as available.

Table 7 Molecular Biology (amended 19 February 2015)

System	Test	Component	Method	Unit	Number of subject	Laboratory [4]
Urine	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	All subjects[1]	DDL
Urine	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	All Newborns	BARC
Saliva	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	Subset[3]	DDL
Saliva	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	All Newborns	BARC
Plasma/ buffy coat	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	Subset[3]	DDL
TBD[2]	Multi strain typing	CMV DNA	CMV strain typing	NA	Subset[3]	TBD
Whole blood	RNA transcript	TBD	mRNA microarray or qPCR	NA	Subset[3]	TBD

Note: Most of the read-outs described in this table might be done if deemed necessary.

[1] In seronegative subjects, testing of viral load will only be done upon seroconversion.

[2] The matrix will be determined based on the results obtained with the PCR and the availability of the assay.

[3] Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.

[4] Refer to APPENDIX B for the laboratory addresses.

TBD = To be determined, mRNA = messenger Ribonucleic acid; PCR = Polymerase Chain reaction; DNA = deoxyribonucleic acid; NA = not applicable.

In case the results from a 4-month Site Visit suggests primary or secondary (re-infection/re-activation) infection, testing related to the tertiary endpoints will be done on selected samples collected during the Sample Collection Visit. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.

If the results from a 4-month Site Visit suggest no primary or secondary (re-infection/re-activation) infection, the samples collected during the Sample Collection Visit period will be stored and used if applicable (e.g. negative control for laboratory validation).

Collected samples will be used for purposes related to the quality assurance of data generated within the scope of this protocol, such as for maintenance of assays described in this protocol and comparison between analytical methods and/or laboratories.

The laboratories that will perform antibody recognition profile, multi strain typing and RNA transcript assays are not yet identified and will be defined as soon as available.

5.6.4. Biological samples evaluation

5.6.4.1. Read-outs

Table 8 Read-outs (amended 19 February 2015)

Sampling time point		Subset Name	No. subjects	Component
Type of contact and time point	**Sampling time point**			
Site visits: (V1 to V10)	Months 0, 4, 8, 12, 16, 20, 24, 28, 32, and 36	All subjects	All	Anti-CMV tegument protein IgG (serum)
		Subset(2)	TBD	Anti-gB IgG (serum)
		All subjects(1)	TBD	CMV DNA (urine)
		Subset(2)	TBD	Anti-CMV tegument protein IgG antibody avidity index
				Anti-gB IgG antibody avidity index
		Subset(2)	TBD	CMV DNA (saliva, blood)
		Subset(2)	TBD	Anti-CMV IgM (serum)
		Subset(2)	TBD	Anti-CMV neutralizing antibodies (multiple cell lines and/or other viral strains)
		Subset(2)	TBD	Antibody recognition profile
		Subset(2)	TBD	Multi strain typing
Site visit (V1 ***and*** V4***)	Months 0 ***and*** 12***	Subset(2)	TBD	RNA transcript
Sample Collection Visits (SCV 1 to SCV 9)	Month 2, 6, 10, 14, 18, 22, 26, 30, 34(3)	Subset(2)	TBD	CMV DNA (urine + saliva)
	Upon delivery	Newborn	TBD	CMV DNA urine and/or saliva

(1) In seronegative subjects, testing of viral load will only be done upon seroconversion.
(2) Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.
(3) Testing will be done on selected samples only if the samples at the following 4-month Site Visit time-point is positive. If the 4-month sample is negative for CMV infection/re-infection, the samples self-collected during the inter-visit period will be stored and be used if applicable. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.
**** Blood sample will be collected until approval of protocol amendment 2.***
DNA = deoxyribonucleic acid; RNA = Ribonucleic acid
IgG = immunoglobulin G; IgM = immunoglobulin M; gB = glycoprotein B; TBD = To be determined.

6. SERIOUS ADVERSE EVENTS

Only SAEs related to study participation will be collected and reported during the entire study period.

The investigator or site staff is/are responsible during the study for the detection and documentation of events meeting the criteria and definition of a SAE as provided in this protocol.

Each subject/subject's parent(s)/LAR(s) will be instructed to contact the investigator immediately should they/the subject manifest any signs or symptoms they perceive as serious.

6.1. Safety definitions

6.1.1. Definition of an adverse event

An adverse event (AE) is any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure.

For an AE to be reportable, there is a minimum set of criteria (as recommended by the Council for International Organizations of Medical Sciences (CIOMS) and ICH) to be considered:

- an identifiable reporter
- an identifiable patient,
- suspect medicinal product (immunization history)
- detailed description of the event
- suspected causal relationship (event is explicitely labelled as "related" by the reporter)

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of a concurrent medication (overdose per se should not be reported as an AE/SAE).

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Example of events to be recorded in the medical history section of the CRF/eCRF:

- Pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e. prior to enrolment in an observational study).

6.1.2. Definition of a serious adverse event

A SAE is any AE that:

a. Results in death,

b. Is life-threatening,

   NB: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalization or prolongation of existing hospitalization,

   NB: In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting.

   Complications that occur during hospitalization are also considered adverse events. If a complication prolongs hospitalization or fulfills any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the adverse event should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an SAE.

d. Results in disability/incapacity, OR

   NB: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza like illness, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect in the offspring of a study subject.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

6.1.3. Clinical laboratory parameters and other abnormal assessments qualifying as serious adverse events

Abnormal laboratory findings (e.g. clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as SAEs if they meet the definition of an SAE, as defined in Section 6.1.2.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as SAEs.

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

6.2. Detecting and recording serious adverse events

6.2.1. Time period for detecting and recording serious adverse events

In order to fulfill international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) will be collected and recorded from the time the subject consents to participate in the study until she is discharged.

6.2.2. Evaluation of serious adverse events

6.2.2.1. Active questioning to detect serious adverse events

Each subject/subject's parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs and symptoms she perceives/they perceive as serious.

All SAEs either observed by the investigator or his/her staff or reported by the subject/subject's parent(s)/LAR(s) spontaneously or in response to a direct question will be evaluated by the investigator. The nature of each event, data and time (where appropriate) of onset, outcome, and relationship to the study procedures should be established.

When an SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an SAE on the eCRF or SAE Report screens, as applicable. It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK Biologicals instead of the appropriate completed SAE pages on the SAE screens in the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the SAE and not the individual signs/symptoms.

6.2.2.2. Assessment of intensity

The investigator will assess the maximum intensity that occurred over the duration of the event for all SAEs reported during the study. The assessment will be based on the investigator's clinical judgement.

The intensity of each SAE recorded in the eCRF or SAE Report screens, as applicable, should be assigned to one of the following categories:

1 (mild)	=	An SAE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An SAE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An SAE which prevents normal, everyday activities In adults/adolescents, such an SAE would, for example, prevent attendance at work/school and would necessitate the administration of corrective therapy.

6.2.2.3. Assessment of causality

The investigator should assess the causality of each SAE. The investigator will use clinical judgment to determine the relationship of SAEs to study procedures. Alternative causes, such as natural history of the underlying diseases, concomitant therapy and other risk factors will be considered and investigated.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE information accordingly.

If an event meets the criteria to be determined 'serious' (refer to Section 6.1.2), it will be examined by the investigator to the extent to enable determination of all contributing factors applicable to each SAE.

Possible contributing factors include:

- Medical history.
- Concomitant medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Other cause (specify).

6.2.2.4. Assessment of outcomes

Outcome of any SAE reported during the entire study will be assessed as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal.

6.3. Reporting of serious adverse events

6.3.1. Prompt reporting of serious adverse events related to a study procedure to GSK Biologicals

SAEs that occur in the time period defined in Section 6.2.1 will be reported promptly to GSK within the timeframes described in Table 9 once the investigator determines that the event meets the protocol definition of a SAE.

Table 9 Timeframes for submitting SAEs

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to a study procedure	24 hours(1)	SAE screen	24 hours(1)	SAE screen

(1) Timeframe allowed after receipt or awareness of the information.

6.3.2. Contact information for reporting serious adverse events and other events to GSK Biologicals

Please see the Sponsor Information Sheet for contact details.

Back-up Study Contact for Reporting SAEs
GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD 24/24 hour and 7/7 day availability

6.3.3. Completion and transmission of SAE reports related to a study procedure to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the SAE screens of the eCRF WITHIN 24 HOURS. The SAE screens will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the SAE screens should still be completed within 24 hours. Once additional information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

6.3.3.1. Back-up system in case the electronic SAE reporting system does not work

If the electronic SAE reporting system does not work, the investigator (or designate) must complete, then date and sign a SAE Report Form and fax it to the GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic SAE reporting system is not working and NOT if the system is slow. As soon as the electronic SAE reporting system is working again, the investigator (or designate) must complete the SAE screens in the eCRF within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

6.3.3.2. Updating of SAE information after freezing of the subject's eCRF

When additional information is received on a SAE after freezing of the subject's eCRF, new or updated information is to be recorded on a SAE Report Form, with all changes signed and dated by the investigator. The updated form should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) WITHIN 24 HOURS of receipt of the follow-up information.

6.4. Follow-up of serious adverse events

After the initial SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition to GSK Biologicals.

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

Investigators will follow-up subjects with SAEs until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such abnormalities noted for any subject must be made available to the Site Monitor.

GSK Biologicals may request that the investigator performs or arranges for the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period,

GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

7. SUBJECT COMPLETION AND WITHDRAWAL

7.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

7.2. Subject withdrawal

Subjects who are withdrawn because of SAE must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn as result of a SAE until resolution of the event (see Section 6.3).

From an analysis perspective, a 'withdrawal' from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject herself, by the subject's parent(s) or LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE.
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event.
- Moved from the study area.
- Lost to follow-up.
- Death.
- Other (specify).

8. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

8.1. Endpoints

8.1.1. Primary endpoints

- Occurrence of CMV secondary infections determined in all seropositive subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*

8.1.2. Secondary endpoints

- Occurrence of CMV primary infection determined in all seronegative subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*

8.1.3. Tertiary endpoints (amended 19 February 2015)

- Further characterization of primary infection in initially seronegative subjects during the 4-month Site Visits until study conclusion (testing done upon seroconversion) ***might be done***:
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
- Further characterization of primary and /or secondary CMV infections in a subset of subjects on selected samples of the Sample Collection Visits***** ***might be done***.
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR).*

** In case of a positive sample at a 4-month Site Visit time point, testing-**might** be done on* selected *samples collected during the Sample Collection Visit.* The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits ***might be done***:
  - *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody concentration in serum (ELISA).*
  - *Anti-gB IgG antibody avidity index in serum (ELISA).*
  - *Anti-CMV IgM antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva and blood (qPCR).*
  - *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*

- Development of assays that will allow differentiating re-infection from re-activation from primary infection in a subset of subjects ***might be done***.
  - *Characterization of CMV strains by genotyping/sequencing.*
  - *Exploring the CMV strain specific antibody profile using peptide microarrays.*
- Assessment of the expression of host genes* using techniques such as qPCR or mRNA microarray in a subset of subjects ***might be performed***.

  * excluding genes related to hereditary characteristics of the subject.
- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection ***might be performed***.
- Occurrence of congenital CMV infection in newborns of subjects who become pregnant during the study ***might be evaluated:***
  - *Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes)* ***might be tested****.*

8.2. Sample size consideration

The sample size for this study is not based on formal statistical considerations. The-target is to recruit approximately 200 evaluable seropositive adolescent females. Considering a conservative incidence rate of CMV infection of 2% per year, it is expected that approximately 12 seropositive subjects will be re-infected during the study period.

A target number of approximately 200 evaluable seropositive adolescent females will be analyzed. Considering a 15-20% drop out rate during the 3-year study period, approximately 240 seropositive subjects will be enrolled.

Some laboratory assays will be performed on a subset. The subset will be defined based on the outcome of the primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.

8.3. Classification of cases (amended 19 February 2015)

- A **primary infection** is defined as the first infection with CMV in subjects who were seronegative at enrolment and will be evaluated by:
  - Appearance of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  - Appearance of anti-gB IgG antibodies in serum

  WITH or WITHOUT

  - Presence of CMV DNA in urine.

- CMV **re-infection*** with a different CMV strain in subjects who were seropositive at the time of enrolment will be evaluated by:

  – Appearance or increase of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  – Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  – Appearance or increase of CMV DNA in urine

  AND ***(depending on assay availability)*** in the presence of

  – Change in polymorphism in the antibody recognition profile in follow-up serum samples

  AND/OR

  – Genotyping data characteristic of a re-infection

- CMV **re-activation*** of latent CMV in subjects who were seropositive at the time of enrolment will be evaluated by:

  – Appearance or increase of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  – Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  – Appearance or increase of CMV DNA in urine

  AND ***(depending on assay availability)*** in absence of

  – Change in polymorphism in the antibody recognition profile in follow-up serum samples

  AND/OR

  – Genotyping data characteristic of a re-infection

* Depending on the future scientific knowledge and the data generated in this study multiple case definition might be explored.

8.4. Study cohorts to be evaluated

8.4.1. Total cohort

The **Total cohort** will include all subjects enrolled in the study.

8.4.2. According-*to*-*p*rotocol cohort (amended 19 February 2015)

The **according-to-protocol (ATP) *c*ohort** will include all subjects who meet all inclusion criteria and no exclusion criteria for the study, who do not have any elimination criteria during the study (Section 5.5.2.9) and who comply with the study procedures.

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

8.5. Derived and transformed data

Immunogenicity

The cut-off value is defined by the laboratory before the analysis and is described in Table 6.

- A seronegative subject is a subject whose titer is below the cut-off value.
- A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.

For seronegative subjects, seroconversion is defined as the appearance of antibodies (i.e. titer greater than or equal to the cut-off value).

The geometric mean titers (GMTs) calculations will be performed by taking the anti-log of the mean of log titer transformations. Antibody titers below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.

Virology

For seronegative subjects, CMV DNAeamia is defined as the detection of CMV DNA (i.e. number of CMV DNA copies/mL is greater than or equal to the cut-off value).

8.6. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

8.6.1. Sequence of analyses (amended 19 February 2015)

- The final analyses on all endpoints will be done when all data are available for Visit 10 (Month 36), and provided in a full study report.
- All analyses based on tertiary endpoints not available at the time of the writing of the full report, will be provided in annex reports.

All analyses will be performed on clean data.

8.7. Statistical methods

Statistical analyses will be described in detail in a separate document, the statistical analysis plan (SAP).

The primary analysis will be performed on the ATP cohort(s). Analysis on the Total ***c***ohort may be performed to complement the primary analysis. **(Amended 19 February 2015)**

8.7.1. Analysis of demographics/baseline characteristics

Demographic (e.g. age at study entry in years, race) and baseline characteristics will be summarized using descriptive statistics.

- Frequency tables will be generated for categorical variable such as race.
- Mean, median, standard error will be provided for continuous data such as age.

8.7.2. Analysis of primary objective

Descriptive statistics will be provided for each primary endpoint at all available time-points and will include (not exhaustive) the following tabulations (with 95% confidence interval [CI]):

- The percentage of CMV seropositive subjects with appearance or increase of anti-CMV tegument protein IgG antibodies in serum.
- The percentage of CMV seropositive subjects with appearance or increase of anti-gB IgG antibodies in serum when applicable.
- The percentage of CMV seropositive subjects with appearance or increase of CMV DNA in urine.

8.7.3. Analysis of secondary objective

Descriptive statistics will be provided for each secondary endpoint at each timepoint for which a sample is available time-points and will include (not exhaustive) the following tabulations (with 95% CI):

- The percentage of CMV seronegative subjects with appearance of anti-CMV tegument protein IgG antibodies in serum.
- The percentage of CMV seronegative subjects with appearance of anti-gB IgG antibodies in serum when applicable.

8.7.4. Tertiary objectives (amended 19 February 2015)

Based on the results of primary and secondary endpoints, following statistical analyses on tertiary endpoints might be done:

Descriptive statistics will be provided for the number of CMV DNA copies in urine at each timepoint for which a sample is available time-points and the percentage of CMV seronegative subjects with presence of CMV DNA in urine will be tabulated (with 95% CI).

For all results of assays performed for the further characterization of primary and/or secondary CMV infections, descriptive statistics will be provided for each assay at each timepoint for which a sample is available time-points.

Analysis performed for the evaluation of the impact of demographic, social or behavioral factors upon CMV infection will be described in a separate document (SAP).

Analysis of results following genotyping/sequencing, mRNA microarray and peptide microarrays will also be described in a separate document (SAP).

The number and percentage of CMV infected newborns will be tabulated and descriptive statistics will be provided for the number of CMV DNA copies.

9. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled.

9.1. Remote Data Entry instructions

Remote Data Entry (RDE), a validated computer application, will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designate. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the clinical study report is complete and approved by all parties.

9.2. Monitoring by GSK Biologicals

Monitoring visits by a GSK Site Monitor are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with GCP and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), verifying that the site staff and facilities continue to be adequate to conduct the study).

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a RDE review and a Source Document Verification (SDV). By SDV we understand verifying CRF/ RDE entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the RDE. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the RDE will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For RDE, the monitor will mark completed and approved screens at each visit

In accordance with applicable regulations, GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the CRF entries will serve as the source document.

GSK will monitor the study to verify that, amongst others, the:

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

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

9.3. Archiving of data at study sites

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the

investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by ICH GCP any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to, the following: archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

9.4. Audits

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

9.5. Posting of information on public registers

Study information from this protocol will be posted on public registers (e.g. GSK Clinical Study Register, clinicaltrials.gov) before enrolment of subjects begins as applicable.

9.6. Ownership, confidentiality and publication

9.6.1. Ownership

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) are the sole property of GSK.

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of GSK, and are hereby assigned to GSK.

If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between GSK and the study site, that contract's ownership provisions shall apply rather than this statement.

9.6.2. Confidentiality

Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the

investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (i) information which becomes publicly available through no fault of the investigator or site staff; (ii) information which it is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study; (iii) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (iv) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

9.6.3. Publication

For multi-center studies, the first publication or disclosure of study results shall be a complete, joint multi-center publication or disclosure coordinated by GSK. Thereafter, any secondary publications will reference the original publication(s).

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a 'Publication'), the investigator shall provide GSK with a copy of the proposed Publication and allow GSK a period to review the proposed Publication (at least twenty-one working days, or at least fifteen working days for abstracts/posters/presentations). Proposed Publications shall not include either GSK confidential information other than the study results or personal data on any subject, such as name or initials.

At GSK's request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow GSK to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract's publication provisions shall apply rather than this statement.

9.6.4. Provision of study results to investigators and publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

10. COUNTRY SPECIFIC REQUIREMENTS

Not applicable

11. REFERENCES

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APPENDIX A LABORATORY ASSAYS

Note that some assays, the general principles of which are described below, are still in development or planned for development at GSK laboratories or delegate and that it cannot be assumed that all of them will be successfully developed due to possible technical constraints. It is also assumed that some already available assays can be slightly modified in order to improve robustness and quality of these assays.

1. Anti-CMV tegument proteins IgG ELISA (biotest like assay)

The assay is an indirect solid-phase ELISA for the detection of specific IgG antibodies using two highly purified, autologous fusion proteins, CG1 and CG2, each combining two immunodominant fragments from HCMV tegument (pp150; CG1:UL32, amino acids (aa) 495-691 and 862-1048; CG2:UL32, aa 695-854) and the delayed-early DNA binding protein (pp52; UL44, aa 297-433). If the sample contains specific antibodies, these bind to the recombinant antigens in the microtiter wells. Non-specific antibodies are removed by washing steps. The antibody-antigen complex is detected by peroxidase-labeled anti-human IgG antibodies. The presence of bound antibodies is demonstrated by adding the chromogen-substrate solution.

2. Anti-gB IgG ELISA

Anti-HCMV gB antibody concentrations are measured by ELISA using recombinant gB as coating antigen on 96-well microplates. After washing, the wells are blocked with ***a*** saturating reagent. Two dilutions of sera, in duplicate, as well as controls are incubated onto the coated wells to allow the specific antibodies, present in the sample, to react with gB. Non-specific antibodies are removed by washing steps. The antibody-antigen complex is detected by peroxidase-labeled anti-human IgG antibodies. The presence of bound antibodies is demonstrated by adding the chromogen-substrate solution.

3. Anti-CMV IgM ELISA

The method used for qualitative determination of specific IgM antibodies to cytomegalovirus in human serum is an immunoenzymatic assay based on the antibody capture ELISA technique. The removable polystyrene wells of the 96 well microplates are precoated with IgG mouse monoclonal to human IgM. During the first step of the immunological reaction, diluted serum samples and controls (negative, positive, cut-off control and diluent) are incubated into the pre-coated plate. The plates are washed to remove non-specific reactants, and a mix of enzyme-labeled detection antibody (IgG to CMV conjugated to horseradish peroxidase) and CMV antigen is added. The presence of specific IgM to CMV allows the tracer to bind to the solid phase through the presence of CMV antigen. After a washing step to remove excess of conjugate, the chromogen-enzyme substrate is added to measure the enzyme activity. After incubation at room temperature away from direct light the enzymatic reaction is stopped by the addition of Stop Reagent. The intensity of the resultant color change is measured by a spectrophotometer. The optical density (OD) is proportional to the concentration of the anti-CMV IgM present in the sample. The IgM concentration in the cut-off control is calibrated in order to provide a cut-off, unknown samples with an OD above the cut-off OD + 10% cut-off OD are positive, those with an OD below the cut-off OD – 10% cut-

off OD are negative. In the greyzone around the cut-off OD (cut-off OD +/- 10% cut-off OD) samples are retested.

4. Anti-CMV tegument protein IgG avidity index

This assay will be performed using an elution step with a chaotropic agent to remove low-avidity antibodies bound to CG1 and CG2 fusion proteins. Briefly, each diluted serum is first added to wells of plates coated with CG1 and CG2 fusion proteins. After incubation, the unbound antibodies are removed by washing, and a chaotropic agent is then added in the coated wells to dissociate low-avidity antibodies. The microplate is then washed, and horseradish peroxidase (HRP)-conjugated anti-human IgG antibodies is added. After incubation, unbound antibodies are removed by washing and the chromogen-substrate solution is added to reveal the enzyme activity. The color reaction is stopped by addition of a stop reagent and the resulting color is measured spectrophotometrically. An avidity index is then calculated.

5. Anti-gB IgG avidity index

This assay will be performed according to [De Souza, 2003] using an elution step with urea to remove low-avidity antibodies from gB antigen. Briefly, each diluted serum is first added to wells of plates coated with gB antigen. After incubation, the unbound antibodies are removed by washing, and 8M urea in phosphate buffered saline is then added in the coated wells to dissociate low-avidity antibodies. The microplate is then washed, and HRP-conjugated anti-human IgG antibodies are added. After incubation, unbound antibodies are removed by washing and the chromogen-substrate solution is added to reveal the enzyme activity. The color reaction is stopped by addition of a stop reagent and the resulting yellow color is measured spectrophotometrically.

The avidity index is calculated as the mean absorbance of reactions in which the immune complexes are exposed to urea divided by the mean absorbance of reactions in which the immune complexes are not exposed to urea, expressed as a percentage.

6. Anti-CMV neutralisation assay

The microneutralization test uses MRC-5 human lung embryo fibroblast cells as the infection target. The different serum sample dilutions are mixed with a fixed amount of virus at different dilutions and the serum-virus mixture is incubated at 37°C with MRC-5 cells. Infected cells are then fixed with acetone. The degree to which neutralizing antibodies decrease CMV infection of cells in the assay is determined by counting the number of nuclei expressing the CMV 72 kDa IE1 protein. Nuclear IE1 is detected using highly sensitive immunoperoxidase staining. The stained nuclei are counted. The neutralization titer is expressed as the reciprocal of the serum dilution that reduced by 50% the number of infected cells as compared to a control without serum.

Additional microneutralization assays using other cell types such as epithelial, trophoblast cells as the infection targets and other virus strains may also be performed.

7. Antibody recognition profile

The exact methodology to perform antibody recognition profiling is not yet decided but the type of assay may be derived from classical high or low density peptide arrays. The peptide –microarray is a technology allowing the simultaneous measurement of serum reactivity (IgG, IgM, IgA or IgE) to hundreds or thousands of peptides in a single biological sample. Briefly, solid support spotted/coated with CMV peptides are incubated with serum dilutions. Slides are then washed to remove unbound antibodies and incubated with labeled anti-human Immunoglobulin. After a washing step, antibody recognition profile against different peptides is revealed by fluorescence or other means following the technology platform selected.

8. Detection of CMV-DNA (qPCR)

Quantitative PCR provides a rapid and sensitive method for the diagnosis of CMV infection. The CMV detection test used by GSK Biologicals is designed to detect the pp65 gene.

Real-time PCR technology allows the determination of the copy number of sequence-specific nucleic acids target molecules. The amplification of the target sequence is detected using the 5' nuclease assay based on the Taqman chemistry. In those PCR experiments, the formation of a PCR product is monitored in real-time during amplification by means of a fluorogenic probe that binds specifically to the amplified product. The reporter fluorophore is at the 5' end of the Taqman probe and the quencher is at the 3' end. As long as the probe is intact, no fluorescence is observed from the fluorophore. During the polymerization step, the Taq DNA polymerase displaces the Taqman probe by 3-4 nucleotides, and the 5' nuclease activity of the DNA polymerase separates the fluorophore from the quencher. The fractional cycle number at which the generated fluorescence passes a fixed threshold above the baseline defines a "Threshold Cycle" (Ct value). A standard curve can be generated from the log of the starting copy number of DNA references against the measured Ct value.

In the context of this study, the qPCR technology is used to detect CMV DNA in different matrix (blood, urine and saliva) following adapted purification methodology.

9. CMV strain typing

In addition to direct detection of CMV through PCR, the company is seeking to develop, evaluate and validate next generation sequencing based assay and/or other technologies aiming to characterize CMV genomic diversity that allow discriminating re-infection or re-activation to primary infection.

10. mRNA microarray /qPCR

Transcriptional profiling in the blood consists of measuring RNA abundance in circulating nucleated cells. Changes in transcript abundance can result from exposure to host or pathogen-derived immunogenic factors and/or changes in relative cellular composition. Techniques such as mRNA microarrays of qPCR may be used to evaluate these changes, if available.

APPENDIX B CLINICAL LABORATORIES

Table 10 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Global Vaccine Clinical Laboratory, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Global Vaccine Clinical Laboratory, North America-Laval	Biospecimen Reception - Clinical Serology 525 Cartier blvd West - Laval - Quebec - Canada - H7V 3S8
GSK Biologicals Global Vaccine Clinical Laboratory, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 11 Outsourced laboratories

Laboratory	Address
DDL Diagnostic Laboratory B.V.	Fonteijnenburghlaan 7 Voorburg Netherland
BARC USA Inc	5, Delaware Drive Lake Success NY 11042-1114 USA
CERBA European labs	7/11 Rue de l'Equerre, Parc d'activités 'les Béthunes' 95310 Saint Ouent l'Aumone France

APPENDIX C AMENDMENTS TO THE PROTOCOL

GlaxoSmithKline Biologicals Clinical Research & Development **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Amendment number:	Amendment 1
Amendment date:	31-JUL-2013
Co-ordinating author:	PPD, GSK Biologicals'

Rationale/background for changes:

Initially, the study was set-up, as a preparation for large phase III trials, to assess with good precision the rates of CMV re-infections and reactivations in CMV seropositive adolescents from different countries or areas with presumably high CMV seroprevalence. Additionally, the study aimed at collecting human biological samples to support the development of new laboratory assays that would be both specific and sensitive for the detection of these biological events but also that would facilitate the conduct of large efficacy trials using non/less-invasive sampling methods.

Since the initial discussions on the CMV-014 study, the company has progressed on its reflection of a clinical program needed for the development of a CMV vaccine. Over time it appeared clearer that the success of a phase III trial necessitated a better understanding of the complex virology and immune parameters in CMV seropositive subjects. Consequently, the Company has decided to switch the CMV program from a classical development strategy to a more experimental, clinical research status.

The CMV-014 EXPLO study has been redesigned to better align with this new scope and will serve the design of future vaccine research studies in terms of scientific questions to be addressed, like assays to be used and developed. The protocol amendment of the study CMV-014 EXPLO is reflecting these strategic changes : the incidence of CMV primary and secondary infection in adolescent females will be evaluated using less stringent assumptions on CMV re-infection and reactivation attack rates . Assays primarily intended to evaluate the feasibility of sample collection and testing in the context of large phase III trials were removed from the present study that will focus more on the development of assays used to better decipher the complex virological and immune parameters associated to CMV infection.

In consequence:

- The planned number of seropositive subjects was decreased from 475 to 240, with an estimated total enrolled subjects of 400 instead of 800 which is still considered sufficient to evaluate the incidence of CMV primary and secondary infection.
- The objectives and endpoints of the study were revised:
  - The anti-tegument IgG ELISA testing in saliva was removed, as it was more aimed to address operational questions in anticipation of Phase III clinical trials rather than to address the actual evaluation of the incidence of CMV primary and secondary infections. Indeed, in case of good correlation between the anti-tegument IgG ELISA in serum and in saliva, the anti-tegument IgG ELISA in saliva was intended to be used as a more practical diagnostic readout to evaluate CMV infection in large Phase III trials.
  - Similarly, the qPCR on dry saliva and dry urine samples were removed. They were initially included to assess the feasibility to collect some samples using more practical sampling devices in large Phase III trials.
  - The analysis of anti-gB IgG ELISA in serum was moved to tertiary endpoint because the anti-tegument IgG ELISA in serum (which should provide equivalent results) is already tested as per primary and secondary endpoints. The anti-gB ELISA might only be used in a subset of subjects to confirm results generated with the anti-tegument IgG ELISA and/or to confirm the case definition.
  - The occurrence of congenital CMV infections of newborns from subjects who became pregnant during the study will still be evaluated. Nevertheless deep genotypic characterization activities will be reserved to CMV strains infecting the mothers and not the babies.
  - The evaluation of T-cell proliferation (mentioned as a tertiary endpoint) was removed as it was finally decided not to collect PBMC in this study.

Note that all samples will still be used, except the dry urine and dry saliva samples which are prepared from the collected urine and saliva samples collected from the subjects (no change of procedure for the subjects). Some dry urine samples were already prepared for the enrolled subjects and will not be used. No dry saliva samples were already prepared. Dry saliva and dry urine samples will not be prepared from upcoming samples. The section on the laboratory assays was updated accordingly.

- The list of laboratories to perform the tests was updated, as the collaboration with ImmuneHealth will be stopped before the first testing of CMV-014 samples. A service provider was selected for the development of the qPCR in saliva, urine and blood and for the associated clinical testing. Some other laboratories are still to be identified.
- Appendix A was slightly reworded to give a more general description of the principle of the assays. Indeed, some of these assays are still in development or are susceptible to improvement for robustness and quality purposes.
- Some minor updated were made
- The list of contributing authors was updated following changes in the team members/ function names.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Co-ordinating author:

PPD (Keyrus Biopharma contractor for GSK Biologicals), PPD, Scientific Writers

Contributing authors:

- PPD, PPD, Clinical Development Manager
- PPD, PPD, ***Study Delivery Lead*** ~~Global Study Manager~~
- PPD, PPD, Project Statistician
- PPD, PPD, PPD ***(Keyrus Biopharma contractor for GSK Biologicals)***, ***Study Data Manager*** ~~Clinical Data Coordinator~~
- PPD, PPD, Safety Representative (Valesta contractor for GSK Biologicals)

Synopsis

Rationale for the study design

- ~~To evaluate the operational feasibility for future studies.~~

Tertiary objectives

- ~~To evaluate the degree of matching of the detected CMV strain(s) in newborn(s) and the mother.~~

Synopsis Table 1: study groups foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)
S+	+/- ~~475~~ ***240*** seropositive subjects	10 – 17 years
S-	Estimated approximate number of seronegative subjects[1]: ~~325~~***160***	10 – 17 years

[1] As subjects will be enrolled consecutively without previous CMV screening with a target number of ~~475~~ ***240*** seropositive subjects, the number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries.

Discussion of study design

The study sites will be selected in ***multiple countries, including countries where the*** ~~high~~ CMV prevalence ***is assumed to be high*** in order to enroll primarily CMV seropositive adolescent females to allow the evaluation of CMV secondary infections. Since enrolment will proceed without previous CMV screening, some of the enrolled subjects will be seronegative. The enrolled seronegative subjects will allow the estimation of the incidence of CMV primary infections in the adolescent population.

To increase the probability to capture all CMV infections (primary and secondary infections), regular sample collection time-points have been arranged:

- **Site Visits**: Subjects will be asked to perform a Site Visit approximately every 4 months for 3 years (10 Site Visits in total). At Site Visits, blood, urine, ~~dry urine~~, ***and*** saliva, ~~and dry saliva~~ will be collected.

Number of subjects

Subjects will be enrolled consecutively without previous CMV screening. Enrolment will be terminated once approximately ~~475~~ ***240*** seropositive subjects are included in the trial. The number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries. It is estimated that approximately ~~800~~ ***400*** subjects will be enrolled in total.

Primary endpoint

- Occurrence of CMV secondary infections determined in all seropositive subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*
  - ~~*Anti-gB IgG antibody concentration in serum (ELISA).*~~
  - ~~*Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~
  - ~~*Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*~~

Secondary endpoints

- Occurrence of CMV primary infection determined in all seronegative subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*

~~– *Anti-gB IgG antibody concentration in serum (ELISA).*~~

~~– *Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~

Tertiary endpoints

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on ~~all available~~ ***selected*** samples of the Sample Collection Visits*.

  ~~– *Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~

  – *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*

  – *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR).*

* In case of a positive sample at a 4-month Site Visit time point, testing will be done on ~~all available~~ ***selected*** samples collected during the Sample Collection Visit***. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits***.*

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits:

  – *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*

  – *Anti-gB IgG antibody avidity index in serum (ELISA).*

  – ***Anti-gB IgG antibody concentration in serum (ELISA).***

  – *Anti-CMV IgM antibody concentration in serum (ELISA).*

  – *Number of CMV DNA copies (pp65 or other genes) in saliva, ~~dry saliva, dry urine~~, blood (qPCR).*

  – *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*

- Development of assays that will allow differentiating re-infection from re-activation from primary infection ***in a subset of subjects***

  – *Characterization of CMV strains by genotyping/sequencing.*

  – *Exploring the CMV strain specific antibody profile using peptide microarrays.*

- Assessment of the expression of host genes[*] ~~related to immunity~~ ***using techniques such as qPCR or*** mRNA microarray ***in a subset of subjects***, ~~and/or other functions such as T-cell proliferation.~~

  [*] ***excluding genes related to hereditary characteristics of the subject.***

- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection.

- Occurrence ~~and characterization~~ of congenital CMV infection in newborns of subjects who become pregnant during the study:

  – *Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes).*

~~– *Characterization of strains by genotyping/sequencing in newborns from re-infected mothers.*~~

1.2. Rationale for the study

~~To evaluate the operational feasibility for future studies.~~

2.3. Tertiary objectives

- ~~To evaluate the degree of matching of the detected CMV strain(s) in newborn(s) and the mother~~.

3. Study design overview

Time point							
	Year 1						
Month	**M0**	**M2**	**M4**	**M6**	**M8**	**M10**	**M12**
Site Visits	**V1**		**V2**		**V3**		**V4**
Sample Collection Visits		**SCV1**		**SCV2**		**SCV3**	
	Year 2						
Month		**M14**	**M16**	**M18**	**M20**	**M22**	**M24**
Site Visits			**V5**		**V6**		**V7**
Sample Collection Visits		**SCV4**		**SCV5**		**SCV6**	
	Year 3						
Month		**M26**	**M28**	**M30**	**M32**	**M34**	**M36**
Site Visits			**V8**		**V9**		**V10**
Sample Collection Visits		**SCV7**		**SCV8**		**SCV9**	
Samples:							
Blood	✔		✔		✔		✔
Saliva	✔	✔	✔	✔	✔	✔	✔
~~Dry Saliva~~	~~✔~~		~~✔~~		~~✔~~		~~✔~~
Urine	✔	✔	✔	✔	✔	✔	✔
~~Dry Urine~~	~~✔~~		~~✔~~		~~✔~~		~~✔~~

In case of pregnancy resulting in live birth: Urine and or saliva from the newborn (please refer to Table 3) will be collected (if possible) as per standard of care in the center where the subject has delivered or through a home visiting nurse or alternatively, the subject may be asked to come back to the study center within 10 days of delivery.

- **Number of subjects and study groups:**

  At Visit 1, subjects will be enrolled regardless of their seropositivity status. Subjects will be allocated to the CMV seropositive (S+) or the CMV seronegative (S-) groups based on the local laboratory results (alternatively, a GSK designated and validated central laboratory can perform the analyses). Enrolment will be terminated when approximately ~~475~~ ***240*** CMV seropositive females have been enrolled.

Table 1: Study groups foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)
S+	+/- ~~240~~ ***240*** seropositive subjects	10 – 17 years
S-	Estimated approximate number of seronegative subjects(1): ~~160~~ ***160***	10 – 17 years

(1) As subjects will be enrolled consecutively without previous CMV screening with a target number of ~~475~~ ***240*** seropositive subjects, the number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries.

- **Time points and biological samples**
  - Blood, ~~dry saliva and dry urine~~: approximately every 4 months;
  - Saliva and urine: approximately every 2 months;
  - Urine and /or saliva of newborn (in case of pregnancy): within 10 days post-delivery.

3.1. Discussion of the study design

The study sites will be selected in ***multiple countries, including countries where the*** ~~high~~ CMV prevalence ***is assumed to be high*** in order to enroll primarily CMV seropositive adolescent females to allow the evaluation of CMV secondary infections. Since enrolment will proceed without previous CMV screening, some of the enrolled subjects will be seronegative. The enrolled seronegative subjects will allow the estimation of the incidence of CMV primary infections in the adolescent population.

- **Site Visits**: Subjects will be asked to perform a Site Visit approximately every 4 months for 3 years (10 Site Visits in total). At Site Visits, blood, urine, ~~dry urine~~, ***and*** saliva ~~and dry saliva~~ will be collected.

4.1. Number of subjects/ centers

The study will be conducted world-wide in countries with a high seroprevalence. Considering a 15-20% drop-out rate over the study period it is estimated that approximately ~~475~~ ***240*** seropositive subjects should be enrolled to obtain approximately ~~400~~***200*** evaluable seropositive subjects.

Overview of the recruitment plan

Subjects will be enrolled consecutively without previous CMV screening. Enrolment will be terminated once approximately ~~475~~ ***240*** seropositive subjects are included in the trial, based on the local laboratory results (or alternatively, based on the GSK designated and validated central laboratory results) obtained from the sample collected at Visit 1. The number of seronegative subjects enrolled will depend on the seroprevalence of the participating countries. It is estimated that approximately ~~800~~ ***400*** subjects will be enrolled in total.

5.4. Outline of study procedures

Table 2: List of study procedures

Age	10-17 years							
Epoch	001							
	Year 1							
Time-point (Months)	M0	M2	M4	M6	M8	M10	M12	
Visit/Sample Collection Visit	V1	SCV1	V2	SCV2	V3	SCV3	V4	
	Year 2							
Time-point (Months)		M14	M16	M18	M20	M22	M24	
Visit/Sample Collection Visit		SCV4	V5	SCV5	V6	SCV6	V7	
	Year 3							
Time-point (Months)		M26	M28	M30	M32	M34		M36
Visit/Sample Collection Visit		SCV7	V8	SCV8	V9	SCV9		V10
CMV serostatus[2] (~~2~~ ***~3.5 to 5*** mL)	●							
Blood sample for gene expression signature (***qPCR or*** RNA microarray; 2.5 mL)	●						●	●
~~Dry Urine~~	~~●~~		~~●~~		~~●~~		~~●~~	~~●~~
Saliva	●	●	●	●	●	●	●	●
~~Dry saliva~~	~~●~~		~~●~~		~~●~~		~~●~~	~~●~~

5.5.2.4. CMV serostatus

Collect a blood sample (approximately ~~2~~ ***3.5 to 5*** mL) at Site Visit 1 to determine the subject's serostatus by the local laboratory (or alternatively, by a GSK designated and validated central laboratory). Record results of the test in the eCRF.

5.5.2.5. Blood sampling

- A volume of approximately 2.5 mL of whole blood should be drawn from all subjects for ribonucleic acid (RNA) assays using ***qPCR or*** microarray and exploratory testing at Site Visits 1 (Month 0), 4 (Month 12), 7 (Month 24), and 10 (Month 36).

5.5.2.6. Urine ~~and dry urine~~ sampling

~~Urine and dry urine will be collected for virology analysis and exploratory testing at each time-point specified in the list of study procedure (Table 2).~~

Refer ~~to the module on Biospecimen Management in the SPM for general handling of dry urine samples and~~ to the module on Biospecimen Management in the SPM and the sample collection booklet for the general handling of urine samples.

5.5.2.7. Saliva ~~and dry saliva~~ sampling

~~Saliva and dry saliva will be collected for virology analysis and exploratory testing at each time-point specified in the list of study procedure (Table 2).~~

Saliva will be collected from all subjects using a specific device at each Site Visit by the investigator or designate. ~~A dry saliva sample will also be collected at each visit site.~~

Refer ~~to the module on Biospecimen Management in the SPM for general handling of dry saliva samples and~~ to the module on Biospecimen Management in the SPM and the sample collection booklet for general handling of saliva samples.

5.5.3. Procedure during study participation for the newborns (in case of pregnancy and delivery during the study period)

If the delivery occurs during the study period, the pregnancy and, if the subject and/or subject's parent(s)/LAR(s) has given her/his/their consent, information regarding CMV infection of the newborn will be documented (see Section 5.5.3) in the ~~subject~~ ***newborn***'s eCRF.

5.5.3.2. Record CMV infection status

~~If available~~, The CMV infection status (positive or negative) of the newborns will be recorded in the ***newborn***'s eCRF ~~of his/her mother~~.

5.5.3.3. Assessment of CMV disease at birth

Assessment of CMV disease (if applicable, symptomatic or asymptomatic) will be performed for newborns diagnosed as CMV-positive and record in the ***newborn***'s eCRF ~~of his/her mother~~. The investigator or designate (such as a pediatrician) will perform the assessment him/herself during the Site Visit which should occur within 10 days of delivery or will contact the center where the subject has delivered to obtain a copy of the medical dossier.

5.6.2. Biological samples

Table 5: Biological samples

Sample type	Quantity	Time point	Priority rank
~~Dry urine~~	~~NA~~	~~Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36~~	~~2~~
~~Dry saliva~~	~~NA~~	~~Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36~~	~~2~~

5.6.3. Laboratory assays

Table 6: Humoral immunity (antibody determination

System	Component	Method	Kit / Manufacturer	Unit	Cut-off	Number of subject	Laboratory *(3)*
Serum(1)	Anti-CMV tegument protein IgG	ELISA	Biotest like assay	EU/mL	TBD	All subjects	~~Immune Health~~ ***TBD***
Serum(1)	Anti-gB IgG	ELISA	In house	EU/mL	54	~~All subjects~~ ***Subset(2)***	~~Immune Health~~ ***TBD***
~~Saliva(1)~~	~~Anti-CMV tegument proteins IgG~~	~~ELISA~~	~~TBD~~	~~TBD~~	~~TBD~~	~~All subjects~~	~~Immune Health~~
Serum(1)	Anti-CMV tegument protein avidity index	ELISA	Biotest like assay	NA	NA	Subset(2)	~~Immune Health~~ ***TBD***
Serum(1)	Anti-gB IgG avidity index	ELISA	In house	NA	NA	Subset(2)	~~Immune Health~~ ***TBD***
Serum(1)	Anti-CMV IgM	ELISA	***Diasorin or equivalent***	***NA***	***NA***	Subset(2)	~~Immune Health~~ ***TBD***
Serum	Anti-CMV neutralizing antibodies (multiple cell lines) and /or other virus strains	Seroneutralisation	In house	ED_{50}	***TBD***	Subset(2)	~~Immune Health~~ ***TBD***
Serum(1)	Antibody recognition profile	Peptide microarray	TBD	NA	NA	Subset(2)	TBD

(1) Coating Ag will be defined based on available commercial kits or in house developed assays (such as whole virus proteins or tegument proteins or others to be defined).
(2) Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.
(3) Refer to APPENDIX B for the laboratory addresses.
TBD = To be determined, gB = glycoprotein B; Ig = immunoglobulin, ELISA = Enzyme-linked immunosorbant assay, EU = ELISA unit, NA = not applicable; ED_{50} = effective dose 50, Ag = antigen.

The laboratories that will perform antibody determination are not yet identified and will be defined as soon as available.

Table 7: Molecular Biology

System	Test	Component	Method	Unit	Number of subject	Laboratory *(4)*
Urine	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	All subjects(1) ~~Newborns~~	~~TBD~~ ***DDL***
Urine	***Viral load detection***	***CMV DNA***	***quantitative PCR***	***Copy/mL***	***All Newborns***	***BARC***
Saliva	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	Subset(3) + ~~Newborns~~	~~TBD~~ ***DDL***
Saliva	***Viral load detection***	***CMV DNA***	***quantitative PCR***	***Copy/mL***	***All Newborns***	***BARC***
~~Dry Urine~~	~~Viral load detection~~	~~CMV DNA~~	~~quantitative PCR~~	~~Copy/mL~~	~~Subset(3)~~	~~TBD~~
~~Dry Saliva~~	~~Viral load detection~~	~~CMV DNA~~	~~quantitative PCR~~	~~Copy/mL~~	~~Subset(3)~~	~~TBD~~
Plasma/ buffy coat	Viral load detection	CMV DNA	quantitative PCR	Copy/mL	Subset(3)	~~TBD~~ ***DDL***
TBD(2)	Multi strain typing	CMV DNA	CMV strain typing	NA	Subset(3) ~~+ Newborns (urine only)~~	TBD
Whole blood	RNA transcript	TBD	mRNA microarray ***or qPCR***	NA	Subset(3)	TBD

(1) In seronegative subjects, testing of viral load will only be done upon seroconversion.
(2) The matrix will be determined based on the results obtained with the PCR ***and the availability of the assay***.
(3) Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.
(4) Refer to APPENDIX B for the laboratory addresses.
(TBD = To be determined, mRNA = messenger Ribonucleic acid; PCR = Polymerase Chain reaction; DNA = deoxyribonucleic acid; NA = not applicable.

In case the results from a 4-month Site Visit suggests primary or secondary (re-infection/re-activation) infection, testing ***related to the tertiary endpoints*** will be done on ~~all available~~ ***selected*** samples collected during the Sample Collection Visit. ***The selection of samples to be tested will be based on the qPCR results obtained following the Site visits***.

The laboratories that will perform antibody recognition profile, multi strain typing and RNA transcript assays are not yet identified and will be defined ~~before study start~~ ***as soon as available***.

5.6.4.1. Read-outs

Table 8: Read-outs

Sampling time point		Subset Name	No. subjects	Component
Type of contact and time point	**Sampling time point**			
Site visits: (V1 to V10)	Months 0, 4, 8, 12, 16, 20, 24, 28, 32, and 36	All subjects	All	Anti-CMV tegument protein IgG (serum ~~+ saliva~~)
		Subset[2]	***TBD***	Anti-gB IgG (serum)
		All subjects[1]	~~All~~ ***TBD***	CMV DNA (urine)
		Subset[2]	TBD	Anti-CMV tegument protein IgG antibody avidity index
				Anti-gB IgG antibody avidity index
		Subset[2]	TBD	CMV DNA (saliva, ~~dry saliva, dry urine~~, blood)
		Subset[2]	TBD	Anti-CMV IgM (serum)
		Subset[2]	TBD	Anti-CMV neutralizing antibodies (multiple cell lines ***and/or other viral strains***)
		Subset[2]	TBD	Antibody recognition profile
		Subset[2]	TBD	Multi strain typing
Site visit (V1, V4, V7, V10)	Months 0, 12, 24, and 36	Subset[2]	TBD	RNA transcript
Sample Collection Visits (SCV 1 to SCV 9)	Month 2, 6, 10, 14, 18, 22, 26, 30, 34[3]	~~Subset[2]~~	~~TBD~~	~~Anti-CMV tegument proteins IgG (saliva)~~
		Subset[2]	TBD	CMV DNA (urine + saliva)
	Upon delivery	Newborn[4]	TBD	CMV DNA urine and/or saliva ~~Multi-strain typing~~

[1] In seronegative subjects, testing of viral load will only be done upon seroconversion.
[2] Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.
[3] Testing will be done ***on selected samples*** only if the samples at the following 4-month Site Visit time-point is positive. If the 4-month sample is negative for CMV infection/re-infection, the samples self-collected during the inter-visit period will be stored and be used if applicable. ***The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.***
[4] ~~Not done in seronegative mothers who remain seronegative during pregnancy~~
DNA = deoxyribonucleic acid; RNA = Ribonucleic acid
IgG = immunoglobulin G; IgM = immunoglobulin M; gB = glycoprotein B; TBD = To be determined.

8.1.1. Primary endpoint

- Occurrence of CMV secondary infections determined in all seropositive subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*
  - ~~*Anti-gB IgG antibody concentration in serum (ELISA).*~~
  - ~~*Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*

8.1.2. Secondary endpoints

- Occurrence of CMV primary infection determined in all seronegative subjects on samples collected during the 4-month Site Visits until study conclusion:
  - *Anti-CMV tegument protein IgG antibody concentration in serum (ELISA).*
  - ~~*Anti-gB IgG antibody concentration in serum (ELISA).*~~
  - ~~*Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~

Tertiary endpoints

- Further characterization of primary infection in initially seronegative subjects during the 4-month Site Visits until study conclusion (testing done upon seroconversion):
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
- Further characterization of primary and /or secondary CMV infections in a subset of subjects on ~~all available~~ ***selected*** samples of the Sample Collection Visits*.
  - ~~*Anti-CMV tegument protein IgG antibody concentration in saliva (ELISA).*~~
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR*

** In case of a positive sample at a 4-month Site Visit time point, testing will be done on ~~all available~~ **selected** samples collected during the Sample Collection Visit.* ***The selection of samples to be tested will be based on the qPCR results obtained following the Site visits****.*

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits:
  - *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*
  - ***Anti-gB IgG antibody concentration in serum (ELISA).***
  - *Anti-gB IgG antibody avidity index in serum (ELISA).*
  - *Anti-CMV IgM antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva, ~~dry saliva, dry urine,~~ blood (qPCR).*
  - *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*
- Development of assays that will allow differentiating re-infection from re-activation from primary infection ***in a subset of subjects***
  - *Characterization of CMV strains by genotyping/sequencing.*
  - *Exploring the CMV strain specific antibody profile using peptide microarrays.*

- ***Assessment of the expression of host genes* ~~related to immunity~~ using techniques such as qPCR or*** mRNA microarray ***in a subset of subjects***, ~~and/or other functions such as T-cell proliferation~~.

  * ***excluding genes related to hereditary characteristics of the subject.***

- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection.
- Occurrence ~~and characterization~~ of congenital CMV infection in newborns of subjects who become pregnant during the study:
  - *Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes).*
  - ~~*Characterization of strains by genotyping/sequencing in newborns from re-infected mothers.*~~

8.2. Sample size consideration

The sample size for this study is not based on formal statistical considerations. ~~A~~ ***The*** target ***is to recruit*** ~~number of~~ approximately ~~400~~ ***200*** evaluable seropositive adolescent females ~~is considered sufficient to obtain a reasonable number of CMV infections during the study~~. Considering a conservative incidence rate of CMV infection of ~~1~~ ***2***% per year, it is expected that approximately 12 seropositive subjects will be re-infected during the study period.

A target number of approximately ~~400~~ ***200*** evaluable seropositive adolescent females will be analyzed. Considering a 15-20% drop out rate during the 3-year study period, approximately ~~475~~ ***240*** seropositive subjects will be enrolled.

8.3. Classification of cases

- A **primary infection** is defined as the first infection with CMV in subjects who were seronegative at enrolment and will be evaluated by:
  - Appearance of anti-CMV tegument protein IgG antibodies in serum ~~and/or saliva~~

  AND/OR

  - Appearance of anti-gB IgG antibodies in serum

  WITH or WITHOUT

  - Presence of CMV DNA in urine.

- CMV **Re-infection*** with a different CMV strain in subjects who were seropositive at the time of enrolment will be evaluated by:

  – Appearance or increase of anti-CMV tegument protein IgG antibodies in serum ~~and/or saliva~~

  AND/OR

  – Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  – Appearance or increase of CMV DNA in urine

  AND in the presence of

  – Change in polymorphism in the antibody recognition profile in follow-up serum samples (assay to be further developed)

  AND/OR

  – Genotyping data characteristic of a re-infection (assay to be further developed)

- CMV **re-activation*** of latent CMV in subjects who were seropositive at the time of enrolment will be evaluated by:

  – Appearance or increase of anti-CMV tegument protein IgG antibodies in serum ~~and /or saliva~~

  AND/OR

  – Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  – Appearance or increase of CMV DNA in urine

  AND in absence of

  – Change in polymorphism in the antibody recognition profile in follow-up serum samples (assay to be further developed)

  AND/OR

  – Genotyping data characteristic of a re-infection (assay to be further developed)

* Depending on the future scientific knowledge and the data generated in this study multiple case definition might be explored.

8.7. Statistical methods

The primary analysis will be performed ~~on the Total Cohort. Analyses~~ on the ATP cohort(s). ***Analysis on the Total Cohort*** may be performed to complement the primary analysis.

8.7.2. Analysis of primary objective

Descriptive statistics will be provided for each primary endpoint at all available time-points and will include (not exhaustive) the following tabulations (with 95% confidence interval [CI]):

- The percentage of CMV seropositive subjects with appearance or increase of anti-gB IgG antibodies in serum ***when applicable***.
- ~~The percentage of CMV seropositive subjects with appearance or increase of anti-CMV tegument protein IgG antibodies in saliva.~~

8.7.3. Analysis of secondary objectives

Descriptive statistics will be provided for each secondary endpoint at ~~all~~ ***each timepoint for which a sample is*** available time-points and will include (not exhaustive) the following tabulations (with 95% CI):

- The percentage of CMV seronegative subjects with appearance of anti-CMV tegument protein IgG antibodies in serum.
- The percentage of CMV seronegative subjects with appearance of anti-gB IgG antibodies in serum ***when applicable***.
- ~~The percentage of CMV seronegative subjects with appearance of anti-CMV tegument protein IgG antibodies in saliva.~~

8.7.4. Tertiary objectives

Descriptive statistics will be provided for the number of CMV DNA copies in urine at ~~all~~ ***each timepoint for which a sample is*** available time-points and the percentage of CMV seronegative subjects with presence of CMV DNA in urine will be tabulated (with 95% CI).

For all results of assays performed for the further characterization of primary and/or secondary CMV infections, descriptive statistics will be provided for each assay at ~~all~~ ***each timepoint for which a sample is*** available time-points.

The number and percentage of CMV infected newborns will be tabulated and descriptive statistics will be provided for the number of CMV DNA copies. ~~Characterization of CMV strains in newborns will be described~~.

Appendix A: Laboratory assays

Note that some assays, the general principles of which are described below, are still in development or planned for development at GSK laboratories or delegate and that it cannot be assumed that all of them will be successfully developed due to possible technical constraints. ***It is also assumed that some already available assays can be slightly modified in order to improve robustness and quality of these assays.***

1. Anti-CMV tegument proteins IgG ELISA (biotest like assay)

The assay is an indirect solid-phase ELISA for the detection of specific IgG antibodies using two highly purified, autologous fusion proteins, CG1 and CG2, each combining two immunodominant fragments from HCMV tegument (pp150; CG1:UL32, amino acids (aa) 495-691 and 862-1048; CG2:UL32, aa 695-854) and the delayed-early DNA binding protein (pp52; UL44, aa 297-433). If the sample contains specific antibodies, these bind to the recombinant antigens in the microtiter wells. Non-specific antibodies are removed by washing steps. The antibody-antigen complex is detected by peroxidase-labeled anti-human IgG antibodies. The presence of bound antibodies is demonstrated by adding the chromogen-substrate solution ~~(TMB and H202)~~.

2. Anti-gB IgG ELISA

Anti-HCMV gB antibody concentrations are measured by ELISA using recombinant gB as coating antigen on 96-well microplates. After washing, the wells are blocked with ***a saturating reagent*** ~~bovine serum albumin~~. Two dilutions of sera, in duplicate, as well as controls are incubated onto the coated wells to allow the specific antibodies, present in the sample, to react with gB. ***Non-specific antibodies are removed by washing steps. The antibody-antigen complex is detected by peroxidase-labeled anti-human IgG antibodies. The presence of bound antibodies is demonstrated by adding the chromogen-substrate solution.***

~~Non-specific reactants are removed by washing, and peroxidase-conjugated anti-human IgG polyclonal antibodies are added to bind with anti-gB IgG antibodies. Excess conjugate is removed by washing. Enzyme substrate solution (TMB) is added, and the blue color is allowed to develop. The reaction is stopped by addition of sulphuric acid, resulting in a color change to yellow. The intensity of the color is quantified and expressed in EU/mL. The optical density (OD) is proportional to the concentration of anti gB antibodies present in the sample. Titers of each serum dilution are calculated by reference to a standard serum using the 4-parameters equation (mean OD), and the final titer of a serum is the mean of the titers falling in the proportional part of the reference curve.~~

3. Anti-CMV IgM *ELISA*

The method used for qualitative determination of specific IgM antibodies to cytomegalovirus in human serum is an immunoenzymatic assay based on the antibody capture ELISA technique. The removable polystyrene wells of the 96 well microplates are precoated with IgG mouse monoclonal to human IgM. During the first step of the immunological reaction, diluted ~~(1:101)~~ serum samples and controls (negative, positive,

cut-off control and diluent) are incubated into the pre-coated plate. The plates are washed to remove non-specific reactants, and a mix of enzyme-labeled detection antibody (IgG to CMV conjugated to horseradish peroxidase) and CMV antigen is added. The presence of specific IgM to CMV allows the tracer to bind to the solid phase through the presence of CMV antigen. After a washing step to remove excess of conjugate, the chromogen-enzyme substrate ~~solution containing 3, 3', 5, 5'-tetramethyl benzidine and hydrogen peroxide~~ is added to measure the enzyme activity. After incubation at room temperature away from direct light the enzymatic reaction is stopped by the addition of Stop Reagent ~~(sulphuric acid solution)~~. The intensity of the resultant color change is measured by a spectrophotometer ~~at a defined wavelength (450 nm) against a reference wavelength (630 nm)~~. The optical density (OD) is proportional to the concentration of the anti-CMV IgM present in the sample. The IgM concentration in the cut-off control is calibrated in order to provide a ~~clinical~~ cut-off, unknown samples with an OD above the cut-off OD + 10% cut-off OD are positive, those with an OD below the cut-off OD – 10% cut-off OD are negative. In the greyzone around the cut-off OD (cut-off OD +/- 10% cut-off OD) samples are retested.

4. Anti-CMV tegument protein IgG avidity index

This assay will be performed using an elution step with a chaotropic agent to remove low-avidity antibodies bound to CG1 and CG2 fusion proteins. Briefly, each diluted serum is first added to wells of plates coated with CG1 and CG2 fusion proteins. After incubation, the unbound antibodies are removed by washing, and a chaotropic agent is then added in the coated wells to dissociate low-avidity antibodies. The microplate is then washed, and horseradish peroxidase (HRP)-conjugated anti-human IgG antibodies is added. After incubation, unbound antibodies are removed by washing and ***the chromogen-substrate solution is*** ~~TMB and oxygen peroxide are~~ added to reveal the enzyme activity. The color reaction is stopped by addition of ***a stop reagent*** ~~sulphuric acid~~ and the resulting ~~yellow~~ color is measured spectrophotometrically. An avidity index is ***then*** calculated.

5. Anti-gB *IgG* avidity index

This assay will be performed according to [De Souza, 2003] using an elution step with urea to remove low-avidity antibodies from gB antigen. Briefly, each diluted serum is first added to wells of plates coated with gB antigen. After incubation, the unbound antibodies are removed by washing, and 8M urea in phosphate buffered saline is then added in the coated wells to dissociate low-avidity antibodies. The microplate is then washed, and HRP-conjugated anti-human IgG antibodies are added. After incubation, unbound antibodies are removed by washing and ***the chromogen-substrate solution is*** ~~TMB and oxygen peroxide are~~ added to reveal the enzyme activity. The color reaction is stopped by addition of ~~sulphuric acid~~ ***a stop reagent*** and the resulting yellow color is measured spectrophotometrically.

The avidity index is calculated as the mean absorbance of reactions in which the immune complexes are exposed to urea divided by the mean absorbance of reactions in which the immune complexes are not exposed to urea, expressed as a percentage.

6. Anti-CMV neutralisation assay

The microneutralization test uses MRC-5 human lung embryo fibroblast cells as the infection target. The different serum sample dilutions are mixed with a fixed amount of virus at different dilutions and the serum-virus mixture is incubated ~~overnight~~ at 37°C with MRC-5 cells. Infected cells are then fixed with acetone. The degree to which neutralizing antibodies decrease CMV infection of cells in the assay is determined by counting the number of nuclei expressing the CMV 72 kDa IE1 protein. Nuclear IE1 is detected using highly sensitive immunoperoxidase staining. The stained nuclei are counted ~~using a manual reading method~~. The neutralization titer is expressed as the reciprocal of the serum dilution that reduced by 50**%** the number of infected cells as compared to a control without serum. ~~Other target cells and clinical virus isolates obtained from the urine of pregnant women may be used to explore more deeply the neutralization function of the anti-CMV antibodies and its possible association with the virus genotype as determined by DNA sequencing.~~

Additional microneutralization assays using other cell types such as epithelial, trophoblast cells as the infection targets and other virus strains may also be performed.

7. Antibody recognition profile

The exact methodology to perform antibody recognition profiling is not yet decided but the type of assay may be derived from classical high or low density peptide arrays. ~~Whatever the technology used~~, The peptide –microarray is a technology allowing the simultaneous measurement of serum reactivity (IgG, IgM, IgA or IgE) to hundreds or thousands of peptides in a single biological sample. Briefly, solid support spotted/coated with ***CMV*** peptides ~~selected based on their capacity to discriminate the antibody profiles induced either by the vaccine or by reinfection with a different CMV strains,~~ are incubated with serum dilutions. Slides are then washed to remove unbound antibodies and incubated with labeled anti-human Immunoglobulin. After a washing step, antibody recognition profile against different peptides is revealed by fluorescence or other means following the technology platform selected.

8. Detection of CMV-DNA (qPCR)

Quantitative PCR provides a rapid and sensitive method for the diagnosis of CMV infection. The CMV detection test used by GSK Biologicals is designed to detect the pp65 gene.

Real-time PCR technology allows the determination of the copy number of sequence-specific nucleic acids target molecules. The amplification of the target sequence is detected using the 5’ nuclease assay based on the Taqman chemistry. In those PCR experiments, the formation of a PCR product is monitored in real-time during amplification by means of a fluorogenic probe that binds specifically to the amplified product. The reporter fluorophore is at the 5’ end of the Taqman probe and the quencher is at the 3’ end. As long as the probe is intact, no fluorescence is observed from the fluorophore. During the polymerization step, the Taq DNA polymerase displaces the Taqman probe by 3-4 nucleotides, and the 5’ nuclease activity of the DNA polymerase separates the fluorophore from the quencher. The fractional cycle number at which the

generated fluorescence passes a fixed threshold above the baseline defines a "Threshold Cycle" (Ct value). A standard curve can be generated from the log of the starting copy number of DNA references against the measured Ct value.

In the context of this study, the qPCR technology is used to detect CMV DNA in different matrix (blood, urine, ~~dry urine~~, ***and*** saliva, ~~and dry saliva~~) following adapted purification methodology.

9. CMV strain typing

In addition to direct detection of CMV through PCR, the company is seeking to develop, evaluate and validate next generation sequencing based assay ***and/or other technologies aiming to characterize CMV genomic diversity that*** ~~to~~ allow discriminating re-infection or re-activation to primary infection.

10. mRNA microarray /*qPCR*

Transcriptional profiling in the blood consists of measuring RNA abundance in circulating nucleated cells. Changes in transcript abundance can result from exposure to host or pathogen-derived immunogenic factors and/or changes in relative cellular composition. ***Techniques such as mRNA microarrays of qPCR may be used to evaluate these changes, if available.***

~~The study of transcriptomics may be explored to examine the expression level of mRNAs related to immune genes by using high-throughput techniques based on DNA microarray technology.~~

Appendix B: Clinical laboratories

Table 11: Outsourced laboratories

Laboratory	Address
~~Immune Health~~	~~Rue A. Bolland~~ ~~B-6041 Charleroi~~ ~~Belgium~~
DDL Diagnostic Laboratory B.V.	***Fonteijnenburghlaan 7*** ***Voorburg*** ***Netherland***
BARC USA Inc	***5, Delaware Drive*** ***Lake Success*** ***NY 11042-1114*** ***USA***
CERBA European labs	***7/11 Rue de l'Equerre, Parc d'activités 'les Béthunes'*** ***95310 Saint Ouent l'Aumone*** ***France***

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 2

eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Amendment number:	Amendment 2
Amendment date:	19-FEB-2015
Co-ordinating author:	PPD (XPE Pharma & Science for GSK Biologicals)

Rationale/background for changes:
Protocol amendment 2 was prepared:

- To cancel the planned interim analyses and to keep the final analysis only: The need to validate and develop some assays has led to a delay in the release of data on time. Since no vaccine is administered in the CMV-014 EXPLO study and as the interim analyses were not linked to subjects' safety or efficacy, but rather to exploring the natural history of CMV disease, it was decided to group the planned interim analysis after Year 1 and Year 2 and to have a single final analysis of the total follow-up at the end of the study.
- To clarify that the tertiary endpoints (i.e. exploratory tests) and the subcohort selected for testing will be based on the evaluations of primary and secondary endpoints.
- To stop collection of blood samples for gene expression signature (qPCR or RNA microarray) as of protocol amendment 2 approval: A Year 1 dataset will be used for analysis of gene expression in a subset of subjects.

In addition, the list of contributing authors and the Sponsor Signatory Approval page have been updated following changes in the team members/ function names. The notation of the terms "ATP cohort" and "Total cohort" were adapted to maintain consistency throughout the protocol.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Contributing authors:

- PPD, PPD, Clinical ***Research and*** Development ***Leads*** ~~Manager~~
- PPD ***, Study Delivery Manager***
- PPD, PPD, Project Statistician
- PPD, PPD, PPD ***(Business & Decision Life Sciences for GSK Biologicals),*** ~~Clinical Immunology~~ ***GVCL*** Representatives
- PPD, PPD, PPD (Keyrus Biopharma contractor for GSK Biologicals), PPD ***(TCS Consultant for GSK Biologicals),*** PPD **,** Study Data Manager
- PPD ***, Project Data Manager***
- PPD, PPD, PPD **,** Safety Representative***s*** ~~(Valesta contractor for GSK Biologicals)~~
- PPD, Epidemiologist

Sponsor Signatory Approval:

Sponsor signatory	Jeanne-Marie Devaster ***Director*** Clinical ***Research and Translational Science*** ~~Development Director~~

List of abbreviations

ATP	~~A~~***a***ccording-~~T~~***t***o-~~P~~***p***rotocol

Synopsis

Tertiary endpoints

- Further characterization of primary infection in initially seronegative subjects during the 4-month Site Visits until study conclusion (testing done upon seroconversion) ***might be done***:
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
- Further characterization of primary and /or secondary CMV infections in a subset of subjects on selected samples of the Sample Collection Visits***** ***might be done***.
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR).*

** In case of a positive sample at a 4-month Site Visit time point, testing ~~will~~ **might** be done on selected samples collected during the Sample Collection Visit. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.*

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits ***might be done***:
  - *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody concentration in serum (ELISA).*
  - *Anti-CMV IgM antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva and blood (qPCR).*
  - *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*
- Development of assays that will allow differentiating re-infection from re-activation from primary infection in a subset of subjects ***might be done***.
  - *Characterization of CMV strains by genotyping/sequencing.*
  - *Exploring the CMV strain specific antibody profile using peptide microarrays.*
- Assessment of the expression of host genes* using techniques such as qPCR or mRNA microarray in a subset of subjects ***might be performed***.

  * excluding genes related to hereditary characteristics of the subject.
- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection ***might be performed***.
- Occurrence of congenital CMV infection in newborns of subjects who become pregnant during the study ***might be evaluated***:

Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes) ***might be tested****.*

Section 5.4 Outline of study procedures

Table 2 List of study procedures

Age	10-17 years							
Epoch	001							
	Year 1							
Time-point (Months)	M0	M2	M4	M6	M8	M10	M12	
Visit/Sample Collection Visit	V1	SCV1	V2	SCV2	V3	SCV3	V4	
	Year 2							
Time-point (Months)		M14	M16	M18	M20	M22	M24	
Visit/Sample Collection Visit		SCV4	V5	SCV5	V6	SCV6	V7	
	Year 3							
Time-point (Months)		M26	M28	M30	M32	M34		M36
Visit/Sample Collection Visit		SCV7	V8	SCV8	V9	SCV9		V10
Informed consent	●							
Check inclusion/exclusion criteria	●							
Record demographic data	●							
Record social and behavioral data(1)	●						●	●
Medical history	●							
CMV serostatus(2) (~3.5 to 5 mL)	●							
Blood sample(3) - for humoral immunity (10 mL) - for molecular biology (4 mL)	●		●		●		●	●
Blood sample for gene expression signature (qPCR or RNA microarray; 2.5 mL)***(4)***	●						●	~~●~~
Urine sample ~~(4)~~ ***(5)*** (~10 mL)	●	●	●	●	●	●	●	●
Saliva	●	●	●	●	●	●	●	●
Subject sample collection booklet distribution~~(5)~~ ***(6)***	○							
Check elimination criteria	●		●		●		●	●
Record any concomitant medication/vaccination~~(6)~~ ***(7)***	●		●		●		●	●
Reporting of SAEs related to study participation	●		●		●		●	●
Reporting of pregnancy and outcome	●		●		●		●	●
Study conclusion								●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF
~~The double line border indicates that an interim analysis will be performed on clean data each year (Month 12 and Month 24).~~

(1) Social and behavioral data will be recorded at inclusion (Visit 1 [Month 0]), at Visit 4 (Month 12), Visit 7 (Month 24) and Visit 10 (Month 36).

(2) CMV serostatus, for allocation to a study group (S+ or S-), will be determined by the local laboratory as per local practices or by a GSK designated and validated central laboratory.

(3) CMV serostatus for endpoints analysis will be determined by GSK Biologicals or designated laboratory at Visit 1 (Month 0).

(4) These blood samples will be collected until approval of protocol amendment 2.

~~(4)~~ ***(5)*** Prepared from the 20 mL urine samples collected from the subject.

~~(5)~~ ***(6)*** The sample collection booklet will instruct the subjects of the study procedures to be performed at the Sample Collection Visits. The sampling can be done through self-collection, through a home-visiting nurse or the subject may be asked to come back to the study center.

~~(6)~~ ***(7)*** Only concomitant medication/vaccination related to inclusion/exclusion and elimination criteria will be recorded.

Footnote of Table 4 Interval between study Site Visits/Sample Collection Visits

(2) Subjects will not be eligible for inclusion in the according-to-protocol (ATP) cohort if they make the study visit outside this interval (See Section 8.4.2 for definition of the ATP cohort).

Section 5.5.2.5. Blood sampling

- A volume of approximately 10 mL of whole blood should be drawn from all subjects for analysis of humoral immune response ***at each Site Visit. This blood sample might also be used for exploratory testing.*** ~~and exploratory testing at each Site Visit.~~
- A volume of approximately 4 mL of whole blood should be drawn from all subjects for molecular biology tests ***at each Site Visit. This blood sample might also be used for exploratory testing.*** ~~and exploratory testing at each Site Visit~~.
- A volume of approximately 2.5 mL of whole blood should be drawn from all subjects for ribonucleic acid (RNA) assays using qPCR or microarray at Site Visits 1 (Month 0) ***and*** 4 (Month 12). ***This blood sample might also be used for exploratory testing.*** ~~and exploratory testing~~ at Site Visits 1 (Month 0)~~,~~ 4 (Month 12)~~, 7 (Month 24), and 10 (Month 36).~~

Section 5.6.2. Biological samples

Table 5 Biological samples

Sample type	Quantity	Time point	Priority rank
Blood for humoral immunity determination	Approximately 10 mL	Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36	1
Blood for molecular biology	Approximately 4 mL	Months 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36	1
Blood for gene signature expression (RNA transcript)	Approximately 2.5 mL	Months 0 ***and*** 12~~, 24* , and 36~~	2
Urine	Approximately 10 mL	Months 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36	1
Saliva	NA	Months 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36	1

**** Blood samples will be collected until approval of protocol amendment 2.***

Section 5.6.3 Laboratory assays

- **Footnote of Table 6 Humoral Immunity (Antibody determination)**

Note: Most of the read-outs described in this table might be done if deemed necessary.

- **Footnote of Table 7 Molecular Biology**

Note: Most of the read-outs described in this table might be done if deemed necessary.

Section 5.6.4.1. Read-outs

Table 8 Read-outs

Sampling time point		Subset Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Site visits: (V1 to V10)	Months 0, 4, 8, 12, 16, 20, 24, 28, 32, and 36	All subjects	All	Anti-CMV tegument protein IgG (serum)
		Subset(2)	TBD	Anti-gB IgG (serum)
		All subjects(1)	TBD	CMV DNA (urine)
		Subset(2)	TBD	Anti-CMV tegument protein IgG antibody avidity index
				Anti-gB IgG antibody avidity index
		Subset(2)	TBD	CMV DNA (saliva, blood)
		Subset(2)	TBD	Anti-CMV IgM (serum)
		Subset(2)	TBD	Anti-CMV neutralizing antibodies (multiple cell lines and/or other viral strains)
		Subset(2)	TBD	Antibody recognition profile
		Subset(2)	TBD	Multi strain typing
Site visit (V1 ***and***~~,~~ V4~~, V7, V10~~)*	Months 0 ***and*** 12*~~, 24, and 36~~	Subset(2)	TBD	RNA transcript
Sample Collection Visits (SCV 1 to SCV 9)	Month 2, 6, 10, 14, 18, 22, 26, 30, 34(3)	Subset(2)	TBD	CMV DNA (urine + saliva)
	Upon delivery	Newborn	TBD	CMV DNA urine and/or saliva

(1) In seronegative subjects, testing of viral load will only be done upon seroconversion.
(2) Subset will be defined based on the outcome of primary/secondary endpoints or the development of the assay. The subset is the minimum number of subjects that will be tested. If applicable, all subjects could be tested.
(3) Testing will be done on selected samples only if the samples at the following 4-month Site Visit time-point is positive. If the 4-month sample is negative for CMV infection/re-infection, the samples self-collected during the inter-visit period will be stored and be used if applicable. The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.
**** Blood sample will be collected until approval of protocol amendment 2.***
DNA = deoxyribonucleic acid; RNA = Ribonucleic acid
IgG = immunoglobulin G; IgM = immunoglobulin M; gB = glycoprotein B; TBD = To be determined.

Section 8.1.3 Tertiary endpoints

- Further characterization of primary infection in initially seronegative subjects during the 4-month Site Visits until study conclusion (testing done upon seroconversion) ***might be done***:
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on selected samples of the Sample Collection Visits* ***might be done***.
  - *Number of CMV DNA copies (pp65 or other genes) in urine (qPCR).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva (qPCR).*

** In case of a positive sample at a 4-month Site Visit time point, testing ~~will~~* ***might*** *be done on* selected *samples collected during the Sample Collection Visit****.*** The selection of samples to be tested will be based on the qPCR results obtained following the Site visits.

- Further characterization of primary and /or secondary CMV infections in a subset of subjects on all available samples of the 4-month Site Visits ***might be done***:
  - *Anti-CMV tegument protein IgG antibody avidity index in serum (ELISA).*
  - *Anti-gB IgG antibody concentration in serum (ELISA).*
  - *Anti-gB IgG antibody avidity index in serum (ELISA).*
  - *Anti-CMV IgM antibody concentration in serum (ELISA).*
  - *Number of CMV DNA copies (pp65 or other genes) in saliva and blood (qPCR).*
  - *Assessment of anti-CMV neutralizing antibodies on different target cells and/or other virus strains.*
- Development of assays that will allow differentiating re-infection from re-activation from primary infection in a subset of subjects ***might be done***.
  - *Characterization of CMV strains by genotyping/sequencing.*
  - *Exploring the CMV strain specific antibody profile using peptide microarrays.*
- Assessment of the expression of host genes***** using techniques such as qPCR or mRNA microarray in a subset of subjects ***might be performed***.

  * excluding genes related to hereditary characteristics of the subject.
- Evaluation of the impact of demographic, social or behavioral factors upon CMV infection ***might be performed***.
- Occurrence of congenital CMV infection in newborns of subjects who become pregnant during the study ***might be evaluated:***
  - *Evidence of CMV DNA in urine and/or saliva of newborns within 10 days of delivery by using qPCR (pp65 or other genes)* ***might be tested****.*

Section 8.3 Classification of cases

- A **primary infection** is defined as the first infection with CMV in subjects who were seronegative at enrolment and will be evaluated by:
  - Appearance of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  - Appearance of anti-gB IgG antibodies in serum

  WITH or WITHOUT

  - Presence of CMV DNA in urine.
- CMV **re-infection*** with a different CMV strain in subjects who were seropositive at the time of enrolment will be evaluated by:
  - Appearance or increase of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  - Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  - Appearance or increase of CMV DNA in urine

  AND ***(depending on assay availability)*** in the presence of

  - Change in polymorphism in the antibody recognition profile in follow-up serum samples ~~(assay to be further developed)~~

  AND/OR

  - Genotyping data characteristic of a re-infection ~~(assay to be further developed)~~
- CMV **re-activation*** of latent CMV in subjects who were seropositive at the time of enrolment will be evaluated by:
  - Appearance or increase of anti-CMV tegument protein IgG antibodies in serum

  AND/OR

  - Appearance or increase of anti-gB IgG antibodies in serum

  AND/OR

  - Appearance or increase of CMV DNA in urine

  AND ***(depending on assay availability)*** in absence of

  - Change in polymorphism in the antibody recognition profile in follow-up serum samples ~~(assay to be further developed)~~

  AND/OR

  - Genotyping data characteristic of a re-infection ~~(assay to be further developed)~~

Section 8.4.2 According-t~~T~~o-p~~P~~rotocol cohort

The **according-to-protocol (ATP) ~~C~~*c*ohort** will include all subjects who meet all inclusion criteria and no exclusion criteria for the study, who do not have any elimination criteria during the study (Section 5.5.2.9) and who comply with the study procedures ~~indicated below~~.

~~The **ATP Cohort Year 1** will include all subjects who attend the 4 Site Visits (Visit 1 to Visit 4) and have samples collected from at least 2 Sample Collection Visits during Year 1.~~

~~The **ATP Cohort Year 2** will include all subjects who attend the 3 Site Visits (Visit 5 to Visit 7) and have samples collected from at least 2 Sample Collection Visits during Year 2.~~

~~The **ATP Cohort Year 3** will include all subjects who attend the 3 Site Visits (Visit 8 to Visit 10) and have samples collected from at least 2 Sample Collection Visits during Year 3.~~

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

Section 8.6.1 Sequence of analyses

~~The analyses will be performed stepwise:~~

- ~~An interim analysis will be done every 12 months (Month 12 and Month 24). No study report will be written.~~

~~Section 8.6.2 Statistical considerations for interim analyses~~

~~In this study no treatment is given to the subjects. An interim analysis can therefore be performed every 12 months (Month 12 and Month 24) on all available results without adjustment of the sample size.~~

Section 8.7 Statistical methods

Statistical analyses will be described in detail in a separate document, the statistical analysis plan (SAP).

The primary analysis will be performed on the ATP cohort(s). Analysis on the Total ~~C~~*c*ohort may be performed to complement the primary analysis.

Section 8.7.4 Tertiary objectives

Based on the results of primary and secondary endpoints, following statistical analyses on tertiary endpoints might be done:

Descriptive statistics will be provided for the number of CMV DNA copies in urine at each timepoint for which a sample is available time-points and the percentage of CMV seronegative subjects with presence of CMV DNA in urine will be tabulated (with 95% CI).

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	115639 (CMV-014 EXPLO)
Date of protocol amendment	Protocol Amendment 2 Final: 19 February 2015
Detailed Title	A study to explore cytomegalovirus primary infection, re-activation and re-infection in an adolescent female population.
Sponsor signatory	Jeanne-Marie Devaster
(Amended 19 February 2015)	***Director*** Clinical ***Research and Translational Science***
Signature	PPD
Date	24 Feb 2015

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