

QuantiFERON-CMV (QuIC)

QuantiFERON-CMV (QuIC) Study: Utilising QuantiFERON-CMV to personalise CMV management in haemopoietic stem cell transplant patients

QuantiFERON-CMV (QuIC) Clinical Study Protocol

Study title:	QuantiFERON-CMV (QuIC) Study: Utilising QuantiFERON-CMV to personalise CMV management in haemopoietic stem cell transplant patients
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1 AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.1	23 Apr2025	Dami Collier and QuIC study coordination	Section 6 - Secondary objectives 2, 4, and 5 have been reclassified as exploratory objectives. Section 9.2 and 10.2 - Removed secondary objectives 2, 4, and 5, as they have been moved to exploratory objectives. Sections 9.3, 10.3 and 18.2.3 added - Moved to these sections the secondary objectives 2, 4, and 5, now designated as exploratory objectives and outcomes, and clarified the second objective - diagnostic accuracy of the in-house whole blood CMV-specific IFN-γ assay compared with the QuantiFERON-CMV ELISA and FluoroSpot assays. Section 12.5 - Removed reference to remote consent. Section 12.8 - Clarified patients who withdraw from the study but had samples collected and analysed at weeks 6 and 8 will still be included in the study analysis.

				Sections 13.2 and 14.2 - Added the windows for patients visits. Section 13.2- Corrected the 99 pan CMV peptide mix assay for the Whole blood IFNγ assay to determine CMV-specific T cells responses. Section 13.4 - Clarified wording. Section 14.1 - Removed height, weight and BMI and clarified the demographics to collect are sex, age and ethnicity). Clarified the disease and transplant data to collect. Removed the HLA matching to donor range. Replaced '12 months' with '52 weeks' throughout the document to ensure consistency in wording. Updated Appendix 1 to revise the 52-week follow-up visit window from 1 month to 4 weeks. Added Baseline visit demographics and disease transplant assessments. Updated the immunosuppressant medication assessment to be collected at all visits. Removed medical history, and therapy review from this assessment.
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2 SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Signature:

Date:...../...../.....

.....

Name: (please print):

.....

3 STUDY MANAGEMENT GROUP

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5 LIST OF ABBREVIATIONS

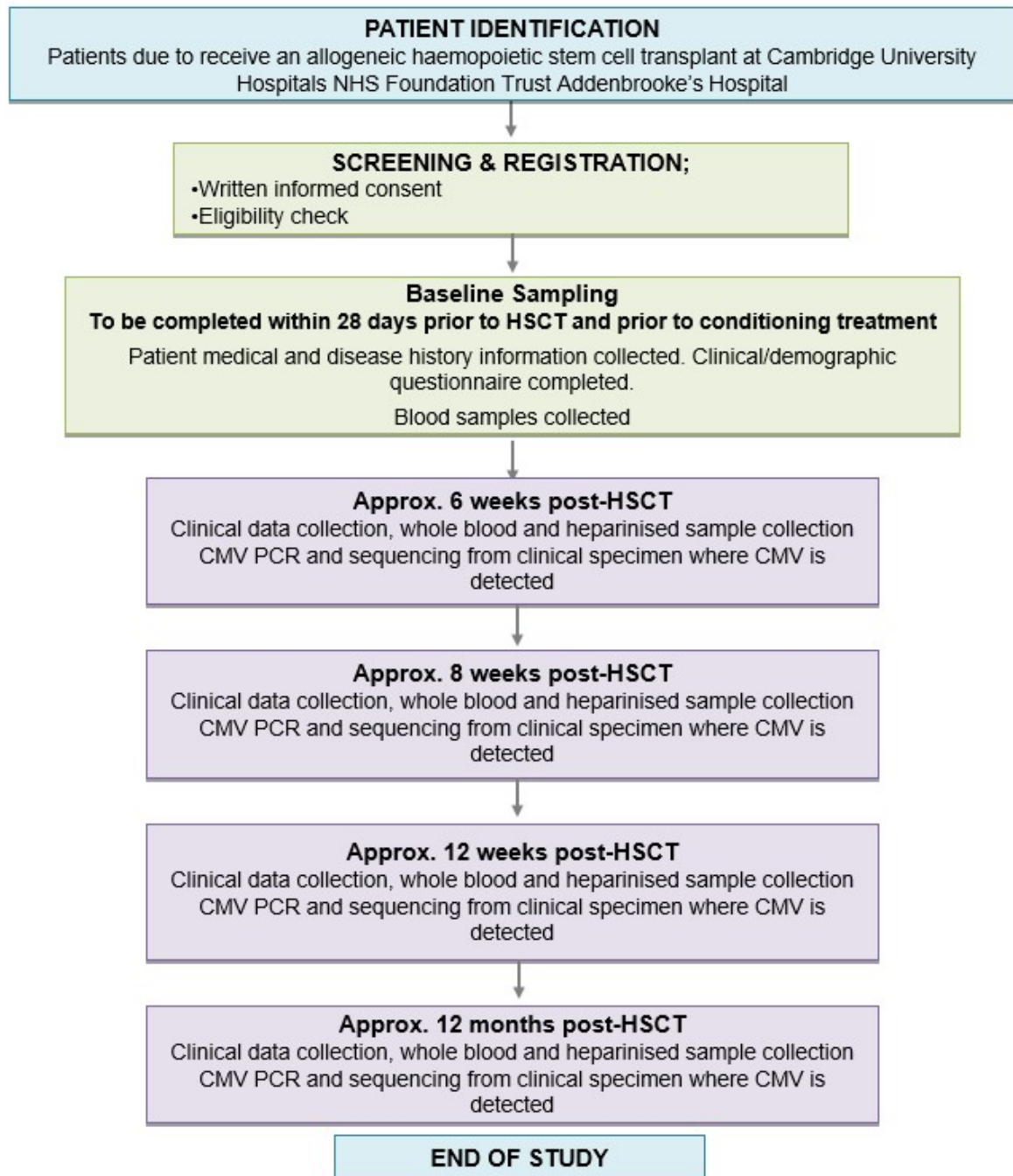
ACT	Addenbrooke's Charitable Trust
BMI	Body Mass Index
CCTU-CT	Cambridge Clinical Trials Unit – Cancer Theme
CMV	Cytomegalovirus
CUH	Cambridge University Hospitals NHS Foundation Trust
D+/R-	Donor+ / Recipient-
(e)CRF	(Electronic) Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
GP	General Practitioner
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GvHD	Graft versus Host Disease
HLA	Human Leukocyte Antigens
HRA	Health Research Authority
HSCT	Haemopoietic stem cell transplant
ID	Identification
IFN γ	Interferon-gamma
IgG	Immunoglobulin G
ISF	Investigator Site File
NHS	National Health Service
NPV	Negative Predictive Value
PBMCs	Peripheral Blood Mononuclear Cells
PIS	Patient Information Sheet
PPV	Positive Predictive Value
QF-CMV	QuantiFERON-CMV
R&D	Research and Development
REC	Research Ethics Committee
PCR	Polymerase Chain Reaction
SOPs	Standard Operating Procedures
SMG	Study Management Group
TMF	Trial Master File
UKHSA	United Kingdom Health Security Agency

6 SYNOPSIS

Study Title	QulC Study: Utilising Quant iFERON- CMV to personalise CMV management in haemopoietic stem cell transplant patients
Sponsor Name	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge
Study Design	Longitudinal cohort study
Study Patients	Allogeneic haemopoietic stem cell transplant patients
Planned Sample Size	A total of 60 evaluable patients
Follow-up duration (if applicable)	Patients will exit the study at 52 weeks following Haemopoietic stem cell transplant (HSCT)
Planned Study Period	24 months
Primary Objective	To determine, in the context of prophylaxis with Letermovir, the prevalence of stable CMV-specific T cell immune reconstitution, defined as 2 reactive QuantiFERON-CMV (QF-CMV) responses at 6 week and 8 weeks post allogeneic-HSCT measured by the QuantiFERON-CMV ELISA assay (Qiagen)
Secondary Objectives	1. To explore the association between stable CMV-specific T cell immune reconstitution and clinical outcomes (including CMV viraemia, CMV disease, CMV mutation and death) at 52 weeks. 2. Evaluate the cost savings if Letermovir is stopped after 8 weeks rather than 100 days in those with stable CMV-specific T cell immune reconstitution
Exploratory Objectives	1. Develop an in-house whole blood CMV-specific IFNγ assay that may be transferable to clinical practice 2. Determine the diagnostic accuracy of the in-house whole blood CMV-specific IFNγ assay compared with the QuantiFERON-CMV ELISA assay (Qiagen) and FluoroSpot assay 3. Identify any CMV mutations emerging in first 52 weeks following allogeneic-HSCT

7 STUDY FLOW CHART

Population: 60 evaluable patients who are due to receive allogeneic haemopoietic stem cell transplants
Setting: Cambridge University Hospitals NHS Foundation Trust



8 BACKGROUND AND RATIONALE

Human cytomegalovirus (CMV) infection is a common complication following haemopoietic stem cell transplant (HSCT) and is an important cause of morbidity and mortality in these patients. CMV IgG positive, allogeneic transplant recipients, regardless of their donor's CMV status, are at the highest risk of CMV reactivation. The period of highest risk of CMV disease is between 1 and 3 months after transplant.

Approximately a third of allogeneic-HSCT patients who develop a detectable CMV viraemia will show spontaneous recovery from CMV infection. In the absence of CMV-specific T-cell immunity there is failure to clear CMV viraemia post HSCT (Herling et al. 2016; Król et al. 2011). Patients with CMV-specific T-cell immunity can clear reactivation/viraemia quicker and are more likely to have asymptomatic CMV compared to those without CMV-specific immunity (Tey et al. 2013). Furthermore, persistent viraemia in the presence of drug treatment with ganciclovir risks development of resistance (Herling et al. 2016).

CMV-specific T cell response has been measured in some cohort studies using the QuantiFERON-CMV (QF-CMV) assay (Qiagen, USA) and has shown reactive QF-CMV at 100 days post allogeneic-HSCT when one would expect engraftment and CMV-specific T-cell reconstitution (Thompson et al. 2022). It has 80% sensitivity and 100% specificity in a healthy population to measure CMV immunity (Among donor+/recipient- (D+/R-) solid organ transplant patients QF-CMV was able to predict those who would go on to develop CMV disease. Those with a non-reactive or indeterminate QF-CMV were 4 times more likely to develop CMV disease (Manuel et al. 2013). In addition, measuring immune response may identify patients with rapid immune reconstitution who do not need prophylaxis.

The use of ganciclovir as prophylaxis in this population is limited due to unacceptable myelosuppression and therefore a pre-emptive monitoring and treatment strategy has been adopted. However, this has changed since the advent of Letermovir as prophylaxis. Letermovir has been shown in randomised clinical trials to prevent clinically acquired CMV infection when used in the first 100 days of transplantation (Marty et al. 2017). It is relatively well tolerated without the myelotoxic and nephrotoxic effects associated with ganciclovir. Letermovir's use in this population is also associated with lower all-cause mortality. However, it is expensive, currently costing £4000 per patient per month. Letermovir has been introduced as prophylaxis against CMV in the first 100 days post-transplant in CMV seropositive donor or recipient at Addenbrooke's Hospital since 2019. If reconstitution of CMV-specific T cell response can be determined, then it may be possible to stop Letermovir at 60 days following allogeneic-HSCT. This would constitute a considerable cost-saving for the hospital.

The aim of this study is to determine, in the context of prophylaxis with Letermovir, the prevalence of stable CMV-specific T-cell immune reconstitution, defined as 2 reactive QF-CMV responses at 6 week and 8 weeks post allogeneic-HSCT, and measured by the QuantiFERON-CMV ELISA assay (Qiagen). Secondary outcomes of the study include exploring the association between stable CMV-specific T-cell immune reconstitution and clinical outcomes at 52 weeks, developing an in-house whole blood CMV-specific interferon- γ (IFN γ) assay that may be transferable to clinical practice, and evaluating the cost savings if Letermovir is stopped after 8 weeks rather than 100 days in those with stable CMV-specific T-cell immune reconstitution.

9 OBJECTIVES

9.1 Primary Objective

To determine, in the context of prophylaxis with Letermovir, the prevalence of stable CMV-specific T-cell immune reconstitution.

9.2 Secondary Objectives

- Explore the association between stable CMV-specific T-cell immune reconstitution and clinical outcomes (including CMV viraemia, CMV disease, CMV mutation and death) at 12 months
- Evaluate the cost savings if Letermovir is stopped after 8 weeks rather than 100 days in those with stable CMV-specific T-cell immune reconstitution

9.3 Exploratory Objectives

- Develop an in-house whole blood CMV-specific IFN γ assay that may be transferable to clinical practice
- Determine the diagnostic accuracy of the in-house whole blood CMV-specific IFN γ assay compared with the QuantiFERON-CMV ELISA assay (Qiagen) and Fluorospot assay
- Identify any CMV mutations emerging in the first 52 weeks following allogeneic-HSCTs

10 STUDY OUTCOME MEASURES

10.1 Primary Outcome

Prevalence of stable CMV-specific T-cell immune reconstitution in the context of Letermovir prophylaxis, defined as 2 reactive QF-CMV responses at 6 weeks and 8 weeks post allogeneic-HSCT, measured by the QuantiFERON-CMV ELISA assay (Qiagen).

10.2 Secondary Outcomes

1. Summarise CMV viraemia, disease, mutation and mortality at 52 weeks and analyse the association between these outcomes and stable CMV-specific T cell immune reconstitution
2. Cost savings if Letermovir is stopped after 8 weeks rather than 100 days in those with stable CMV-specific T-cell immune reconstitution

10.3 Exploratory Outcomes

1. Development of an in-house whole blood CMV-specific IFN γ assay that may be transferable to clinical practice
2. Measurement of the diagnostic accuracy of an in-house whole blood CMV-specific IFN γ assay to detect stable CMV-specific T-cell immune reconstitution in allogeneic-HSCT patients, in comparison to the QuantiFERON-CMV ELISA assay (Qiagen) and FluoroSpot assay.
3. Identification of any CMV mutations emerging in the first 52 weeks following allogeneic-HSCTs

11 STUDY DESIGN AND METHODS

11.1 Statement of Design

This is a longitudinal cohort study.

11.2 Number of Centres

This study will be conducted at Cambridge University Hospitals NHS Foundation Trust.

11.3 Number of Patients

A total of 60 evaluable patients are required for this study.

11.4 Patient Study Duration

The patient study duration is 52 weeks from the day of the patient undergoing an allogeneic HSCT.

12 STUDY PATIENTS SELECTION

12.1 Inclusion Criteria

To be included in the study, the patient must meet all of the following criteria:

- Written informed consent form
- Aged 16 years old or above
- Due to undergo an allogeneic-HSCT at Cambridge University Hospitals NHS Foundation Trust Addenbrooke's Hospital
- Confirmed determination of donor AND recipient CMV status by serology
- Due to take Letermovir prophylaxis following the allogeneic-HSCT
- Willing and able to comply with scheduled visits, treatment plans, sample collections and other study procedures for the duration of the study

12.2 Exclusion Criteria

The patient may not enter the study if ANY of the following apply:

- Patient is CMV seronegative
- Patient is in receipt of virus-specific T cell therapy

12.3 Co-enrolment Guidance

Enrolment into other ethically approved non-interventional clinical trial(s) or studies is allowed, where co-enrolment is permitted as part of the corresponding trial or study protocol provided there is no conflict of interest to the QuIC study. It is the responsibility of the participating clinical Study Investigator to ensure oversight of co-enrolment throughout the QuIC study. Questions regarding co-enrolment should be directed to a member of the QuIC Study Management Group.

12.4 Patient Identification and Recruitment

Patients will be identified by liaising with the HSCT team and service providers at Cambridge University Hospitals NHS Foundation Trust in order to capture any patients that may be eligible. The responsible clinical team will alert the study research team to these individuals. The study research team will verify the eligibility criteria of all referred patients and informed consent will be obtained before enrolment into the study. Patients will be asked to give consent for their clinical records to be obtained from their normal provider through electronic medical records.

12.5 Patient Informed Consent

The Patient Informed Consent Form will be approved by the Research Ethics Committee (REC) and must follow Good Clinical Practice (GCP), local regulatory requirements and legal requirements. The Investigator or designee must ensure that each study patient is fully informed about the nature and objectives of the study and possible risks associated with their participation.

The Investigator or designee will obtain written informed consent from each patient before any study-specific activity is performed. The Investigator will retain a printed copy of each patient's signed informed consent form in the Investigator Site File (ISF).

As this is a low risk, non-interventional study, patients may consent to take part on the same day as being offered the opportunity to participate. However, they must be given sufficient time to read the Patient Information Sheet (PIS), consider taking part in the study and ask any questions before consenting. The Investigator may delegate responsibility for consenting patients to appropriately trained healthcare professionals (including Research Nurses and Practitioners) who may not be medical staff if this is accepted local practice.

Should a patient require a written or verbal translation of the study documentation, it is the responsibility of the individual investigator to use locally approved translators. Copies of translated documents must be provided to the central coordination team for filing in the Trial Master File (TMF).

Any new information which becomes available, which might affect the patient's willingness to continue participating in the study, will be communicated to the patient as soon as possible. This will be via email or via telephone. A follow-up letter may be posted to the patient depending on the nature of the information to be communicated.

Study-specific procedures will only be performed after patients have consented to participation and signed the informed consent form.

12.6 Screening Evaluation

The following screening and eligibility assessments should be completed before the patient is registered into the study:

- Details of consent (incl. sex, date of consent, age at consent)
- Patients must give informed consent prior to any other study-specific procedures being undertaken
- Confirmation by serology of the patient's and their allogeneic-HSCT donor's CMV status

- Verify that the patient satisfies all protocol eligibility criteria (section 12.1 and 12.2) by reviewing their medical history and concomitant medications

The eligibility criteria will be verified for all study patients by a delegated member of the research team.

12.7 Patient Registration

Eligibility requirements documented in Section 12.6 must be met before registering a patient in this study. Only consenting patients who meet the eligibility requirements of the study at screening will be registered.

The following data will be required in order to enrol the patient into the study:

- Confirmation that the patient satisfies all of the eligibility criteria
- Age at consent
- Sex (Male or Female)
- Date of signed informed consent

Once it has been confirmed by the Investigator or suitably trained and delegated member of the study team that a patient meets the eligibility criteria, the Investigator or designee must complete the electronic Case Report Form (eCRF) in the REDCap® database (see the eCRF completion guidelines for instructions).

A unique study ID will be assigned by the REDCap® database to the patient upon registration, and this should be used in all future correspondence and on all patient-related documents.

12.8 Patient Withdrawal and Replacement Criteria

Patients may withdraw from the study at any time at their own request, without prejudice; or may be withdrawn from the study at any time at the discretion of the Investigator or Sponsor for safety, behavioural or administrative reasons.

Any samples collected as part of this study prior to patient withdrawal will be retained, analysed and used by the study team. However, if a patient explicitly requests that their sample(s) are destroyed, this will be undertaken by the study team.

Both non-evaluable patients and patients who withdraw from the study before the 8 week follow-up timepoint will be replaced. Evaluability criteria are outlined below.

All patients will be used to assess the study outcomes unless one of the following occurs:

- The patient's collected samples at the 6 and/ or 8 week follow-up time points are of inadequate volume or quality, as determined by the laboratory processing the samples

- At the time of the patient's withdrawal, the patient withdraws consent to allow for any prior samples collected to be used for the study and their samples from the 6 and 8 week timepoints have not yet been analysed.

13 STUDY PROCEDURES

Refer to Section 26.1: Appendix 1 for Schedule of Assessments.

Patients will be clinically assessed throughout the study as per local practice. Clinical assessments may be done remotely by telephone or videoconferencing, as appropriate and as per local practice.

Wherever possible, study procedures should be arranged to coincide with usual clinic attendance and blood sampling schedule. If this is not possible, study-specific visits should be arranged.

Sample collection will be done in the hospital in an appropriate clinical setting.

13.1 Baseline Procedures

The baseline visit will be on a suitable date prior to the commencement of conditioning treatment and within 4 weeks prior to the allogeneic-HSCT date. Conditioning treatment usually occurs 5-10 days prior to the allogeneic-HSCT.

The following procedures should be completed after the patient has been registered on to the study:

- Blood samples will be collected for the following assays:
 - QuantiFERON-CMV ELISA assay (Qiagen) – this assay will be used to measure T-cell immune reconstitution.
 - FluoroSpot assay - In parallel, a gold standard FluoroSpot assay will be used to measure T-cell response to CMV.
 - Whole blood IFN γ assay - In addition, an in-house whole blood IFN γ assay using a 99 pan-CMV peptide mix (JPT) will be developed and evaluated.

13.2 Follow-Up On Study Procedures

Follow-up visits will occur at 6 weeks (+/- 1 week), 8 weeks (+/- 1 week), 12 weeks (+/- 14 days) and 52 weeks (+/- 4 weeks) post-allogeneic-HSCT.

During the follow-up visits, all patients will have repeat blood sampling for QuantiFERON-CMV, FluoroSpot and Whole blood IFN γ assays to determine CMV-specific T cells responses.

13.3 CMV Review

If patients develop CMV viraemia or disease during the course of study follow-up, information about their CMV disease type, treatment, treatment duration and treatment response will be collected from the patient's medical record by the study team. Clinical samples will also be obtained for CMV sequencing. This will be done by using residual clinical samples from Pathology services at Cambridge University Hospitals NHS Foundation Trust Addenbrooke's Hospital. If residual clinical samples are not available, additional samples will not be collected.

13.4 End of Study

Patients will reach the end of study approximately 52 weeks after their allogeneic-HSCT.

13.5 Adverse Event Monitoring and Management

It is considered unlikely that adverse events related to study procedures will occur during the study, but if patients do experience an adverse event in relation to the study procedures this will be managed according to local guidelines.

Expected adverse events for this study would include:

- Temporary discomfort and/or pain related to the blood collection
- Bleeding or bruising in the skin around the blood collection site
- Infection at the blood collection site

This study is non-interventional, so information regarding adverse events will not be routinely collected as part of this protocol.

13.5.1 Safety Reporting for Non-CTIMP Studies to REC

Only reports of Serious Adverse Events (SAEs) occurring to a patient that in the opinion of the Chief investigator are considered:

- related to the study (i.e., they resulted from administration of any of the research procedures) and
- unexpected (i.e., not listed in the protocol as an expected occurrence)

Should be emailed to the REC using the Non-CTIMP safety report to REC form. These should be sent within 15 days of the chief investigator becoming aware of the event.

A serious adverse event is any untoward medical occurrence that:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

14 COLLECTION OF CLINICAL DATA

Patients will be followed up during their routine clinic visits/consultations where possible, with study data collection time points at baseline, and approximating 6 weeks (± 7 days), 8 weeks (± 7 days), 12 weeks (± 14 days), and 52 weeks (± 4 weeks) post-allogeneic-HSCT. Additional clinic assessments may be required per local practice, but study data collection will only occur at the approximate aforementioned time points.

14.1 Baseline Data

Information about the patient's medical and disease history will be collected. This will include:

- Demographic data (sex, age and ethnicity)
- Disease and transplant details:
 - Donor and recipient pre-transplant CMV serostatus
 - Primary haematological diagnosis (i.e. reason for transplant)
 - Type of conditioning treatment
 - Donor type
 - Type of transplant
 - Start & End dates of conditioning treatment
 - Actual date of transplant
- HLA matching to donor
- Type of immunosuppression taken by patient, corticosteroids, Sirolimus, Tacrolimus, Ciclosporin, Ruxolitinib etc.

- Start/stop dates for each immunosuppressant taken by the patient
- Baseline concomitant medications
- Routine blood test results (white cell counts, neutrophil and lymphocyte differential)

14.2 On-Study Data Collection

At approximately 6 weeks (+/- 7 days), 8 weeks (+/- 7 days), 12 weeks (+/- 14 days), and 52 weeks (+/- 4 weeks), post-allogeneic-HSCT, the following clinical data will be collected, to include:

- All post-transplant CMV PCR and quantification results up to 52 weeks post-transplant (as well as the date they were performed)
- All post-transplant CMV sequencing data up to 52 weeks post-transplant (as well as the date the sequencing was performed)
- Name and start/stop dates of any antivirals used to treat CMV disease
- Clinical outcome at 52 weeks post-transplant, i.e. remission of cancer, recurrence cancer, dead
- Date of death
- Whether Graft vs Host Disease (GvHD) developed
- Onset/end date and severity of GvHD
- Start/stop date of Letermovir prophylaxis
- QuantiFERON-CMV test result at each specified study time point
- Routine blood test results (white cell counts, neutrophil and lymphocyte differential) at each specified study time point
- Name and start/ stop dates of any immunosuppression medication used

Should the patient develop CMV viraemia or disease during study follow-up, the following clinical data related to CMV disease status will be collected as part of the CMV review:

- Date of onset and resolution of CMV viraemic episodes ($>10^4$ iu/ml)
- Clinical outcome of any CMV viraemic episodes ($>10^4$ iu/ml), i.e. undetectable, died, persistent
- Date of CMV disease diagnosis and resolution
- Clinical outcomes of CMV disease (recovered /died /persisted)
- CMV disease type:
 - 1) Reactivation (i.e. detectable viraemia $>10^4$ on 2 consecutive occasions)
 - 2) Disease with end organ manifestation (e.g. symptomatic colitis, pneumonitis, retinitis, neurological, CMV syndrome) and name of organ involved (e.g. eye, brain, lung)
- CMV treatment details

15 SAMPLE COLLECTION

Blood samples will be collected as detailed in the laboratory manual (see also Appendix 1). Full details of sample collection and transportation can be found in the QulC laboratory manual. The main aspects are summarised here.

15.1 QuantiFERON-CMV ELISA assay (Qiagen)

Assay will be used to measure T-cell immune reconstitution. 1ml of whole blood will be collected in 3 collection tubes: nil Control tube, CMV antigen tube, and Mitogen tube. The Mitogen tube is used in the QuantiFERON-CMV test as a positive control.

The whole blood samples will not be stored long term.

15.2 FluoroSpot assays

FluoroSpot assays will be used to measure T cell response to CMV. 30ml of whole blood will be collected and PBMCs will be prepared and stored in 1 million cell aliquots in a nominal - 80°C freezer

15.3 Whole Blood IFN γ assay using a 99 pan-CMV peptide mix (JPT):

5ml of heparinised blood will be collected from the study patient. 0.5 ml aliquots of blood will either be treated with CMV peptide pools, mock treated as a negative control or treated with PHA as a positive control. Plasma will be collected and stored in a nominal - 80°C freezer. IFN γ or IL-2 readout will be done by ELISA.

15.4 CMV Sequencing

In patients who develop CMV viraemia or disease, residual clinical samples will be obtained from Pathology services at Cambridge University Hospitals NHS Foundation Trust Addenbrooke's Hospital where possible for CMV sequencing. These residual samples may include EDTA blood, bronchoalveolar lavage, curls from fixed histological samples etc.

Any specimens that are stored will be kept in a -80°C freezer in an access-controlled biorepository.

16 PATIENT WITHDRAWAL

Patients may withdraw from the study at any time at their own request, or they may be withdrawn from the study at any time at the discretion of the Investigator or Sponsor for safety, behavioural or administrative reasons.

If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document relevant patient

outcomes. The Investigator will enquire the reason for withdrawal. If the patient explicitly states they no longer wish to contribute further data to the study, the Investigator should inform the co-ordinating centre in writing and the withdrawal of consent should be documented in the patient's medical records and in the eCRF. However, data collected up to the time of consent withdrawal will be included in the data reported for the study.

17 FORMS AND PROCEDURES FOR COLLECTING DATA

All data will be transferred into an eCRF by the participating site. Data on the eCRF will be linked to the patient's data using their age and sex. All study data in the eCRF must be extracted from and be consistent with the relevant source documents. The eCRFs will be completed and held in a secure electronic data capture system (REDCap®). It remains the Investigator's responsibility for the timing, completeness and accuracy of the eCRF. The eCRF will be accessible to Trial Coordinators, Data Managers, Investigators, Clinical Trial Monitors, Auditors and Inspectors as required. Paper CRFs will be used as a backup system. For further information, please refer to the Case Report Form Guidelines document.

The Investigators must ensure that the CRFs and other study-related documentation sent to the study Coordination Centre contain no patient-identifiable data.

17.1 Source Data

To enable monitoring, audit and/or inspection, the Investigator must agree to keep the records of all patients (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and a copy of each CRF.

Source data include, but are not limited to:

- Patient Medical Records
- Online Medical Records (e.g. medical records, prescribing records, results/reports from clinical investigations such as blood tests or scans)
- Signed and dated informed consent forms
- Worksheets and forms for sample collection, processing, storage and shipment
- NHS databases e.g. NHS Spine

17.2 Data Protection & Patient Confidentiality

All Investigators and study site staff involved in this study must comply with the requirements of the EU General Data Protection Regulations (GDPR) and the Data Protection Act 2018 and Trust Policy with regard to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles.

In particular, the Investigator and site staff must ensure where possible that no patient-identifiable information (including name, address, and hospital number) is transmitted to the Study Team or Sponsor. Every patient will be allocated a unique Study ID that will link all of the clinical information held about them on the study database. It will also be used in all correspondence with the participating clinical study site. At no point in presentations or publications of study data will individual patients be identified. It is possible that the residual samples taken from CUH Pathology services for CMV sequencing may include patient-identifiable information. This information cannot be removed from pathology samples but will be covered when sent for analysis.

The Investigator will be provided with an Investigator Site File prior to opening to recruitment. This file contains all essential documentation pertaining to the study and must be kept up to date during the course of the study.

All essential sources and study documentation must be securely archived after the Sponsor has confirmed in writing that the study has ended. Archiving should be for a period of at least 5 years, or the length of time specified by current, applicable legislation, whichever is longer.

The Investigator must not destroy any documents or records associated with the study without written approval from the Sponsor.

18 STATISTICS

18.1 The Number of Patients

The primary endpoint is the prevalence of stable CMV-specific T-cell immune reconstitution. A sample size of 60 evaluable patients provides an accurate 95% confidence interval (i.e. using $\alpha=0.05$) for the prevalence of stable CMV-specific T-cell immune reconstitution with maximal width of 0.25. If the observed prevalence is 75% then the interval estimate will be approximately (0.65, 0.85).

18.2 Analysis of Endpoints

18.2.1 Primary Outcome

Statistical analyses will be done using stata, R and Prism. Summary statistics will be used to describe the population. The prevalence of stable CMV-specific T-cell immune reconstitution will be summarised using the sample proportion and the exact (Clopper-Pearson) 95% confidence interval. The study is not powered for comparison of groups but the differences between the group with stable CMV-specific T-cell immune reconstitution and the group

without will be analysed using Chi-square test for categorical data and T-test and Mann-Whitney U test for parametric and non-parametric quantitative data respectively.

18.2.2 Secondary Outcomes

Outcomes at 52 weeks , including CMV viraemia, CMV disease, CMV mutation and death, will be summarised. Time to event analysis will be performed for these outcomes and presented as Kaplan-Meier plots. Cox proportional hazards model will be used to analyse the association between stable CMV-specific T-cell immune reconstitution and the stated outcomes at 52 weeks and presented as hazard ratios with 95% confidence intervals. Confounders such as age at consent, sex, graft versus host disease (GvHD) and haematological disease reoccurrence will be accounted for in the model.

The cost savings if Letermovir is stopped after 8 weeks rather than 100 days in patients with stable CMV-specific T-cell immune reconstitution will also be determined.

18.2.3 Exploratory Outcomes

An in-house whole blood CMV-specific IFN assay that may be transferable to clinical practice will be developed. The diagnostic accuracy of the in-house whole blood IFN γ assay using a 99 pan-CMV peptide mix (JPT) will be assessed using; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) tested against the QuantiFERON-CMV ELISA (Qiagen) and FluoroSpot assays.

Any CMV mutations emerging in the first 52 weeks following allogeneic-HSCT will also be identified.

19 DEFINITION OF THE END OF THE STUDY

The end of the study will be declared 6 months after the last patient's last visit which will allow sufficient time for the sample analysis to be investigated and the data cleaned for primary analyses and reports.

20 OVERSIGHT COMMITTEE

The Study Management Group will meet at least every six months to review the progress of this study.

21 ETHICAL AND REGULATORY COMPLIANCE

21.1 Ethical Committee Review

Before the start of the study or implementation of any amendment we will obtain approval of the study protocol, protocol amendments, informed consent forms and other relevant documents e.g., advertisements and GP information letters if applicable, from the REC. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

Annual reports will be submitted to the REC in accordance with national requirements. It is the Chief Investigator's responsibility to produce the annual reports as required.

21.2 Regulatory Compliance

The protocol and study conduct will comply with all national regulatory research requirements. It is the Chief Investigator's responsibility to produce any annual reports as required.

21.3 Protocol Amendments

Protocol amendments will be reviewed, and agreement received from the Sponsor for all proposed amendments prior to submission to the HRA and REC.

21.4 Declaration of Helsinki and Good Clinical Practice

The study will be performed in accordance with the spirit and the letter of the Declaration of Helsinki, the conditions and principles of Good Clinical Practice (GCP), the protocol and applicable local regulatory requirements and laws.

21.5 GCP Training

All research staff must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this study. This training should be updated every 3 years or in accordance with local Trust's policy.

22 SPONSORSHIP, FINANCIAL AND INSURANCE

The study is sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge, and is funded by Addenbrooke's Charitable Trust (ACT).

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to patients in the clinical study caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a patient be harmed through participation in the study, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising through participation in the clinical study.

There are no participation payments planned for this study, and there are no additional provisions for patients' travel or sustenance expenses.

23 MONITORING, AUDIT & INSPECTION

Should a monitoring visit or audit be requested, the Investigator must make the study documentation, source data and related records available to the Sponsor's representative. All patient data must be handled and treated confidentially.

The Sponsor's monitoring frequency will be determined by an initial risk assessment performed prior to the start of the study. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the study. Throughout the course of the study, the risk assessment will be reviewed and the monitoring frequency adjusted as necessary.

24 PROTOCOL COMPLIANCE AND BREACHES OF GCP

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Studies and must not be used.

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. They can happen at any time but are not planned. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor where required

Deviations from the protocol which are found to occur constantly again and again will not be accepted, will require immediate action and could potentially be classified as a serious breach.

Any potential/suspected serious breaches of GCP must be reported immediately to the Sponsor without any delay.

25 PUBLICATIONS POLICY

Ownership of the data arising from this study resides with the Study Management Group (SMG). On completion of the study the data will be analysed and tabulated, and a Final Report will be prepared.

It is anticipated any results from this study will be submitted to peer reviewed journals for publication and presented at national and international scientific meetings. All presentations and publications relating to the study must be authorised by the SMG and any other parties where authorisation forms part of a legally binding funding award or agreement.

Publications will be made Open Access in line with University of Cambridge policies. All publications will acknowledge the support of the Cambridge Cancer Trials Centre and Addenbrooke's Charitable Trust

In addition, patients who have consented to receive the study results will be provided with these via the investigator study team.

26 REFERENCES

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27 APPENDICES

27.1 Appendix 1: Schedule of Assessments:

	Screening	Baseline	6 Week Follow-up	8 Week Follow-up	12 Week Follow-up	52 Week Follow-up	CMV Review ^a
		Prior to conditioning treatment and up to 28 days prior to HSCT	± 7 days	± 7 days	± 14 days	± 4 weeks	N/A
Informed consent	X						
Eligibility criteria review	X						
Demographics		X					
Disease and Transplant Details		X					
Concomitant medication		X					
Immunosuppressant and Letermovir therapy review		X	X	X	X	X	X
Routine blood test results ^a		X	X	X	X	X	X
Research blood sample collection ^b		X	X	X	X	X	
Survival status			X	X	X	X	X
CMV sequencing ^c							X
CMV disease status ^c			X	X	X	X	X
CMV treatment details ^c			X	X	X	X	X

^aTo include white cell counts, neutrophil and lymphocyte differential

^b See laboratory manual for details

^c If patient develops CMV viraemia or disease