Study Protocol

Full Title: An observational study to correlate IMMUNE cell biology with Dilated CardioMyopathy patient characteristics

Short Title/Acronym: IMMUNE-DCM

Protocol Version Number & Date: V 1.2 25-April-2024

RESEARCH REFERENCE NUMBERS

IRAS Number:	**334984**
NHS REC Reference:	
Research Registry & References:	

RESEARCH SPONSOR

Sponsor Name:	**The Newcastle upon Tyne Hospitals NHS Foundation Trust**
Sponsor Reference:	**10686**

RESEARCH FUNDER(S)

Funder Name:	**AstraZeneca UK Ltd**
Funder Reference:	

PROTOCOL APPROVAL 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, and the agreed SOPs.

By signing, 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.

Representative of the Research Sponsor

Name:

Position: Regulatory Compliance Officer

Signature: Date:

Chief Investigator

Name: Professor Ioakim Spyridopoulos

Position: Professor of Cardiology and Cardiovascular Gerontology, Newcastle University

Signature: Date:

PROTOCOL ACCEPTANCE SIGNATURE PAGE

Short Study Title: IMMUNE-DCM

Principal Investigator

I have carefully read and understood protocol version 1.0 dated 12 December 2023.

Name:

Position:

Signature: Date:

KEY STUDY CONTACTS

Chief Investigator *	Professor Ioakim Spyridopoulos Professor of Cardiology and Cardiovascular Gerontology Vascular Biology & Medicine, Translational and Clinical Research Institute, Newcastle University Newcastle Upon Tyne, NE1 3BZ Email: Ioakim.spyridopoulos@newcastle.ac.uk Tel. 07932 745476
Principal Investigator *	Dr David Austin, Consultant Cardiologist Academic Cardiovascular Unit, South Tees Hospitals NHS Foundation Trust The James Cook University Hospital Marton Road, Middlesbrough, TS4 3BW Email: David.Austin@nhs.net Tel: 01642 850 850 ext 55909 Mobile: 07832 137739
Study and Data Management *	Academic Cardiovascular Unit The James Cook University Hospital, South Tees Hospitals NHS Foundation Trust Marton Road, Middlesbrough, TS4 3BW Email: stees.acu@nhs.net Contacts: Dr Lisa Chang, Principal Research Manager Rebecca Maier, Head of the Academic Cardiovascular Unit
Clinical Research Fellow	Dr Visvesh Jeyalan, Cardiology Research Fellow, Academic Cardiovascular Unit, The James Cook University Hospital, Marton Road, Middlesbrough, TS4 3BW
Sponsor	Newcastle upon Tyne Hospitals NHS Foundation Trust Regulatory Compliance Manager Regent Point Level 1 Newcastle Upon Tyne NE3 3HD Email: tnu-tr.sponsormanagement@nhs.net
Funder(s)	AstraZeneca The Darwin Building, 310 Milton Rd, Milton, Cambridge CB4 0WG Tel: 01582 837837

Collaborators

***Rebecca Maier**, Head of the Academic Cardiovascular Unit, The James Cook University Hospital rebecca.maier2@nhs.net

***Members of the Study Management Group**

STUDY SUMMARY

Study Title	An observational study to correlate immune cell biology with Dilated CardioMyopathy (DCM) patient characteristics.
Short Title	IMMUNE-DCM
Lay Title	Evaluating immune expression in patients with DCM as well understanding as the feasibility of a future clinical drug trial.
Summary of Study Design	Multicentre, observational, feasibility study
Summary of Participant Population	Patients with DCM aged 18 and above with left ventricular systolic dysfunction on optimised medical therapy (OMT) for at least 3 months.
Planned Sample Size	One hundred (100) evaluable participants in total. Fifty (50) with an ejection fraction of less than or equal to 35% and fifty with an ejection fraction of more than 35%, which are the two major phenogroups. The planned sample size was decided in order to secure the recruitment of at least 50 patients in each of the two major phenotypes and within feasible study timelines. Participants will be recruited from two sites in north-east England.
Funding Duration	28 months

Main Objectives	**Objectives:** 1. To investigate whether there is a correlation between the clinical phenotypes (PG1-PG4) and immune phenotype in the peripheral blood. 2. To investigate whether there is a correlation between cardiac MRI phenotype and immune phenotype in the peripheral blood. 3. To explore if CMV seropositivity has an impact on cardiac MRI parameters or clinical phenotype. 4. To explore if CX_3CR1 expression on leukocyte subsets correlates with CMV expression, the 4 clinical phenotypes (PG1 – PG4) or cardiac MRI findings. 5. To investigate whether Clonal haematopoiesis of indeterminate potential (CHIP) in patients with DCM is related to MRI phenotype or immune phenotype in the peripheral blood 6. To use digital technologies (wearable devices) and questionnaires to assess the arrhythmia burden, level of

	physical activity, quality of life and develop potential related study endpoints for possible future interventional studies.
Main Study Endpoints:	1. Difference in CD3+CX3CR1+ T cell levels and CX3CR1 leukocyte population 2. Correlation between CD3+CX3CR1+ T cell levels and the cardiac MRI parameters 3. Link between CMV seropositive status on; a) clinical phenotype b) cardiac MRI parameters 4. Correlation between CMV seropositive status and the concentration of CX3CR1 positive leukocyte populations (cells per ul peripheral blood), and CX3CR1 expression on: a) total T-cells and their main subsets (CD3, CD4 and CD8), b) CD4 effector memory cells (CD4+CCR7-), c) CD8 effector memory cells (CD8+CCR7-), d) NK cells (CD3-CD16+), e) monocytes (CD3-CD19-CD14+/CD16+), and monocytes subsets (classical, intermediate and non-classical (using CD14/CD16). 5. Correlation between the presence of the CHIP mutations (DNMT3A and TET2) with the MRI parameters and CD3+CX3CR1+ T cell expression. 6. Assessment of type, duration and frequency of arrhythmia at baseline and 6 months. 7. Assessment of level of physical activity of the major clinical phenogroups using a multiparameter remote patient monitoring device, and the findings from the sit to stand test. 8. KCCQ-23 score at baseline and 6 months.

Contents

GLOSSARY OF ABBREVIATIONS

Abbreviation	Meaning
ACU	Academic Cardiovascular Unit, South Tees Hospitals NHS FT
BMI	Body Mass Index
CHIP	Clonal Haematopoiesis of undetermined Potential
CI	Chief Investigator
CMR	Cardiac MRI
CMV	Cytomegalovirus
DCM	Dilated cardiomyopathy
EF	Ejection fraction
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
ICD	Implantable Cardioverter Defibrillator
ICI	Immune Checkpoint Inhibitors
ICM	Ischaemic Cardiomyopathy
ICMJE	International Committee of Medical Journal Editors
IFN	Interferon
irRAE	Immune related adverse events
ISF	Investigator Site File
JVP	Jugular Venous Pressure
KCCQ	Kansas City Cardiomyopathy Questionnaire
LGE	Late Gadolinium Enhancement
LVEDP	Left ventricular end diastolic pressure
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
PG	Phenogroups
PI	Principal Investigator
PIS	Patient information sheet
pPCI	Primary percutaneous coronary intervention
REC	Research Ethics Service
SLE	Systemic Lupus Erythematosus
SMG	Study Management Group
SOP	Standard Operating Procedure
STEMI	ST elevation myocardial infarction
TGF	Transformation Growth Factor
TNF	Tumour Necrosis Factor
Treg	Regulatory T cells

1. BACKGROUND AND RATIONALE

1.1 Dilated CardioMyopathy

Dilated cardiomyopathy (**DCM**) is a cardiac condition with structural and functional impairment, where either the left ventricle or both left and right ventricular chambers are enlarged, causing reduced systolic function (reduced ejection fraction, rEF). Clinically patients present with signs of heart failure in the absence of coronary artery disease, valvular or congenital heart disease. The European Society of Cardiology (ESC) classifies DCM as either genetic (familial) or acquired (non familial).(1) The prevalence of DCM is more than 1 in 250 individuals, and mortality is largely due to heart failure (70%) and sudden cardiac death (30%).(2) Despite advances in treating heart failure with medication and devices (implantable defibrillators), there still remains substantial morbidity and mortality with a 5 year survival rate of 50% .(3) Enlargement of the ventricular chambers is a result of remodelling and fibrosis, where scar tissue leads to a spherical shape of the left ventricle. This process causes a decrease in stroke volume, cardiac output, impaired ventricular filling and increase in left ventricular end diastolic pressure (LVEDP). The gradual progression of DCM makes the potential for early intervention before symptomatic heart failure has occurred challenging.

Damage to the myocardium, whether from a genetic or environmental cause, triggers inflammation and recruits immune cells to repair the myocardium; the most common causes of inflammatory DCM are infections and autoimmunity.(4) Examination of myocardial biopsy tissue often reveals an inflammatory cell infiltrate, such as CD3 T lymphocyte infiltration, or other activated immune cells like M2 macrophages and B lymphocytes in the case of autoimmune diseases. All of these have the capacity to release cytokines, such as transforming growth factor-β1 (TGFβ1), IL-4, IL-1β, IL-17A, IL-33 and tumour necrosis factor (TNF), which can promote remodelling, collagen deposition and fibrosis.(5) Fibrosis is a consequence of inflammation at the site of tissue damage and is the characteristic pathological feature of DCM aside from dilation.(6)

Similar to postinfarction healing, fibrotic scar tissue eventually replaces the inflamed tissue over time, thereby stiffening the heart and further amplifying progressive dilation and heart failure. There are no specific treatments, making more clinical research imperative. Viral infections are a frequent cause of myocarditis that can lead to inflammatory DCM in some individuals.(7)

While DCM accounts for half of non-ischemic cardiomyopathies, we are specifically interested in patients with idiopathic DCM (30-40%) and auto-immune disease background (5-10%), which do not respond well to standard guideline-directed medical therapy for heart failure.(8) Both are thought to have a strong inflammatory component that could be targeted by specific immune modulators. An enhanced myocardial immune response in DCM is linked to a worse prognosis over time.(9)

1.1.1 Phenotypic clustering of dilated cardiomyopathy patients

The recently published study from the Maastricht Cardiomyopathy Registry included 795 consecutive DCM patients who underwent in-depth phenotyping, comprising extensive clinical data on aetiology and comorbidities, imaging and endomyocardial biopsies.(10) The authors found four mutually exclusive phenogroups (PG) that were identified based upon unsupervised hierarchical clustering of principal components:

1. PG1 included 331 patients with mild systolic dysfunction,
2. PG2 included 83 patients with auto-immune disease background
3. PG3 included 165 patients with cardiac arrhythmias (mainly atrial fibrillation and ventricular tachycardias), including patients with genetic causes (Familial cardiomyopathy)
4. PG4 included 216 patients with severe systolic dysfunction.

RNA-sequencing of cardiac biopsy samples (n = 91) revealed a distinct underlying molecular profile per PG: pro-inflammatory (PG2, auto-immune), pro-fibrotic (PG3; arrhythmia), and metabolic (PG4, low EF) gene expression. Furthermore, event-free survival differed among the four phenogroups, even when corrected for well-known clinical predictors. Decision tree modelling identified four clinical parameters (auto-immune disease, EF, atrial fibrillation, and kidney function) by which every DCM patient could be placed.(10)

1.1.2 Aetiologies of dilated cardiomyopathy

1.1.2.1 Familial cardiomyopathy

Although a genetic basis for familial DCM is well established, most cases of DCM seem to be sporadic. So, even when family members of an index case of idiopathic DCM are clinically screened, most family members have no evidence of DCM. Therefore, individuals are eventually diagnosed with nonfamilial (sporadic) DCM. To date, there are no published large multicentre study of families whose members have been systematically clinically screened for DCM and have also undergone exome or genome sequencing to identify a possible genetic cause. Family-based studies have established that 15–30% of patients with DCM may be diagnosed with familial DCM if their family members undergo clinical screening.(4) Large multigenerational familial DCM pedigrees historically have been the starting point for most DCM-associated gene discovery. Such multigenerational pedigrees have provided robust statistical genetic evidence for variant causality in DCM-associated genes. The genes most commonly known to cause DCM, which include LMNA44, MYH7, TNNT2, TTN46, RBM20, BAG3 and others, were identified initially in large DCM pedigrees.(4) TTN truncating mutations are a common cause of DCM, occurring in ~25% of familial cases of DCM and in 18% of sporadic cases.(11)

1.1.2.2 Autoimmune myocarditis

Autoimmune myocarditis is a condition where self-reactive immune cells induce damage to the heart muscle. This condition is generally associated with many predisposing genetic factors and environmental triggers. Different systemic autoimmune diseases, such as sarcoidosis or systemic lupus erythmatosus (SLE), can impair heart function through various mechanisms.(12) Patients with either of these conditions generally have a poor prognosis. However, early administration of glucocorticosteroids and immunosuppressive agents has been shown to improve patients' conditions. One of the main therapeutic approaches for autoimmune disease is restoring the balance between autoimmunity and immune tolerance, which Th17 and regulatory T cells (Tregs) regulate, respectively.(13) Several studies have consistently found a defect in the amount or function of Treg cells in autoimmune diseases.(13) These cells can inhibit autoreactive T cells. Hence, Treg administration has shown to be a promising therapy for various autoimmune disorders including autoimmune myocarditis.(13)

1.1.2.3 Post-viral myocarditis

Myocarditis is often preceded by infection, mainly caused by viruses as the most common agent of this condition.(14, 15) Typically, there are three phases of viral myocarditis. The first phase takes several days, in which the virus gains entry and actively replicates itself. Consequently, the innate immune response will be activated against these foreign materials. During this phase, direct viral load significantly contributes to myocardial injury. The next phase demonstrates excessive immune response as the predominant cause of cardiac injury. T-cell infiltration has been reported during this phase, with a subsequent increase in fibrosis and calcification in areas of the heart. In the final phase, the patients can undergo either remission or further progression to dilated cardiomyopathy (DCM), which depends on the ability of the heart to resolve previous insults of direct viral injury and the persistence of immune response.(14, 15) Different theoretical therapeutic targets for post-viral myocarditis can be considered by intervening in each phase of disease progression.

1.1.2.4 Clonal Haematopoiesis of indeterminate potential (CHIP) and heart failure

An increased somatic blood cell clone is considered to be a sign of clonal haematopoiesis of indeterminate potential (CHIP) in people who do not have known haematological abnormalities.(16) It has been demonstrated that CHIP increases with ageing and is strongly linked to the risk of coronary heart disease. In a cohort study conducted by Dorsheimer and colleagues, 200 congestive heart failure patients were examined, and CHIP prevalence was found to be substantial. The most common driver genes for CHIP, DNMT3A and TET2, were found to be significantly and related with a profound increase in death and rehospitalization for heart failure.(16)

1.2 Role of fractalkine signalling in cardiovascular disease

1.2.1 CX_3CR1 and myocardial infarction

Fractalkine is a 373 amino acid protein and the only member of the CX_3C chemokine subfamily which,(16-20) uniquely, has both membrane bound and soluble forms. The former functions as an adhesion molecule and consists of four sections: an extracellular N-terminal domain, a mucin-like stalk, a transmembrane alpha helix and a short cytoplasmic tail. Fractalkine can be cleaved at the junction of the stalk and transmembrane helix, creating its soluble form which acts as a chemoattractant for monocytes, NK cells and T-lymphocytes. Fractalkine is expressed predominantly on endothelial cells, and is induced by the presence of IFN-g and TNF-a.(21) Its G-protein coupled receptor, CX_3CR1, is found on inflammatory cells such as NK cells, cytotoxic T-lymphocytes and monocytes,(22, 23) but also on cardiomyocytes.(24) Soluble fractalkine attracts these cells along a gradient and endothelial cell membrane-bound fractalkine binds to CX_3CR1 on the inflammatory cell surface to internalise them.

Our own studies suggest that the CX_3CR1 receptor is critical in the recruitment of lymphocytes that contribute to cardiac ischaemia/reperfusion injury, and to subsequent adverse remodeling. Binding of the fractalkine receptor CX_3CR1 expressed on circulating lymphocytes leads to adhesion and marginalisation of a subset of lymphocytes with cytotoxic properties. It is proposed that lymphocyte marginalisation, myocardial injury and myocardial inflammation can be diminished by targeting the chemokine receptor CX_3CR1 through CX_3CR1 inhibitors, such as KAND567.

1.2.2 Fractalkine signalling and heart failure

In a study by Nakayama and colleagues, it was discovered that DCM with higher myocardial immune activation was linked to a poor prognosis.(9) Specifically, patients with higher counts of infiltrating CD3-, CD68-, and CD163-positive cells had significantly poorer outcomes ($P = 0.007$, $P = 0.011$, and $P = 0.022$, respectively). Our own unpublished data show that CX_3CR1 and CX_3CL1 expression is consistently up-regulated in cardiac tissue from heart failure patients with different aetiologies, including end-stage DCM. Elevated CX_3CR1 and CX_3CL1 gene expression have also been observed in cardiac tissue from both DCM and ischaemic cardiomyopathy (ICM) patients.(24) Richter and colleagues determined CX3CL1 plasma levels in 349 patients with advanced systolic heart failure.(25) During a median follow-up of 5 years 55.9% of patients died. Fractalkine was a significant predictor of all-cause mortality ($p<0.001$) with a hazard ratio of 2.78 (95% confidence interval: 1.95-3.95) for the third compared to the first tertile. After multivariable correction for demographics, clinical predictive factors, and N-terminal pro-B-type natriuretic peptide (NT-proBNP, $p=0.008$), this connection remained significant. Patients with ischemic and non-ischaemic HF aetiologies did not substantially differ in terms of the predictive value of fractalkine ($p=0.79$). Additionally, fractalkine was an independent predictor of cardiovascular death. Patients on angiotensin-converting enzyme inhibitor therapy had significantly reduced levels of fractalkine. The authors conclude that circulating fractalkine, which has pro-inflammatory and immunomodulatory properties, is a standalone predictor

of mortality in advanced heart failure patients. Beyond NT-proBNP, fractalkine enhances risk prediction, which may assist in identifying high-risk patients who require specialised care.(25)

1.2.3 Link between CX3CR1 and cytomegalovirus

Cytomegalovirus (CMV) is a ubiquitous herpes virus with seroprevalence greater than 60% in people over 50 in most studies,(26) and 85% in people over 80. CMV causes an asymptomatic or mild initial response in immunocompetent hosts but is never cleared from the body, resulting in lifelong latency with the potential for reactivations.

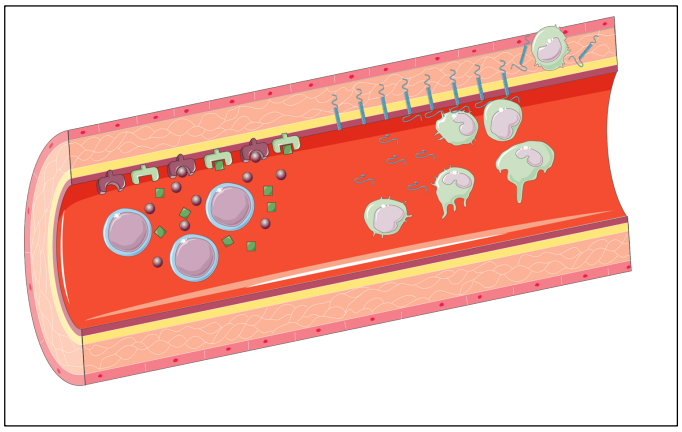


Figure 2. CMV-specific T-lymphocytes produce interferon-g and TNF-a, which induce fractalkine expression on the endothelium. The soluble form attracts monocytes and NK cells, and the membrane-bound form catches and internalises them, removing them from circulation.

CMV seropositivity causes significant changes in the host T-lymphocyte composition. CMV-specific $CD8^+$ and, to a lesser degree, and $CD4^+$ memory T-lymphocytes. CMV-specific $CD4^+$ T-lymphocytes were shown to cause endothelial damage in the presence of viral antigen, with more damage occurring in donors with higher frequencies of CMV-specific $CD4^+$ T-lymphocytes.(27-29) This damage is brought about by T-lymphocyte release of IFN-g and TNF-a at sufficient levels to induce endothelial cell induction of fractalkine, attracting natural killer (NK) cells and monocyte-macrophages - an illustration of this process is given in Figure 2. The same group showed that specific inhibition of CX_3CR1, the fractalkine receptor, attenuated this endothelial damage.

According to a recent meta-analysis, being exposed to CMV infection is linked to a 22% (relative risk: 1.22, 95% CI: 1.07-1.38, P=0.002) higher chance of developing cardiovascular disease in the future. The incidence of cardiovascular disease is thought to be 13.4% attributable to CMV infections,(30) More recently, a prospective epidemiological study in octogenarians by our group found that worse cardiovascular outcomes were predicted by CMV seropositivity, and by higher levels of senescent T-lymphocytes such as T_{EMRA}.(31, 32)

The cardiovascular risks conferred by CMV seropositivity remain controversial. Case-control studies, such as that by Siscovick,(33) have not identified an association between CMV IgG and the development of cardiovascular disease. As CMV and cardiovascular disease are so prevalent, however, this study design probably lacks power to identify this association, as the authors themselves stated. A 2017 meta-analysis of prospective epidemiological studies estimated a 7-38% increased relative risk of cardiovascular disease in seropositive patients, which the authors calculate would account for 13% of the total cardiovascular disease burden, due to the high prevalence of CMV.(30)

From the literature as well as our own data, it seems very likely that the upregulation of CX_3CR1, together with CMV seropositivity, confer to adverse prognosis in patients with dilated cardiomyopathy, potentially through adverse remodelling following cardiac inflammation and subsequent fibrosis.

2 RISK ASSESSMENT

This is a non-interventional study which carries low / negligible risk of serious harm to participants.

3 OBJECTIVES AND OUTCOME MEASURES

3.1. Study Objectives & Endpoints

Table 1: Phenogroups and their major clinical characteristics.(10)

PG1	PG2	PG3	PG4
Mild systolic dysfunction	Autoimmune	Cardiac arrhythmia	Severe systolic dysfunction

Objectives

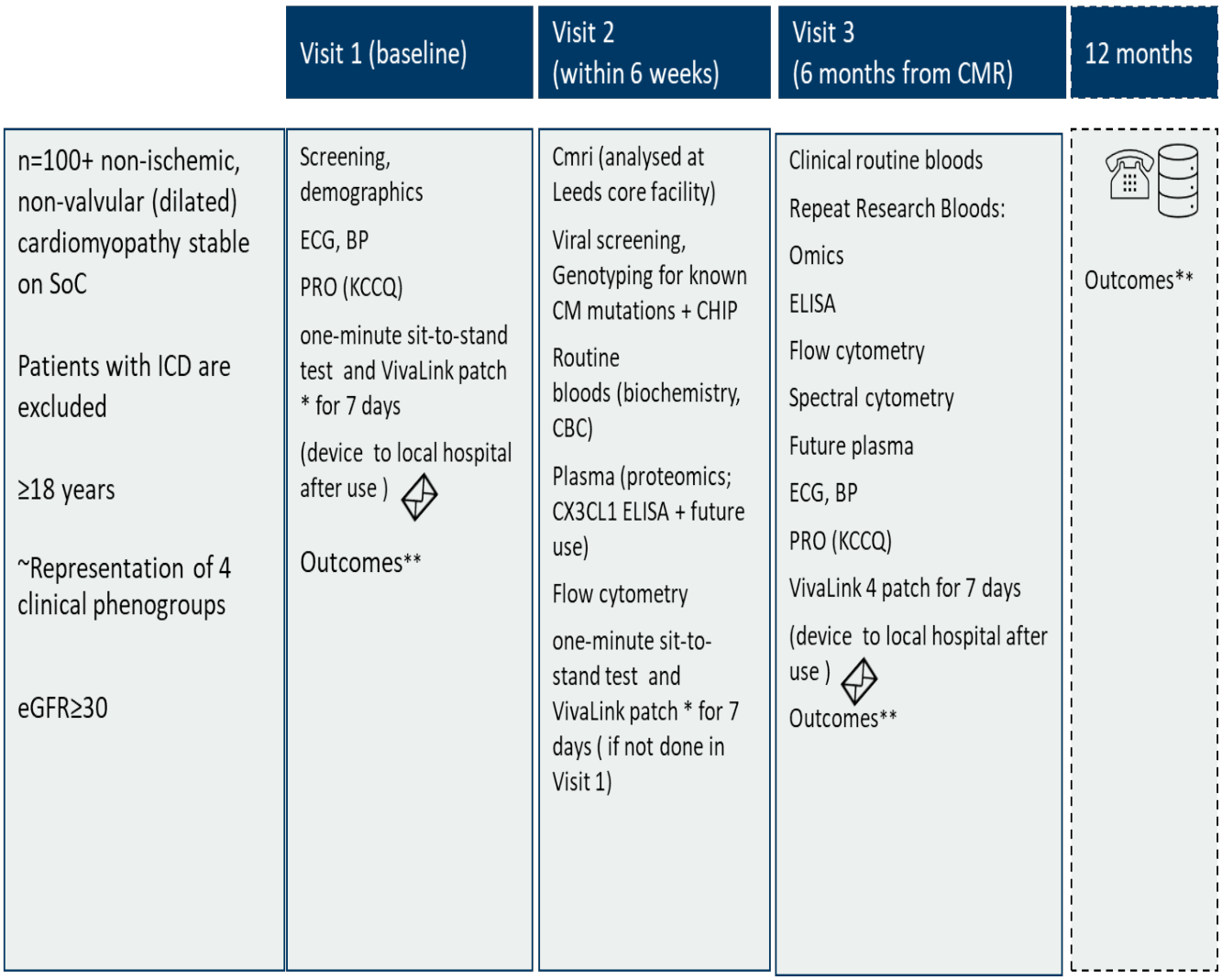
Study Objectives	Study Endpoints
To investigate whether there is a correlation between the major clinical phenotypes (PG1-PG4) and immune phenotype in the peripheral blood	Difference in CD3+CX3CR1+ T cell levels and CX3CR1 leukocyte population
To investigate whether there is a correlation between cardiac MRI phenotype and immune phenotype in the peripheral blood	Correlation between CD3+CX3CR1+ T cell levels and the cardiac MRI parameters
To explore if CMV seropositivity has an impact on cardiac MRI parameters or clinical phenotype	Link between CMV seropositive status on; 1) clinical phenotype 2) cardiac MRI parameters
To explore if CX3CR1 expression on leukocyte subsets correlates with CMV expression	Correlation between CMV seropositive status and the concentration of CX3CR1 positive leukocyte populations (cells per ul peripheral blood), as well as CX3CR1 expression on a) total T-cells and their main subsets (CD3, CD4 and CD8), b) CD4 effector memory cells (CD4+CCR7-), c) CD8 effector memory cells (CD8+CCR7-), d) NK cells (CD3-CD16+), e) monocytes (CD3-CD19-CD14+/CD16+), and monocytes subsets (classical, intermediate and non-classical (using CD14/CD16)
To investigate whether clonal haematopoiesis of indeterminate potential (CHIP) in patients with DCM is related to MRI phenotype or immune phenotype in the peripheral blood	Correlation between the presence of the CHIP mutations (DNMT3A and TET2) with the MRI parameters and CD3+CX3CR1+ T cell expression
To use digital technologies (wearable devices) and questionnaires to assess the arrhythmia burden, level of physical activity, quality of life and develop potential related study endpoints for possible future interventional studies	A) Assessment of type, duration and frequency of arrhythmia at baseline and 6 months B) Assess level of physical activity of both major clinical phenogroups using a multiparameter remote patient monitoring device including the sit to stand test parameters. C) KCCQ-23 score at baseline, 6 months

4. STUDY DESIGN

The study is an observational feasibility study.

One hundred patients with established dilated cardiomyopathy that satisfy the inclusion and exclusion criteria specified in section 6, will be enrolled in the study. The planned sample size is n = 100, assuming that these 100 participants will all complete a baseline blood analysis for serology, virology, immunological assays, a cardiac baseline MRI, and return the wearable devices. Participants who do not undergo these assessments at baseline may be replaced.

Figure 3: Study Participant Flow diagram



* Sit to stand test to be done after the Vivalink patch is attached. Both tests are to be done together on two occasions during the study period

** Subject to further funding and consent, patients will have the following data collected at 12 months: any Heart Failure (HF) hospitalisation, urgent HF visits with requirement for additional loop diuretic treatment, Myocardial Infarction, cardiovascular death, and all-cause mortality recorded from medical notes or over the telephone as appropriate to their current status at the time.

5. STUDY SETTING

This is a multicentre study, recruiting patients with dilated cardiomyopathy in an NHS setting.

6. ELIGIBILITY CRITERIA

Eligibility must be assessed by a medically qualified doctor and this assessment documented in the participant's medical notes. Only personnel formally delegated by the Principal Investigator to assess eligibility may perform this task.

6.1. Inclusion Criteria

Patients are eligible for the study if all the following inclusion and none of the exclusion criteria apply:

1. Established diagnosis of non-ischemic (dilated) cardiomyopathy, who have been receiving guideline-directed standard of care (SoC) pharmacological treatment for at least 3 months;
2. Diagnosis according to ESC guidelines implies left ventricular or biventricular systolic dysfunction and dilatation that are not explained by abnormal loading conditions or coronary artery disease. Systolic dysfunction is defined by abnormal LV ejection fraction, measured using any modality and shown either by two independent imaging modalities or on two distinct occasions by the same technique, preferably echocardiography or CMR;
3. Ability to adhere to study requirements including provision of consent to genetic testing;
4. Aged ≥18 years.

6.2. Exclusion Criteria

1. Heart failure due to either congenital heart disease, hypertensive heart disease, primary valvular heart disease, active acute myocarditis, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, restrictive cardiomyopathy or a known correctable metabolic cause;
2. Active vasculitis;
3. Anyone with known cognitive impairment or unable to provide consent;
4. Serious co-existing medical condition, including but not limited to known hepatic failure, known renal failure with known eGFR <30 mL/min/1.73m2, or severe psychiatric disorder, known at the time of inclusion;
5. Cardiogenic shock, non-compensated acute heart failure and/or pulmonary oedema;
6. Cardiac resynchronization pacemaker implantation within previous 3 months;
7. Active infection at the time of enrolment;
8. History of established coronary disease with known epicardial stenosis of more than 70%;
9. Patients unable to tolerate or undergo MRI scanning including patients with claustrophobia, cardiac pacemaker/defibrillator, ferromagnetic metal implants unless approved for use in MRI scanners or excessive body weight (BMI> 45);
10. Known allergy to gadolinium contrast;
11. Known planned hospitalisations (e.g. elective surgery), or other scheduled treatment for pre-existing conditions during the course of the study that could interfere with clinical assessment;
12. Any known previous diagnosis of invasive cancer within the last 5 years except for treated basal cell carcinoma of the skin;
13. Known pre-existing severe liver disease, including chronic hepatitis or alcohol-dependent liver cirrhosis;
14. Other medical or social reasons for exclusion at the discretion of the investigator.
15. Hypersensitivity to materials included in the patch;

16. Treatment with an investigational drug or other intervention assessment of which has not completed the primary endpoint or that clinically interferes with the present study endpoints will be excluded from this study.

7. STUDY PROCEDURES

7.1. Recruitment

7.1.1. Patient Identification

Potential participants will be identified by their care team at time-points that may include:

1) Inpatient admission

2) Attendance at outpatient cardiology services

3) Identified on each trusts individual heart failure, MRI, and echocardiogram databases.

7.1.2. Screening

Potential participants admitted or attending an outpatient appointment at a study site with an established diagnosis of dilated cardiomyopathy will be initially screened for study participation based on available clinical data. Patients will then be provided with a participant information sheet (PIS). These patients will be provided sufficient time to decide on study participation; if patients express interest to be recruited into the study on the day of initial contact, the written consent form may be signed and research investigations for the baseline visits conducted.

Patients will also be identified in advance of any planned appointment, or through the heart failure, echocardiogram or MRI database, and screened based on available clinical data at each study site. They may then be contacted either via telephone or email. Initial verbal consent will be obtained, and a full information sheet and consent form will be delivered either via email or post. Patients can also agree verbally to study participation and attend for their baseline visit tests during which a written consent form will be signed prior to any assessments.

A screening log will be kept to document details of all patients considered for the study, including those who were not subsequently enrolled and the reason why. Patients will be assigned a screening number consisting of a site number and then a sequential number starting from 001. If the patient is recruited into the study, they will keep this screening number as their Participant ID number.

7.2. Consent

Informed consent will be obtained in every case prior to undergoing any activity related to the study.

The following steps will be followed:

- Consent can only be obtained by a research team member, who will be knowledgeable about the research, its objectives and conduct, and all risks.
- Following verbal explanation of the study and opportunity to ask questions, patients will be provided with a printed copy of the approved PIS and Consent form. Patients will be provided as much time as required to consider participation. This signed form is retained securely in the research office, a copy is retained in the clinical notes, and a second copy is retained by the patient.
- Where a patient does not have capacity to consent, they will not be recruited.
- Participants can withdraw consent at any time during the study.

7.3. Study Assessments

7.3.1 Schedule of Events

Visit Number	1-2		3
Day	Screening Consent	Baseline MRI	6 month visit (6 months from MRI)
Time	Baseline	Within 6 weeks of consent	180 +/- 14 days from MRI
Written Informed Consent	X		
Demographics Weight, height, BMI Medical and surgical history Eligibility Checklist	X		
Bloods – Research * Blood for research Viral screening HbA1c (with FBC together) HS Troponin T Genotyping	X X X X X		X
Bloods – Clinical routine *	X		X
Cardiac MRI with contrast (gadolinium), T1/T2 signalling	X (either visit)		
12-lead ECG, Blood pressure	x		X
Wearable devices (Vivalink wearable patch-7days) and Sit stand test	X (either visit)		X
Concomitant Medication	X		X
Clinical complications	X		X
Kansas City Cardiomyopathy questionnaire (KCCQ-23)	X		X

*To be performed on the same date as the MRI. Full details are in the study laboratory manual.

*Type of tube, samples collected and volume detailed in lab manual

Study assessments will take place during study visits:

Visit 1 (days 0) – Baseline visit for screening, consent, baseline history taking, physical examination, concomitant medication, NYHA class, KCCQ questionnaire, wearable device and site to stand test.

- Visit 2 (0-42 days from baseline visit) - Cardiac MRI, baseline clinical routine bloods, research bloods and genotyping, wearable device provided* (along with sit to stand test*, after MRI). The blood tests will be obtained ideally between 9am and 1pm. Overnight fasting will be recommended to patients.

Visits 1 and 2 can be combined on the same day if organisation of the MRI is possible.

*The wearable device is needed at either visit 1 or visit 2. It is not required both times.

- Visit 3 (day 180 ±14 day from MRI) – 6-month clinical routine and research bloods, 12 lead ECG, Blood pressure concomitant medication, wearable device fitted, clinical complications, NYHA class and KCCQ questionnaire. 7.3.2. Weight and height

Timepoints for assessment: Day 0 - Baseline

Weight and height assessments will be completed during the baseline visit, or the information will be taken from the participant's medical records, if this information is already available. BMI will later be calculated from the height and weight recorded in the eCRF.

7.3.3. Medical and surgical history

Timepoints for assessment: Day 0 - Baseline

Clinically important medical history focussing on the historical work up of the diagnosis of dilated cardiomyopathy will be obtained. The data collected would include but not be limited to previous echocardiographic, cardiac MRI and genetic testing, and cardiology medical history. Clinically significant past and present medical and/or surgical history may also be obtained by interview to verify that the eligibility criteria are met.

7.3.4. Prior and concomitant medications

Timepoints for assessment: Recorded for the duration of the study

Medical history and prior medications used within two weeks of recruitment will be recorded specific to guideline directed heart failure therapy and diuretic therapy.

7.3.5. Clinical complications

Timepoints for assessment: Recorded for the duration of the study

Data will be collected during study visits, through medical records or by phone call to verify HF outcomes such as HF hospitalisation, urgent HF visits with requirement for additional loop diuretic treatment, MI, cardiovascular deaths, and all-cause mortality .

7.3.6 Kansas City Cardiomyopathy Questionnaire

Time-points for assessment: Visit 1: Day 0 - baseline

Visit 3: Day 180 +/- 14 days from CMR

To be assessed and recorded on the KCCQ study pro-forma.

7.3.7. Clinical Assessments

7.3.7.1. ECG

Timepoints for assessment: Visit 1: Day 0 - baseline

Visit 3: Day 180 +/- 14 days from CMR

ECGs will be recorded in supine position using an ECG machine. The ECGs will be assessed by the study team investigators/sub investigators.

7.3.7.2. Blood Pressure

Timepoints for assessment: Visit 1: Day 0 - baseline

Visit 3: Day 180 +/- 14 days from CMR

Diastolic blood pressure will be measured after 5 minutes of rest in supine position.

Blood pressure (BP) will be assessed at baseline, and at each study visit, prior to taking any blood samples. BP needs to be assessed whilst the participant is sitting. If the first reading provides a systolic value greater than 160mmHg, the test should be repeated on two further occasions, with the participant quiet and seated.

7.3.7.3 Respiratory rate

Timepoints for assessment: Visit 1: Day 0 - baseline

Visit 3: Day 180 +/- 14 days from CMR

Respiratory rate assessed at baseline by counting the number of times the chest rises with inspiration in a 30-second window and multiplying by 2.

7.3.7.4 Clinical signs

Timepoints for assessment: Visit 1: Day 0 - baseline

Visit 3: Day 180 +/- 14 days from CMR

Assessment of clinical signs relating to cardiac decompensation will be undertaken such as an assessment of peripheral oedema, assessment of jugular venous pressure (JVP), height, as well as a respiratory and cardiac auscultation.

7.3.7.5 Viral screening

Timepoints for assessment: Visit 1: Day 0 - baseline

A viral throat swab will be done at the baseline visit. Please see the IMMUNE DCM laboratory manual for further details.

7.3.8. Wearable ECG device (Vivalink VV350) and sit-to-stand test

Timepoints for assessment: Visit 1 or Visit 2: Day 0 or within 6 weeks of baseline visit

Visit 3: 180 +/- 14 days from CMR

Patients will receive a VivaLink ECG patch to wear at home for two periods of up to 7 days as shown in the Schedule of Events table. These will be attached by trained research and nursing staff and provided fully charged. An adhesive pad is attached onto the wearable patch and applied to the middle upper left chest area after the surface of the skin is cleaned with an alcohol wipe. The patch is waterproof and participants can shower whilst wearing the patch.

The purpose of wearing the patch is to assess heart rate, rhythm, heart rate variability, breathing rate, as well as activity and posture with its 3-axis accelerometer function. A sit to stand test will be done on the same visit after the vivalink patch is attached (see **Appendix 15.1**). Data collected from the patch will be uploaded to the vendor's server. The results will not be available to participants or treating physicians during the study. Results will be available to the study team at the end of the study.

Instructions for using the VivaLink ECG patch are provided in separate instructions to the patients.

Any device deficiency observed with a third-party medical device will be collected and reported to the manufacturer.

7.3.9. Blood sampling

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Blood samples for analysis of clinical chemistry and haematology will be collected by venepuncture or by an indwelling catheter during the specified visits. Haematology samples will be sent to the local clinical laboratory for analysis by routine analytical methods. Clinical chemistry samples will be

analysed as described in the lab manual. Overnight fasting will be recommended to individual participants. Up to 80mL of blood at each time-point will be needed to undertake the range of analyses required. Blood samples will be collected between 9am-1pm ideally. Timepoint of collection will be documented.

7.3.9.1 HIV and hepatitis B, C, CMV and other cardiotropic viruses

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Blood samples are taken at baseline for testing for HIV, hepatitis B and C and Cytomegalovirus IgG, IGM and titre along with serology for other cardiotropic viruses. These samples will be processed at as per the IMMUNE-DCM Laboratory Manual for details. Results will be entered into the eCRF by study site staff, and results fed back to patients.

7.3.9.2. FICOLL Sampling

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Blood will be taken at baseline and 180 +/- 14 days from MRI, and collected in CPT tubes to perform FICOLL separation. See laboratory manual for details on collection, shipping and processing.

7.3.9.3. Flow cytometry (FACS) and CD3+CX3CR1+ T cells

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Blood will be taken at baseline and at 180 days +/- 14 days from MRI to measure CD3+ CX_3CR1+ T cells. See the laboratory manual for details on collection, shipping and processing.

7.3.9.4. Flow cytometry (FACS) to quantify CX_3CR1^+ leukocyte populations

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Blood will be taken at baseline and at 180 days +/- 14 days from MRI to measure CX3CR1+ cells. See IMMUNE-DCM laboratory manual for details on sampling, processing and shipping.

7.3.9.5. Exploratory analysis: Spectral cytometry

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Cryopreserved PBMC samples will be analysed by spectral cytometry at the Centre for Life at Newcastle University using the panel outlined in Lab Manual.

7.3.9.6. Genomics

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

The mapping of certain relevant genes includes a genetic sample. Collection and storage of DNA samples are intended for genetic research to explore how genetic variations may affect clinical parameters, risk and prognosis of diseases, and the response to medications. This genetic research may lead to better understanding of diseases, better diagnosis of diseases or other improvements in healthcare and to the discovery of new diagnostics, treatments or medications. A blood sample will be collected for DNA analysis from consenting participants.

- This genetic research may consist of the analysis of the structure of the participant´s DNA, i.e, the entire genome.
- The results of genetic analyses may be reported in a separate study summary.
- The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained for up to 5 years after the end of the study at the Centre for Life.
- All participants will be asked to participate in this genetic research. Participation is required and if a participant declines to participate recruitment into the study will not be feasible.

7.3.9.6.1 Cardiac Genetics

DCM genotypes will be analysed as described in the laboratory manual at the Clinical Genetics & Genomics Laboratory, Imperial College London, Royal Brompton Hospital, Sydney Street, London SW3 6NP. All samples will receive a report from a NHS cardiac geneticist at the Royal Brompton, and in cases where clinically relevant information is disclosed, patients will be referred for genetic counselling locally (Centre for Life, Newcastle, NHS Genetics Service).

7.3.9.6.2 CX_3CR1 Genotype

Samples to assess CX3CR1 gene mutations will be collected, processed, stored, analysed and reported as described in the laboratory manual.

7.3.9.6.3 CHIP mutations

CHIP mutations (DNMT3A and TET2) in exons will be collected, processed, stored, and analysed as described in the laboratory manual.

7.3.9.6.4 DNA methylome samples

The mapping of the DNA methylome provides information on which genes are active and inactive (gene activity mapping) enabling pathway analysis. CpG sites in the genome will be profiled with respect to methylation, and specific fingerprints for different types of immune cells will be analysed. DNA methylome samples will be stored processed as per the IMMUNE-DCM Laboratory Manual, frozen and processed later if necessary. They may be sent to a specialised lab within the UK for the purpose of characterising gene activity and performing pathway analysis. Samples will be saved for a maximum period of 5 years following the end of the study and be destroyed after analysis. These DNA methylome results may be analysed within the study and captured in the study database. Processing and analysis is subject to further funding

7.3.9.7 Transcriptomics

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Transcriptomics will be collected, processed, stored, as described in the laboratory manual

7.3.9.8 Immune assay and proteomics

Timepoints for assessment: Visit 1 / 2: Within 6 weeks of baseline visit with CMR

Visit 3: Day 180 +/- 14 days from CMR

Samples for immune assays and proteomics will be stored frozen and sent to a specialised laboratory within the UK or the EU for analysis. Samples will be collected, processed, stored and analysed as described in the laboratory manual

7.3.10 MRI

Timepoint for assessment: Either Visit 1 or 2, and within 6 weeks of baseline visit.

MRI assessments will be completed at the central core lab and at local sites by a trained physicians for detailed analysis of cardiac and non cardiac findings. Prior to the scan, subjects will have a peripheral IV line placed for the administration of gadolinium-based contrast. Gadolinium-enhanced images will be obtained at 7-10 minutes after an IV bolus injection of ionic, non-linear gadolinium contrast agent. Scan duration is estimated to be approximately 60 minutes. The details of the image acquisition protocol will be outlined in a cardiac MRI imaging manual. If the MRI scan is deemed to be of inadequate quality by the (central or local), the subject (may or may not) return for a repeat MRI scan within 3 weeks. Patients will not be recruited into the study if they have had a cardiac MRI within the last month. The MRI scan done will not be part of the patient's routine care.

The following imaging methods and evaluations will be performed:

CINE MRI for quantification of LV function, RV function, and LA function

- LV ejection fraction
- RV ejection fraction
- Left ventricular end diastolic volume
- Left ventricular end systolic volume
- Right ventricular end diastolic volume
- Right ventricular end systolic volume
- LV sphericity Index (LV length and volume)
- End diastolic and end systolic LV wall thickness (global and segmental)
- LV Wall thickening – global and segmental
- LV global longitudinal strain and LV strain rate
- LA volume, strain, and strain rates
- Any additional measurements as described in the IMMUNE-DCM MRI Imaging Manual

Late-gadolinium Enhancement MRI

- LV mass
- T1 mapping
- T2 mapping
- ECV
- Any additional measurements described in the MRI imaging protocol

The results of the analyses will be returned to the study site teams for entry into the study database. Any incidental findings identified on the cardiac MRI scan will be shared with site PI, general practitioners and study participants. Please see IMMUNE-DCM MRI Imaging Manual for details of the MRI analyses.

7.4. Withdrawal

7.4.1. Participant Requested Withdrawal

If a participant states they want to withdraw from the study, sites should try to ascertain the reason for withdrawal and document this reason within the eCRF and participant's medical records.

Where a participant withdraws consent or their participation in the study is discontinued, the data and samples collected up to the point of withdrawal will be retained and included in the analyses.

7.4.2 Withdrawal of informed consent for stored biological samples

If a participant withdraws consent to the further use of stored biological samples during the study, the samples already collected will be disposed/destroyed, if not already analysed and documented.

The Principal Investigator will ensure that biological samples from that participant, if stored at the site, are identified within 24 hours disposed/destroyed and the action documented.

7.5. End of Study

The end of study is defined as the date when the last samples for the main study objectives are analysed, excluding samples for further exploratory analysis and the database is agreed as locked for the final analysis.

7.6. Payment

Participants will be offered reasonable travel expense reimbursement.

8. BIOLOGICAL SAMPLES

It is the responsibility of the study site to ensure that samples are appropriately labelled in accordance with the study procedures to comply with the applicable legislation, the UK Data Protection Act 2018. Blood samples will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and 2006 Human Tissue (Scotland) Act.

All shipments of biological samples will be labelled and transferred according to local legislation.

A full chain of custody is maintained for all samples throughout their lifecycle. Please refer to the IMMUNE-DCM Laboratory Manual for further details.

The site is responsible for keeping full traceability of biological samples from the time the samples are collected from the participant, whilst in storage at the site until shipment. The site also needs to keep documentation to confirm receipt of samples shipped to analytical laboratories.

The sample receiver (analytical laboratory) is responsible for keeping full traceability of the samples received, whilst in their storage and during use until analysed, shipped or disposed of.

The samples will be used up or disposed of after analyses or retained for further use as described below.

Samples which have been collected to be used as part of the IMMUNE-DCM study exploratory analysis (those measuring genotype CX3CR1, Immune assays and proteomics, transcriptome analyses and potentially DNA methylome analyses), will be stored , shipped and processed as per the IMMUNE-DCM Laboratory Manual. These samples may also be used for further exploratory analysis. Remaining samples after study analyses may be used for further in vitro testing with drugs to help identify who may benefit from a new heart failure drug; some of these samples may be sent to AstraZeneca for this purpose. Remaining samples will be destroyed after 5 years.

The sponsor will retain oversight of the entire sample lifecycle through monitoring of the study sites and analytical laboratories.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Analyses

Descriptive statistical methods will be used. Tabulations and listings of data for clinical complication of events, vital signs, ECG, physical examinations and clinical laboratory tests will be presented. For clinical laboratory tests, listings of values for each participant will be presented with abnormal or out-of-range values flagged.

Continuous variables will be summarised using descriptive statistics (number of participants, mean, standard deviation, minimum, median, maximum) where appropriate. Categorical variables will be summarised in frequency tables (frequency and proportion). Graphical presentations will be used as appropriate. All changes from baseline endpoints are calculated as the value of the corresponding day minus the value at baseline.

A Statistical Analysis Plan will be written.

9.2. Missing data

A participant who withdraws prior to the last planned observation will be included in the analyses up to the time of discontinuation.

9.3 Sample Size

This is no formal power calculation. The study will recruit 100 participants with evaluable baseline data, 50 to each of the two major phenotypes (participants with an EF >35% and participants with an EF ≤35%).

The sample size has been planned based on feasibility within the study timelines whilst ensuring a reasonable (and equal number) of participants in each group.

It is anticipated that there will be dropouts prior to the collection of the full baseline dataset (including cardiac MRI, ECG, blood sampling). The study will therefore continue recruiting until baseline data is available for 50 participants in each group. It is estimated that 108-112 consenting patients could be required to meet the target of 50 participants in each group

10. DATA HANDLING

10.1. Data Collection Tools

Data will be collected in medical notes and recorded in a web-based eCRF. Participant identification on the eCRF will be through a unique study identifier. The participant's name will be linked to their unique study identifier via a record filed within each site's ISF, which will be stored in a locked room at site.

10.2. Data Handling and Record Keeping

The CI has overarching responsibility for collection, quality and retention of data. Data will be collected by an appropriately qualified and delegated member of site personnel. Data will be handled, computerised, and stored in accordance with the UK Data Protection Act 2018 and the UK GDPR as amended on 01 January 2021 by regulations under the European Union (Withdrawal) Act 2018, to reflect the UK's status outside the EU, the latest GCP Directive (2005/28/EC) and local site policy. Paper copies of study-related documentation will be annotated, signed, dated and filed in the Investigator Site File. Copies of the Summary PIS, PIS, confirmation of verbal consent form, completed written consent form, eligibility forms and letter to GP will be filed in the participant's medical notes.

The CI or designated nominees will continuously monitor completeness and quality of data collected on the study database. Monitoring will include regular correspondence with site staff to ensure missing data is collected wherever possible and ensuring continuous high quality of data capture. Data completeness and progress reports will be generated for regular review at SMG meetings.

10.3. Access to Data

The site PI and staff formally delegated to do so will have access to source data and the ISF to conduct the study.

Access to the study database will be password-limited. The site PI will formally delegate database tasks to site staff, by way of dated signatures on the Site Delegation Log.

ACU study management staff, representatives of the host institution, and sponsor will be granted access to the source data, ISF and study database for the purposes of monitoring, audit and inspection, respectively. Consent will be sought from the participant for access to their medical records and study data for the purposes of monitoring and audit.

Data may be securely downloaded from the study database and released to the Sponsor and study team for analysis. Data release will only take place after documented agreement from key members of the SMG.

Site staff, including the PI may not disclose or use for any purpose other than conduct of the study any data, record or other unpublished confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said information to other parties.

10.4. Archiving

Study documents and data will be archived in accordance with Sponsor and ACU SOPs. All study documentation and data will be archived for 5 years.

11. MONITORING and AUDIT

11.1. Study Management Group (SMG)

The SMG will be responsible for the day-to-day running of the study and will consist of the CI, members of ACU and, as required, other members of the co-applicant team. The SMG will monitor all aspects of the conduct and progress of the study, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the study itself. SMG meetings will occur approximately 4-6 weekly. Progress will be monitored proactively according to agreed study timelines and any issues addressed. The SMG will liaise with the Sponsor study funder (AstraZeneca) providing updates on study progress and highlighting any issues arising.

11.2. Principal Investigator

Each site will be led by a Principal Investigator who will be responsible for study conduct. They will be supported by research nurses.

The Principal Investigator will be responsible for highlighting day-to-day study conduct at site. The ACU team will provide day-to-day support for the site and training, site initiation visits and routine monitoring visits.

11.3. Monitoring

Quality control will be maintained through adherence to appropriate Sponsor and ACU SOPs, study protocol, GCP principles, and research governance.

Monitoring to ensure appropriate study conduct and data collection will be carried out by ACU. Electronic data will be stored in secure, password-protected computers. ACU staff will use a combination of central monitoring, off-site monitoring, and on-site monitoring visits to ensure the study is conducted in accordance with GCP and the study protocol.

All monitoring findings will be reported and followed up with the appropriate persons in a timely manner. The site PIs and institutions will permit study-related monitoring, audits, and regulatory inspections, providing direct access to source data and documents relating to the study. All data will be retained for 5 years.

The CI or designated nominees will continuously monitor completeness and quality of data collected on the study database. Monitoring will include regular correspondence with site staff to ensure missing data is collected wherever possible and ensuring continuous high quality of data capture. Data completeness and progress reports will be generated for regular review at SMG meetings.

A Site Delegation Log will detail the responsibilities of each member of site staff working on the study.

Monitoring will be risk-based as detailed in the study specific monitoring plan. The monitoring plan will be reviewed and amended during the study based on changes to the protocol and identified or perceived risks.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Research Ethics Committee Review and Reports

ACU will obtain a favourable ethical opinion from an NHS Research Ethics Committee (REC) prior to the start of the study. All parties will conduct the study in accordance with this ethical opinion.

ACU will notify the REC of all required substantial amendments to the study and those non-substantial amendments that result in a change to study documentation (e.g., protocol or patient information sheet). Substantial amendments that require a REC favourable opinion will not be implemented until this REC favourable opinion is obtained. ACU will notify the REC of any serious breaches of GCP principles or the protocol.

An annual progress report will be submitted each year to the REC by ACU until the end of the study. This report will be submitted within 30 days of the anniversary date on which the original favourable ethical opinion was granted.

ACU will notify the REC of the early termination or end of study in accordance with the required timelines.

12.2. Peer Review

The protocol has been reviewed by the Sponsor, AstraZeneca, CI, and ACU, and authorised by the Sponsor.

12.3. Public and Patient Involvement

The study results will be presented to a PPI group at conclusion of this study to gain feedback for a potential phase 2 study that may follow.

12.4. Notification of Serious Breaches to GCP and/or the Protocol

A serious breach is a breach which is likely to effect to a significant degree.

(a) the safety or physical or mental integrity of the participants of the study; or (b) the scientific value of the study

The sponsor must be notified immediately of any incident that may be classified as a serious breach. ACU will notify the NHS REC within the required timelines in accordance with the Sponsor and ACU SOP.

12.5. Data Protection and Patient Confidentiality

All investigators and study site staff will comply with the UK Data Protection Act 2018 with regards to the collection, storage, processing, and disclosure of personal information and will uphold the core principles of the legislation.

Study data held on computers will be accessible only by authorised study personnel and will be password protected. Paper records containing personal information will only be accessible by study personnel at each site, central study personnel, monitors from ACU and auditors/inspectors from the Sponsor or regulatory authorities.

12.6. Indemnity

NHS indemnity for clinical studies conducted with HRA approval will apply for clinical negligence that harms individuals towards whom the NHS has a duty of care. Indemnity in respect of protocol authorship will be provided through a combination of NHS schemes (for those protocol authors who have substantive NHS employment contracts) and through Newcastle University's public liability insurance (for those who have their substantive contracts of employment with the University).

There is no provision for indemnity in respect of non-negligent harm. NHS Organisations must ensure that site staff without substantive NHS contracts hold honorary contracts to ensure they can access patients and are covered under the NHS indemnity arrangements.

12.7. Amendments

It is the responsibility of the Research Sponsor to determine if an amendment is substantial or not and study procedures must not be changed without the mutual agreement of the CI, Sponsor, and the Study Management Group.

Substantial amendments will be submitted to the REC will not be implemented until this approval is in place. It is the responsibility of ACU to submit substantial amendments.

Non-substantial amendments will be submitted to the Health Research Authority (HRA) and will not be implemented until authorisation is received.

Substantial amendments and those minor amendments which may impact sites will be submitted to the relevant NHS R&D Departments for notification to determine if the amendment affects the NHS permission for that site. Amendment documentation will be provided to sites by the ACU.

12.8. Access to the Final Study Dataset

Ownership of the data arising from this study resides with the Sponsor. Data will shared with AstraZeneca at the end of the study and kept by the research team and sponsor. On completion of the study, the study data will be analysed and tabulated, and a final report will be prepared.

13. DISSEMINATION POLICY

13.1. End of study reporting

A final report of the study will be provided to the Sponsor, REC, and the study funder, AstraZeneca, within 1 year of the end of the study.

13.2. Authorship policy

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

Authorship eligibility for each manuscript arising from this study will be determined by the Study Management Group. All co-applicants, plus the Study Manager and data, will be eligible for authorship on papers reporting the protocol and main study results, subject to fulfilling the ICMJE authorship criteria. Authorship for other conference abstracts and scientific papers arising from this work will be decided by the Study Management Group.

All outputs from this programme of work will acknowledge AstraZeneca as funder and will specifically acknowledge the Academic Cardiovascular Unit at James Cook Hospital, Newcastle University, and Newcastle-upon-Tyne Hospitals Trust as Sponsor.

13.3. Publication

Subject to the publication provisions of the Agreement, the final clinical study report will be used for publication and presentation at scientific meetings. Study Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).

All information supplied by AstraZeneca in connection with this study shall remain the sole property of AstraZeneca and is to be considered confidential information. No confidential information shall be disclosed to others without prior written consent from AstraZeneca and shall not be used except in the performance of this study**.**

13.4. Public dissemination

The study will be prospectively registered on ISRCTN prior to enrolment of the first participant.

Study results will be made publicly available on the ISRCTN registry within 12 months of the end of the study.

13.5. Data sharing

Until publication of the study results, access to the full dataset will be limited to the Study Management Group and to authors of the publication. At the end of the study, the pseudo anonymised dataset will be prepared and sent to AstraZeneca in accordance with the contract and Newcastle Upon Tyne NHS Foundation Trust as well as being stored by Newcastle Upon Tyne NHS Foundation Trust.

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15. APPENDICES

15.1 Appendix 1 - One Minute Sit to Stand Test Instructions

Instructions to complete the One-Minute Sit to Stand are listed below. A member of the research team will be present and will talk the patient through the test, recording the pertinent information as needed:

1. Place the back of the stand-high chair against a wall to stop it moving whilst doing the test.

2. Before you start, measure the patient's oxygen levels and heart rate using a pulse oximeter and measure the patient's breathlessness using the BORG breathlessness scale. Record these results.

3. Set a timer for one minute.

4. Make sure the patient is sat down in the chair so that their feet are flat on the floor.

5. Then ask them to put their hands on their hips, ensuring that they hang by their sides or they can alternatively hold them loosely together.

6. Ask them to stand up from the chair until their legs are completely straight – making sure that they do not use their hands or arms to help themselves. Then ask them to sit back down again. This counts as one sit to stand.

7. Ask the patient to continue sitting up and down on the chair as many times as they can in one minute.

8. Rest for a few seconds if they need to during the test, and then ask them to carry on if they can.

9. Let the patient know that they can stop the test at any time if they feel unwell, have chest pain, dizziness or severe breathlessness.

10. When the test is finished (after one minute), write down how many sit to stand exercises they have completed.

11. Then measure the patient's heart rate and oxygen levels using the pulse oximeter and their breathlessness using the BORG scale.

12. Write down these results.

Notes: This test is undertaken twice, once at each of the patch fitting assessments. The test should be completed after the patch is fitted.

If a patient does not complete the full minute, record the number of sit to stand repetitions they completed. Do not ask the patient to repeat the test.

The Borg scale:

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (maximal)

15.2 Appendix 2 - Kansas City Cardiomyopathy Questionnaire (KCCQ-23)

This is a questionnaire developed for participants with congestive HF (Green et al. 2000). It is a 23-item, self-administered health status measure that quantifies physical limitations, symptoms, social interference, self-efficacy and quality of life. Results for each domain are summarised and transformed to a score of 0 to 100; higher scores indicate better health status. To summarise the multiple domains of health status quantified by KCCQ, an overall summary score (KCCQ-os), has been developed that includes the physical limitation, symptoms, quality of life and social interference domains of KCCQ. See example below

Date Completed:___ ___/___ ___ ___/___ ___ ___ ___ (dd/MMM/yyyy)

Cardiomyopathy Questionnaire (Kansas City)

The following questions refer to your **heart failure** and how it may affect your life. Please read and complete the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

1. **Heart failure** affects different people in different ways. Some may mainly feel shortness of breath while others mainly fatigue. Please indicate how limited you have been by **heart failure** (for example, shortness of breath or fatigue) in your ability to do the following activities over the past 2 weeks.

Please put an **X** in one box on each line

Activity	**Extremely** limited	**Quite a bit** limited	**Moderately** limited	**Slightly** limited	**Not at all** limited	**Limited for other reasons or did not do the activity**
Dressing yourself	☐	☐	☐	☐	☐	☐
Showering or having a bath	☐	☐	☐	☐	☐	☐
Walking 100 yards on level ground	☐	☐	☐	☐	☐	☐
Doing gardening, housework or carrying groceries	☐	☐	☐	☐	☐	☐
Climbing a flight of stairs without stopping	☐	☐	☐	☐	☐	☐
Jogging or hurrying (as if to catch a bus)	☐	☐	☐	☐	☐	☐

2. Compared with 2 weeks ago, have your symptoms of **heart failure** (for example, shortness of breath, fatigue, or ankle swelling) changed?

My symptoms of **heart failure** are now…

Much worse	Slightly worse	Not changed	Slightly better	Much better	I've had no symptoms over the last 2 weeks
☐	☐	☐	☐	☐	☐

KCCQ - UK/English

Date Completed:___ ___/___ ___ ___/___ ___ ___ ___ (dd/MMM/yyyy)

3. Over the past 2 weeks, how many times have you had **swelling** in your feet, ankles or legs when you woke up in the morning?

Every morning	3 or more times a week, but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐

4. Over the past 2 weeks, how much has **swelling** in your feet, ankles or legs bothered you?

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no swelling**
☐	☐	☐	☐	☐	☐

5. Over the past 2 weeks, on average, how many times has **fatigue** limited your ability to do what you wanted?

All of the time	Several times a day	At least once a day	3 or more times a week but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐	☐	☐

6. Over the past 2 weeks, how much has your **fatigue** bothered you?

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no fatigue**
☐	☐	☐	☐	☐	☐

7. Over the past 2 weeks, on average, how many times has **shortness of breath** limited your ability to do what you wanted?

All of the time	Several times a day	At least once a day	3 or more times a week but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐	☐	☐

KCCQ - UK/English

Date Completed:___ ___/___ ___ ___/___ ___ ___ ___ (dd/MMM/yyyy)

8. Over the past 2 weeks, how much has your **shortness of breath** bothered you?

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no shortness of breath**
☐	☐	☐	☐	☐	☐

9. Over the past 2 weeks, on average, how many times have you been forced to sleep sitting up in a chair or with at least 3 pillows to prop you up because of **shortness of breath**?

Every night	3 or more times a week, but not every night	1-2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐

10. **Heart failure** symptoms can worsen for a number of reasons. How sure are you that you know what to do, or whom to call, if your **heart failure** gets worse?

Not at all sure	**Not very** sure	**Somewhat** sure	**Mostly** sure	**Completely** sure
☐	☐	☐	☐	☐

11. How well do you understand what things you are able to do to keep your **heart failure** symptoms from getting worse (for example, regularly weighing yourself, eating a low salt diet etc.)?

Do not understand at all	Do not understand very well	Somewhat understand	Mostly understand	Completely understand
☐	☐	☐	☐	☐

12. Over the past 2 weeks, how much has your **heart failure** limited your enjoyment of life?

It has **extremely** limited my enjoyment of life	It has limited my enjoyment of life **quite a bit**	It has **moderately** limited my enjoyment of life	It has **slightly** limited my enjoyment of life	It has **not limited** my enjoyment of life at all
☐	☐	☐	☐	☐

KCCQ - UK/English

Date Completed:___ ___/___ ___ ___/___ ___ ___ ___ (dd/MMM/yyyy)

13. If you had to spend the rest of your life with your **heart failure** the way it is right now, how would you feel about this?

Completely dissatisfied	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

14. Over the past 2 weeks, how often have you felt discouraged or down in the dumps because of your **heart failure**?

I have felt that way **all of the time**	I have felt that way **most of the time**	I have **occasionally** felt that way	I have **rarely** felt that way	I have **never** felt that way
☐	☐	☐	☐	☐

15. How much does your **heart failure** affect your lifestyle? Please indicate how your **heart failure** may have limited your participation in the following activities over the past 2 weeks.

Please put an **X** in one box on each line

Activity	**Extremely** limited	**Quite a bit** limited	**Moderately** limited	**Slightly** limited	**Not at all** limited	**Limited for other reasons or did not do the activity**
Hobbies, recreational activities	☐	☐	☐	☐	☐	☐
Working or doing household chores	☐	☐	☐	☐	☐	☐
Visiting family or friends	☐	☐	☐	☐	☐	☐
Intimate or sexual relationships	☐	☐	☐	☐	☐	☐

KCCQ - UK/English

15.3 Appendix 3 - Amendment History

Amendment Number	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	**1.1**	**31/01/2024**	**Dr Visvesh Jeyalan**	**- "X" removed from MRI row of Day 180 visit within schedule of events table as no MRI scan will be done at this time point.** **- A sentence has been added to section "7.3.10 MRI" to clarify that the MRI scan will not be part of the patient's routine care. A sentence has been added to state that a patient will not be recruited if they have had an MRI scan within the last 30 days.**
2	**1.2**	**25/04/2024**	**Dr Visvesh Jeyalan**	**There is a typographical error within the exclusion criteria. The first exclusion has no number associated with it. The first inclusion criteria requires the No.1 and subsequent exclusion criteria needs to be numbered accordingly. There is no change to the individual exclusion criteria itself**

Enter all amendments to the protocol here.