SERCA2a Gene Therapy as an Anti-arrhythmic in Patients with Advanced Heart Failure

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A thesis submitted for the degree of Doctor of Philosophy

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Chapter 0

Declarations, acknowledgements and abstract
0.1 Declaration of Originality

I, Carl Hayward, hereby declare that this thesis contains the results of my own work except where otherwise acknowledged. The studies described in this thesis were conceived and designed with the assistance of my supervisor Dr Alexander Lyon. In addition, I acknowledge the invaluable assistance from Dr Hitesh Patel and Miss Sophie Welch in the recruitment of participants and Dr Jennifer Simonotto in her assistance with Matlab programming. Information derived from the work of others and discussed in this thesis is referenced in the text and listed in the bibliography. No part of this thesis has previously been submitted in application for a higher degree. Publications arising from this work are listed on Page 5.
0.2 Publications arising from this work

Publications:


Abstracts:


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0.4 Acknowledgements

I am grateful to my supervisor Dr Alexander Lyon for his support, guidance and mentorship throughout my studies. I thank the entire team at the Biomedical Research Unit, Royal Brompton Hospital, for their support and expertise. I would like to acknowledge the National Institute of Health Research Cardiovascular Biomedical Research Unit at the Royal Brompton Hospital which funded this body of work. I am grateful to each patient who took part in these studies without whom this thesis would not be possible.

I would like to thank my parents without whom I would not be have been able to become a Doctor. I would like to thank my wife for all of her support during the difficult and busy times. In particular I would like to thank my Mother-in-law and Father-in-law for childcare duties, without whom this PhD would not have been possible.
0.5 Abstract

Despite advances in pharmacological therapy, mortality and morbidity in heart failure remain high. Numerous underlying molecular abnormalities exist in the failing myocyte, of particular importance are those leading to deranged calcium handling. Gene therapy is an approach that can target these molecular abnormalities directly and a target that has received significant interest is sarcoplasmic (endoplasmic) reticulum calcium ATPase 2a (SERCA2a) which is down regulated in heart failure. There is laboratory and early clinical evidence that SERCA2a gene therapy mediated through adeno-associated virus serotype 1 (AAV1) can improve a number of measures of cardiac function, reduce cardiac alternans and reduce ventricular arrhythmias. T wave alternans (TWA) is a non-invasive marker of arrhythmia risk and is linked to abnormalities of calcium handling. As such, TWA may be a useful tool to assess the effect of AAV1-mediated SERCA2a gene therapy (AAV1.SERCA2a) on arrhythmia risk in a heart failure population.

I conducted a number of studies in an attempt to refine the TWA technique with the aim of using TWA as an efficacy measure of AAV1.SERCA2a. The calcium upregulation by percutaneous administration of gene therapy in cardiac disease-2 (CUPID-2) study was a multi-national, multi-centre, placebo controlled randomised study recruiting 250 patients to assess if intracoronary AAV1.SERCA2a was effective at reducing heart failure hospitalisations in advanced heart failure. As a sub-study of the CUPID-2 trial I conducted the CUPID-2 arrhythmia sub-study in patients with an implantable cardioverter defibrillator (ICD). I investigated whether treatment with AAV1.SERCA2a reduced appropriate ICD therapies and separately I examined if AAV1.SERCA2a reduced TWA in those patients recruited at our site. I found that gene therapy trials are challenging to set up as a result of the number of legislative, safety and governance approvals that are required. Furthermore, gene therapy studies using
AAV1 are difficult to recruit to as more than half of patients are ineligible due to the presence of neutralising antibodies to the viral vector. I found that delivering AAV1.SERCA2a could be performed safely at our site. AAV1.SERCA2a gene therapy did not reduce ICD therapy or TWA as was expected after the publication of neutral results in the main CUPID-2 trial. I found that a refinement of the TWA technique of examining multiple leads of the ECG could alter interpretation of TWA and could be tested as an approach to improve the prognostic value of TWA.
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Abbreviations

6MWD 6 minute walk distance.
AADs anti-arrhythmic drugs.
AAV adeno-associated virus.
ABCD alternans before cardioverter defibrillator.
AC adenyl cyclase.
ACEi angiotensin converting enzyme inhibitor.
AE Adverse event.
AECG ambulatory electrocardiogram.
AF atrial fibrillation.
AGENT-HF AAV1.SERCA2a GENe Therapy Trial in Heart Failure.
APD action potential duration.
APDA action potential duration alternans.
AR Adverse reaction.
ARB angiotensin II receptor blocker.
ARNi angiotensin receptor neprilysin inhibitor.
ARs adrenoreceptors.
ATP anti-tachycardia pacing.
ATRAMI Autonomic Tone and Reflexes After Myocardial Infarction.
BNP B-type natriuretic peptide.
bpm beats per minute.
Ca$^{2+}$ calcium.
cAMP cyclic adenosine monophosphate.
CI confidence interval.
CIBIS-II The Cardiac Insufficiency Bisoprolol Study II.
CRT cardiac resynchronisation therapy.
CRT-D cardiac resynchronisation therapy defibrillator.
CRT-P cardiac resynchronisation therapy pacemaker.
CUPID Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease.
CUPID-2 Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease-2.
DANISH  Danish Study to Assess the Efficacy of ICDs in Patients with Non-ischemic Systolic Heart Failure on Mortality.

DBP  diastolic blood pressure.

DCCV DC cardioversion.

DRP  DNase-resistant particles.

ECC  excitation-contraction-coupling.

ECG  electrocardiogram.

EPHESUS  Eplerenone PostAcute Myocardial Infarction Heart Failure Efficacy and Survival Study.

EPS  electrophysiology study.

ESC  European Society of Cardiology.

EVADEF  Evaluation Médico-Economique du Défibrillateur Automatique Implantable.

FFT  Fast Fourier Transform.

FINCAVAS  Finnish Cardiovascular Study.

GMO  genetically modified organism.

GRK  G protein-coupled receptor kinases.

GTAC  Gene Therapy Advisory Committee.

GTN  glycercyl trinitrate.

HR  hazard ratio.

HUT  head-up tilt.

I-1  protein phosphatase inhibitor 1.

ICD  implantable cardioverter defibrillator.

IMP  investigational medicinal product.

KCCQ  Kansas City Cardiomyopathy Questionnaire.

KO  knock-out.

LBBB  left bundle branch block.

LV  left ventricular.

LVAD  left ventricular assist device.

LVEDP  left ventricular end diastolic pressure.

LVEF  left ventricular ejection fraction.

LVESV  left ventricular end systolic volume.

MADIT-II  Multicenter Automatic Defibrillator Implantation Trial II.
MADIT-RIT Multicenter Automatic Defibrillator Implantation Trial-Reduce Inappropriate Therapy.


MHRA Medicines and Healthcare Products Regulatory Agency.

MI myocardial infarction.

MLWHFQ Minnesota Living with Heart Failure Questionnaire.

MMA modified moving average.

MRA mineralocorticoid receptor antagonist.

nAbs neutralising antibodies.

NCX sodium calcium exchange.

NICE National Institute for Health and Care Excellence.

NPV negative predictive value.

NSVT non-sustained VT.

NT-proBNP N-terminal pro B-type natriuretic peptide.

NYHA New York Heart Association.

OPTIC Optimal Pharmacological Therapy in Cardioverter Defibrillator Patients.


PLN phospholamban.

PP1 protein phosphatase 1.

PPV positive predictive value.

QRSd QRS duration.

rAAV recombinant adeno-associated virus.

REC research ethics committee.

REFINE ICD Risk Estimation Following Infarction Noninvasive Evaluation.

RYR ryanodine receptor.

SAR Serious Adverse Reaction.

SBP systolic blood pressure.

SCD sudden cardiac death.

SCD-HeFT Sudden Cardiac Death in Heart Failure Trial.

SDF-1 stromal cell-derived factor 1.

SERCA-LVAD SERCA2a Gene Therapy in LVAD Patients.
**SERCA2a**  sarcoplasmic (endoplasmic) reticulum calcium ATPase 2a.

**SOLVD**  Studies of Left Ventricular Dysfunction.

**SR**  sarcoplasmic reticulum.

**STOP-HF**  Stromal Cell-Derived Factor-1 Plasmid Treatment for Patients with Heart Failure.

**SUMO1**  small ubiquitin-like modifier type 1.

**SUMOs**  Small ubiquitin-like modifiers.

**SUSAR**  Suspected unexpected serious adverse reaction.

**TTT**  tilt table test.

**TWA**  T wave alternans.

**TWAI**  T wave alternans index.

**VF**  ventricular fibrillation.

**VT**  ventricular tachycardia.
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Chapter 1

Introduction

1.1 Chronic Heart Failure

1.1.1 Definition of Chronic Heart Failure

Heart failure is a clinical syndrome comprising typical symptoms, signs and structural and/or functional cardiac abnormalities. Typical symptoms include breathlessness, orthopnoea, paroxysmal nocturnal dyspnoea and peripheral fluid retention manifested as ankle oedema or ascites. Clinical signs include tachycardia, elevated jugular venous pressure, peripheral oedema, pulmonary oedema and a third heart sound. This definition of heart failure, which is included in the European Society of Cardiology (ESC) guidelines [1], excludes asymptomatic patients who have structural and/or functional cardiac abnormalities. These patients are, however, an important cohort as they have poor outcomes and may benefit from treatment [2].

1.1.2 Diagnosis of Chronic Heart Failure

The diagnosis of heart failure is made in the presence of typical clinical signs and symptoms along with an underlying structural or functional abnormality of the heart. Echocardiography
Table 1.1: European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure 2016. Table illustrating the new classifications of heart failure based on ejection fraction (EF). Elevated levels of natriuretic peptides: BNP > 35 pg/ml and/or NT-proBNP > 125 pg/mL. (reproduced with permission).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>HF with reduced EF</th>
<th>HF with mid-range EF</th>
<th>HF with preserved EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Symptoms +/- Signs</td>
<td>Symptoms +/- Signs</td>
<td>Symptoms +/- Signs</td>
</tr>
<tr>
<td>2</td>
<td>LVEF &lt; 40%</td>
<td>LVEF 40-49%</td>
<td>LVEF ≥ 50%</td>
</tr>
<tr>
<td>3</td>
<td>1. Elevated levels of natriuretic peptides; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction</td>
<td>1. Elevated levels of natriuretic peptides; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction</td>
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</tr>
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</table>

is a common modality used to determine if there are underlying structural or functional abnormalities. Furthermore, echocardiography can quantify the severity of any abnormalities and may be able to identify a possible aetiology of heart failure. Echocardiography can also classify the functional abnormalities as predominantly systolic or diastolic and can make an assessment of severity of heart failure. The latter two points are often simplified in to the measurement of the left ventricular ejection fraction (LVEF). The LVEF is the proportion of blood that leaves the heart, relative to end diastole, each heart beat. Until recently, patients with a low LVEF would be classified as having heart failure with reduced ejection fraction (predominantly systolic heart failure) and patients with a “normal” LVEF would be classified as having heart failure with preserved ejection fraction (predominantly diastolic heart failure). The LVEF cut-point used to make this distinction varies. The new ESC guidelines 2016 have redefined this nomenclature [1], as can be seen in Table 1.1, to allow for a third category that used to represent the grey zone between preserved and reduced ejection fraction: heart failure with mid-range EF (LVEF 40-49%).

Elevated natriuretic peptide levels are required as part of the diagnosis of heart failure with mid-range and preserved ejection. Natriuretic peptides are released by the ventricle when the ventricle is subjected to stretch. B-type natriuretic peptide (BNP) is initially synthesised as a
preprohormone (preproBNP) encoded by the gene NPPB. This is converted to proBNP which in turn is cleaved to produce BNP and N-terminal pro B-type natriuretic peptide (NT-proBNP). In addition to contributing to the diagnosis of heart failure with mid-range or preserved ejection fraction BNP and NT-proBNP have clinical utility as a screening test to rule out heart failure when levels are normal, and as a prognostic marker for heart failure. Research studies also commonly use natriuretic peptides as an inclusion criterion and outcome measure.

1.1.3 Epidemiology of Chronic Heart Failure

Assessing the true prevalence of heart failure is challenging because there are differences in the definition of the condition between studies. Estimates in European countries are around 1-2% but rise to over 10% in those over 70 years of age [3, 4].

1.1.4 Pharmacological Treatment of Chronic Heart Failure

The treatment of chronic heart failure is to first address any potentially reversible causes. Following this, there are lifestyle measures, pharmacological therapies and device therapies that are proven to improve outcomes in chronic heart failure. Life style measures that are recommended may include advice to stop or reduce alcohol intake, smoking cessation, healthy diet including reduced salt, weight loss and exercise (possibly in the form of cardiac rehabilitation.

A number of pharmacological therapies are available that have demonstrated significant improvement in survival and/or quality of life. The main therapies available are the angiotensin converting enzyme inhibitor (ACEi), angiotensin II receptor blocker (ARB), β-blocker, mineralocorticoid receptor antagonist (MRA) and most recently the angiotensin receptor neprilysin inhibitor (ARNi) (a combination of an ARB and a neprilysin inhibitor). A summary
of the effect that these therapies on the relative risk of mortality in their respective studies is illustrated in Figure 1.1.

**Figure 1.1:** Relative risk reduction for therapies with proven prognostic value in chronic heart failure. Data derived from: Studies of Left Ventricular Dysfunction (SOLVD) [2], Candesartan in Heart Failure: Assessment of Reduction in Mortality and morbidity-Alternative (CHARM-Alt) [5], Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) [6], Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF) [7], The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) [8], Randomized Aldactone Evaluation Study (RALES) [9], Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) [10], Prospective Comparison of ARNI [Angiotensin ReceptorNeprilysin Inhibitor] with ACEI [Angiotensin-ConvertingEnzyme Inhibitor] to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) [11]. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ARNi, angiotensin receptor neprilysin inhibitor; MRA, mineralocorticoid receptor antagonist. Modified from data presented at the European Society of Cardiology Congress 2014, McMurray.

Relative risk reduction is a useful measure of the effect of a drug in a population compared to placebo or some other comparative treatment. However, presenting the information as displayed in Figure 1.1 may give a misleading comparison of the different therapies with each other. The trials of the different agents were performed in different populations at different baseline risk and taking different medical therapy. For example, in the Studies of Left Ventricular Dysfunction (SOLVD) study testing the effectiveness of enalapril, only 7.6% of patient were already taking β blockers because there were yet to be proven effective. On the other hand, in the The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) trial, 96% of patients were
already taking an ACEi or ARB so the effect of bisoprolol was in addition to that which was already being provided by the concomitant ACEi/ARB. Instead of considering relative risk, one can examine absolute risk and consider differences in baseline therapy for patients in these clinical trials. Figure 1.2 displays the absolute all-cause mortality rate averaged over the respective trial follow-up to create an annual mortality rate for the treatment and control arms of important clinical trials that each demonstrated mortality improvements with their respective treatments. Annotated on the chart are the baseline therapies that patients were receiving as an attempt to aid interpretation of these results in the context of the baseline prognostic treatment the patients were already receiving. For example, it can be seen from the recently published Prospective Comparison of ARNI [Angiotensin ReceptorNeprilysin Inhibitor] with ACEI [Angiotensin-ConvertingEnzyme Inhibitor] to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) study that sacubitril/valsartan demonstrated a relative risk reduction in all-cause mortality of 16% on top of patients receiving good prognostic therapy and there being a low event rate in the control group.

### 1.1.5 Implantable Cardioverter Defibrillators in Chronic Heart Failure

Numerous clinical trials support the use of an implantable cardioverter defibrillator (ICD) to improve mortality in patients with heart failure (Table 1.2). Indications for the implantation of an ICD can be split into primary or secondary prevention. Secondary prevention applies to patients who have survived ventricular fibrillation (VF) or haemodynamically compromising ventricular tachycardia (VT) (outside the context of a clearly reversible cause). Three randomised trials examined the efficacy of ICDs in the setting of secondary prevention [12–14] and when these trials were combined in a meta-analysis, a statistically significant reduction in mortality was demonstrated in those implanted with an ICD [15]. The largest benefit was
Figure 1.2: Averaged annual absolute mortality rate in heart failure trials. Concomitant prognostic treatments prescribed also displayed. Data derived from: Studies of Left Ventricular Dysfunction (SOLVD) [2], The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) [8], Randomized Aldactone Evaluation Study (RALES) [9], Prospective Comparison of ARNI [Angiotensin ReceptorNeprilysin Inhibitor] with ACEI [Angiotensin-ConvertingEnzyme Inhibitor] to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) [11]. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ICD, implantable cardioverter defibrillator; MRA, mineralocorticoid receptor antagonist.

observed in patients with a reduced ejection fraction.

When considering primary prevention of life threatening ventricular arrhythmias the sub group of heart failure patients generally studied are those with severe left ventricular (LV) systolic dysfunction. Two large randomised controlled trials assessing the effect of ICDs in patients with severe LV impairment are the Multicenter Automatic Defibrillator Implantation Trial II (MADIT-II) and the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT). The MADIT-II trialists recruited patients with ischaemic cardiomyopathy and an LVEF <30% and over a
mean follow-up of 20 months demonstrated a 31% relative risk reduction in mortality. The SCD-HeFT trialists recruited patients with ischaemic and non-ischaemic cardiomyopathy with an LVEF < 35% and demonstrated a relative risk reduction of 23% over a median follow-up of 46 months [16, 17]. Patients with ischaemic cardiomyopathy are at greater risk of sudden death than those with dilated cardiomyopathy. Despite this, a meta-analysis of studies examining the efficacy of ICDs in patients with non-ischaemic cardiomyopathy demonstrated a mortality reduction with ICDs [18]. With this in mind, no distinction was made in the most recent National Institute for Health and Care Excellence (NICE) guidelines for indications for ICD implantation between those with ischaemic and non-ischaemic cardiomyopathy. However, a recently published randomised controlled trial assessing the efficacy of ICDs specifically in those with non-ischaemic cardiomyopathy failed to demonstrate a benefit of ICDs [19]. This study will be discussed in more detail later but the likely explanation for the neutral results in this study was the low event rate.

1.1.6 Cardiac Resynchronisation Therapy in Chronic Heart Failure

The first case series assessing the effect of cardiac resynchronisation therapy (CRT) was in the early 1990s [20]. The technique involved implantation of an endocardial right ventricular lead and one epicardial lead on the LV free wall. Techniques and equipment were developed to allow a totally transvenous approach by the late 1990s [21, 22].

Since these early trials, numerous clinical trials now support the use of CRT in selected patients both in the form of a cardiac resynchronisation therapy defibrillator (CRT-D) and cardiac resynchronisation therapy pacemaker (CRT-P) (Table 1.3). Patients targeted for this therapy are those who, despite optimal medical management, have symptomatic severe LV impairment with evidence of electrical dysynchrony [23–28]. Electrical dysynchrony in this context manifests as
<table>
<thead>
<tr>
<th>Study name</th>
<th>Inclusion criteria</th>
<th>Patient characteristics (%)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Secondary prevention trials</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVID (ICD vs amiodarone or sotalol) n=1,016</td>
<td>Resuscitated from VF VT with syncope Sustained VT with LVEF =40% and haemodynamic compromise Excluded if within 72 hours of MI, electrolyte imbalance</td>
<td>Average age 65 years Average LVEF 32% 42% without chronic heart failure</td>
<td>5 year follow-up Improved survival in ICD group (P&lt;0.02) At 1,2 and 3 year follow-up 39%, 27% and 31% reduction in mortality rate ICD group had more use of betablockers</td>
</tr>
<tr>
<td>CIDS (ICD vs amiodarone) n=631</td>
<td>Documented VF Out of hospital arrest requiring defibrillation Sustained VT causing syncope VT &gt;150 bpm with presyncope and LVEF =35% Syncope with induced monomorphic VT</td>
<td>Average age 63 years Average LVEF 33.5% 50% without chronic heart failure</td>
<td>3 year follow-up No significant difference in mortality with ICD vs amiodarone (8.3% vs 10.2% P=0.142)</td>
</tr>
<tr>
<td>CASH (ICD vs amiodarone or metoprolol) n=288</td>
<td>Resuscitated arrest from VT or VF Excluded if within 72 hours of MI, cardiac surgery or electrolyte imbalance</td>
<td>Average age 58 years Average LVEF 46%</td>
<td>Mean follow-up 57 months Non-significant reduction in mortality in ICD group vs medical therapy (36.4% vs 44.4% P=0.081) Significant reduction in sudden death in ICD group (13% vs 33% P=0.005)</td>
</tr>
<tr>
<td><strong>Secondary prevention trials</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MADIT-II (ICD vs medical therapy) n=1,232</td>
<td>Ischaemic cardiomyopathy LVEF =30% =1 month post-MI</td>
<td>Average age 65 years Average LVEF 23%</td>
<td>Mean follow-up 20 months Significant reduction in mortality in ICD group vs medical group (14.2% vs 19.8% P=0.016, HR 0.69)</td>
</tr>
<tr>
<td>SCD-HeFT (ICD vs placebo or amiodarone) n=2,521</td>
<td>Ischaemic and non-ischaemic cardiomyopathy LVEF=35%</td>
<td>Median age 60 years Median LVEF 25%</td>
<td>Median follow-up 46 months Significant reduction in mortality in ICD group (HR 0.77, P=0.007)</td>
</tr>
<tr>
<td>DANISH (ICD vs standard therapy) n=1,116</td>
<td>Non-ischaemic cardiomyopathy</td>
<td>Median age 63 years Median LVEF 25%</td>
<td>Median follow-up 68 months No significant difference in mortality (P=0.28) Significant reduction in sudden cardiac death in the ICD group (HR 0.50, P=0.005)</td>
</tr>
</tbody>
</table>

**Table 1.2:** Summary of clinical trials evaluating implantable cardioverter defibrillators. HR; hazard ratio, ICD; implantable cardioverter defibrillator, LVEF; left ventricular ejection fraction, MI; myocardial infarction, VF; ventricular fibrillation, VT; ventricular tachycardia
<table>
<thead>
<tr>
<th>Trial</th>
<th>Design</th>
<th>n</th>
<th>Population studied</th>
<th>Primary endpoint</th>
<th>Primary endpoint met</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUSTIC 2001</td>
<td>CRT-P/med crossover</td>
<td>29/29</td>
<td>NYHA II-IV</td>
<td>Mean LVEF (%)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean QRSd (ms)</td>
<td>174</td>
</tr>
<tr>
<td>PATH-CHF 2002</td>
<td>RV/LV/CRT-P crossover</td>
<td>41</td>
<td>NYHA III-IV</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>MIRACLE 2002</td>
<td>CRT-P/med</td>
<td>228/225</td>
<td>NYHA III-IV</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>MIRACLE-ICD 2003</td>
<td>CRT-D/ICD</td>
<td>187/182</td>
<td>NYHA III-IV</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>COMPANION 2004</td>
<td>CRT-D/CRT-P/ICD</td>
<td>617/595/308</td>
<td>NYHA III-IV</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>CARE-HF 2005</td>
<td>CRT-P/med</td>
<td>409/404</td>
<td>NYHA III-IV</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>REVERSE 2008</td>
<td>CRT-P/ICD</td>
<td>419/191</td>
<td>NYHA I-II</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>MADIT-CRT 2009</td>
<td>CRT-D/ICD</td>
<td>1,089/731</td>
<td>NYHA I-II</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>162</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.3: Summary of clinical trials evaluating cardiac resynchronisation therapy in heart failure

6MWD; six minute walk distance, CRT-P; cardiac resynchronisation therapy pacemaker, CRT-D; cardiac resynchronisation therapy defibrillator, CV; cardiovascular, HF; heart failure, ICD; implantable cardioverter defibrillator, LVEF; left ventricular ejection fraction, LV; left ventricular, med; medical therapy, QoL; quality of life, NYHA; New York Heart Association

a QRS duration of >120ms, commonly in the form of left bundle branch block.

Clinical trials of cardiac resynchronisation therapy

Moderate to severe heart failure

The safety and efficacy of CRT was rst demonstrated in the early 2000s in patients with moderate-to-severe heart failure. The Pacing Therapies in Congestive Heart Failure (PATH-CHF) [29] and the Multisite Stimulation in Cardiomyopathy (MUSTIC) [30] studies both demonstrated safety and efficacy of CRT in patients with New York Heart Association (NYHA) class 3 or 4 symptoms of heart failure. These studies demonstrated an increase in quality of life scores, walking distance and peak VO2. The Multicenter InSynch Randomised
Clinical Evaluation (MIRACLE) study was the first prospective, randomised, double-blinded CRT trial [23]. Patients were recruited with severe LV impairment (LVEF ≤35%), NYHA class 3 or 4 symptoms of heart failure, and a QRS duration (QRSd) >130ms. Patients in the CRT arm of the trial experienced improvements in 6 minute walk distance (6MWD), NYHA functional class, peak VO2 and LVEF. Furthermore, there was a reduction in hospitalisations for heart failure. This study was expanded to include the addition of CRT to those receiving an ICD [31]. Patients underwent CRT-D implantation and were randomised to having CRT turned on or off. Similar improvements to those seen in the main MIRACLE study were experienced by those with CRT turned on. The Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure (COMPANION) [24] trial compared the efficacy of CRT-P, CRT-D and optimal medical therapy. Patients were recruited with severe LV impairment (LVEF ≤35%), NYHA class 3 or 4 symptoms and a QRSd ≥120ms. Both CRT-D and CRT-P arms demonstrated a significant reduction in the primary composite endpoint of all-cause mortality and all-cause hospitalisation. Finally, the Cardiac Resynchronization in Heart Failure trial (CARE-HF) compared CRT-P with medical therapy [25]. Patients were recruited with severe LV impairment (LVEF ≤35%), NYHA class 3 or 4 symptoms and either a QRSd ≥150ms or a QRSd of 120 to 149ms with echocardiography evidence of dysynchrony. Patients with CRT-P experienced a reduction in all-cause mortality and cardiovascular hospitalisations. Furthermore, CRT was associated with improvements in LVEF and reverse remodelling.

Mild heart failure

The Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction (REVERSE) trial [26] and Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT) [28] trial investigated the benefits of CRT in
patients with less severe heart failure. The REVERSE trial recruited patients with moderate LV impairment (LVEF ≤40%), NYHA class 1 or 2 symptoms of heart failure and QRSd ≥120ms. All patients underwent CRT-D implantation and were randomised to CRT-on or CRT-off. Those with CRT-on demonstrated improved LVEF and a reduction in heart failure events. MADIT-CRT enrolled patients with severe LV impairment (LVEF ≤30%) but only mild symptoms (NYHA class 1 or 2). Patients were also required to have a QRSd ≥130ms. This study demonstrated the superiority of CRT-D over ICD with a significant reduction in non-fatal heart failure events.

Effect of cardiac resynchronisation therapy on ventricular arrhythmias in heart failure

The effect of CRT on ventricular arrhythmias has not been fully elucidated. A combined analysis of two clinical trials of CRT failed to demonstrate any benefit of CRT on appropriate ICD therapy [32]. These studies were, however, limited by short follow-up times. Other reports raise concern about a proarrhythmic effect of CRT implantation [33, 34]. It has, however, been demonstrated that the incidence of appropriate ICD shocks is reduced among those with reverse remodelling compared to those without significant remodelling [35]. As such it might be that only those who reverse remodel benefit from a reduction in ventricular arrhythmias.

The REVERSE trial investigated this and found that CRT did not impact on the incidence of ventricular arrhythmias when examining all patients but patients with reverse remodelling had less VT/VF compared to unpaced control subjects [36]. Conversely, those without evidence of reverse remodelling had increased VT/VF (possibly due to chronic right ventricular pacing). This supports the possibility that the effect of CRT on ventricular arrhythmias depends on the balance of competing risks between the proarrhythmic effect of chronic right ventricular pacing and the anti-arrhythmic effect of LV reverse remodelling.

These findings were supported by data from MADIT-CRT which also found that in patients who
experienced reverse remodelling after CRT implantation there was a significant reduction in the risk of ventricular arrhythmias [37].

**Cardiac resynchronisation therapy in specific patient groups**

*Atrial fibrillation*

The majority of the initial large studies of CRT excluded patients with atrial arrhythmias. There are some small studies that suggest those with heart failure and atrial fibrillation (AF) do benefit from CRT [38, 39]. However, these benefits require a high rate of biventricular pacing. If an adequate amount of biventricular pacing cannot be achieved due to rapidly conducted AF (despite medical treatment) then atrioventricular (AV) node ablation should be considered.

*Bundle branch morphology*

Retrospective analysis of CRT trials suggests the greatest benefit of CRT is in those with left bundle branch block (LBBB) versus right bundle branch block or other non-LBBB conduction abnormalities. However, even patients who do not have a typical LBBB pattern may benefit from CRT [40].

**Current guidelines**

The current recommendations from NICE for defibrillators and CRT are displayed in Table 1.4. These guidelines apply to patients with an LVEF of 35% or less and the decision about device therapy is then based on NYHA classification and QRSd. A distinction is made about the morphology of the bundle branch block dependent on the QRSd i.e. if the QRSd is 150ms or greater then the morphology does not matter but any less than this and the morphology should be LBBB to qualify for CRT. No specific guidance is given for what constitutes high risk of sudden cardiac death (SCD) when considering ICD implantation.
<table>
<thead>
<tr>
<th>QRS interval</th>
<th>NYHA class</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;120ms</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>ICD if there is a high risk of sudden cardiac death</td>
</tr>
<tr>
<td></td>
<td>ICD and CRT not clinically indicated</td>
</tr>
<tr>
<td>120-149ms without LBBB</td>
<td>ICD</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
</tr>
<tr>
<td></td>
<td>ICD</td>
</tr>
<tr>
<td></td>
<td>CRT-P</td>
</tr>
<tr>
<td>120-149ms with LBBB</td>
<td>ICD</td>
</tr>
<tr>
<td></td>
<td>CRT-D</td>
</tr>
<tr>
<td></td>
<td>CRT-P or CRT-D</td>
</tr>
<tr>
<td></td>
<td>CRT-P</td>
</tr>
<tr>
<td>≥150ms with or without LBBB</td>
<td>CRT-D</td>
</tr>
<tr>
<td></td>
<td>CRT-D</td>
</tr>
<tr>
<td></td>
<td>CRT-P or CRT-D</td>
</tr>
<tr>
<td></td>
<td>CRT-P</td>
</tr>
</tbody>
</table>

Table 1.4: NICE guidelines for the implantation of defibrillators and cardiac resynchronisation therapy in patients with a left ventricular ejection fraction of 35% or less. CRT-D; cardiac resynchronisation defibrillator, CRT-P; cardiac resynchronisation pacemaker, ICD; implantable cardioverter defibrillator, LBBB; left bundle branch block, NYHA; New York Heart Association

Non-responders to cardiac resynchronisation therapy

There is no consensus on what constitutes a response to CRT. Commonly considered variables include improvement in LV parameters on echocardiography or an improvement in heart failure symptoms. It is generally considered that approximately 30% of patients are non-responders to CRT. Explanations for non-response could include patient selection (e.g. a patient with severe COPD may be more symptomatic from lung disease rather than heart failure), suboptimal LV lead position and suboptimal proportion of biventricular pacing.

1.1.7 Prognosis of Chronic Heart Failure

The pharmacological therapies for chronic heart failure described above have resulted in a dramatic impact upon morbidity and mortality with standardised death rates reducing by 40% between 1987 and 2008 [41] but these rates remain unacceptably high with 30-40% of patients dying within a year of diagnosis, though thereafter the mortality is less than 10% per year [42,43]. The majority of patients with systolic heart failure die from cardiovascular causes with the two main modes of death being pump failure or sudden death. In mild to moderate heart
failure sudden death is the most common [7, 44] while in advanced heart failure pump failure predominates [9, 25](Table 1.5).

<table>
<thead>
<tr>
<th>Trial</th>
<th>n</th>
<th>Annual Mortality(%)</th>
<th>Cardiovascular Death (% of all deaths)</th>
<th>Sudden Death (% of all deaths)</th>
<th>Pump Failure (% of all deaths)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MERIT-HF</td>
<td>3,991</td>
<td>10</td>
<td>91.4</td>
<td>58.3</td>
<td>24.3</td>
</tr>
<tr>
<td>CHARM</td>
<td>2,548</td>
<td>7.5</td>
<td>82.3</td>
<td>40.3</td>
<td>26.4</td>
</tr>
<tr>
<td>RALES</td>
<td>1,663</td>
<td>23</td>
<td>80.5</td>
<td>28.6</td>
<td>47.1</td>
</tr>
<tr>
<td>CARE-HF</td>
<td>813</td>
<td>12.6</td>
<td>71</td>
<td>33.1</td>
<td>44.1</td>
</tr>
</tbody>
</table>

Table 1.5: Modes of death in four heart failure trials. Both pump failure and sudden death are important modes of death with sudden death being more common in mild-moderate heart failure and pump death being more common in advanced heart failure. MERIT-HF, Metropolol CR/XL Randomized Intervention Trial in congestive Heart Failure; CHARM, Candesartan in Heart Failure Assessment of Reduction in Mortality and morbidity; RALES, Randomized Aldactone Evaluation Study; CARE-HF, CArdiac REsynchronization in Heart Failure.

An alternative way of presenting this information is to display the cause of death based on NYHA classification (Figure 1.3)

Figure 1.3: Mode of death by NYHA classification (Reproduced from Hsia et al [45] Biomed central formal permission not required).

Sudden death has a variety of aetiologies including primary arrhythmic events, myocardial infarction (MI), pulmonary embolism and stroke. The precise number of patients dying from arrhythmic deaths is difficult to determine due to the lack of contemporary cardiac monitoring at the time of death. The ability of an ICD to improve mortality in patients with heart failure [16, 17] suggests that arrhythmic events are important.
1.1.8 Atrial Fibrillation in Chronic Heart Failure

The prevalence of AF in the heart failure population increases with age, as it does in those without heart failure. The prevalence of AF is also associated with NYHA classification. The prevalence of AF in heart failure has been shown to increase from 4% to 40% from NYHA 1 to NYHA 4 [46–50].

A causal relationship is difficult to establish since AF can lead to a decompensation in heart failure and increase in symptoms but conversely, a deterioration in heart failure can increase the chance of going in to AF. This makes it challenging to establish if AF independently predicts survival in heart failure. In both the SOLVD trial [2] and CHARM [44] trial AF was shown to be an independent predictor of all-cause mortality. However, the V-HeFT trial [51] did not show a significant difference in survival at two years for those in AF. It remains unknown whether AF is causative in the increased mortality seen or a marker of more advanced heart failure.

A priority in the management of AF in patients with heart failure is anticoagulation in those suitable patients. A decision about rate or rhythm control is likely to be on a patient by patient basis but the AF and Congestive Heart Failure (AF-CHF) trial suggests that a strategy of rhythm control (amiodarone, sotalol or dofetilide) was not better than rate control in 1,276 patients with left ventricular dysfunction randomised to rate or rhythm control [52].

1.1.9 Ventricular Arrhythmias in Chronic Heart Failure

The true incidence of ventricular arrhythmias in patients with heart failure is challenging to define. While some patients may present overtly with a ventricular arrhythmia, either due to symptoms, resuscitated cardiac arrest or following appropriate ICD therapy, a significant proportion of patients may be asymptomatic during a ventricular arrhythmia and this therefore not be recorded. Alternatively, patients may experience SCD which may or may not be due
to ventricular arrhythmias. While it is thought that a large proportion of heart failure patients who die suddenly, do so secondary to a ventricular arrhythmia, this cannot be proven due to the lack of cardiac monitoring. Furthermore, a post-mortem may not be performed to exclude other causes of sudden death (such as stroke, pulmonary embolism or ruptured aneurysm). Despite this, ventricular arrhythmias are relatively common in heart failure and have been documented in up to 85% of patients (including non-sustained VT (NSVT)) with severe heart failure [53].

As SCD is an important mode of death in heart failure, ventricular arrhythmias are an important element to consider. There are various abnormalities in the failing heart that can predispose to ventricular arrhythmias as summarised in Table 1.6. For the purpose of this thesis, calcium (Ca$^{2+}$) cycling will be elaborated upon but Table 1.6 serves as a reminder that Ca$^{2+}$ handling is only one pathological process underlying the failing myocyte that can result in ventricular arrhythmias.

Considering therapeutic interventions to treat ventricular arrhythmias in heart failure, several randomised controlled clinical trials have assessed the efficacy of various anti-arrhythmic drugs (AADs) [7, 8, 17, 53–61]. A summary of the findings from these studies is displayed in Table 1.7.

AADs lack efficacy for treating ventricular arrhythmias in patients with heart failure and may be poorly tolerated due to side effects. In the Optimal Pharmacological Therapy in Cardioverter Defibrillator Patients (OPTIC)) trial, after one year 23.5% of patients discontinued sotalol and 18.2% stopped amiodarone. The longer term side effects of amiodarone which include pulmonary and thyroid toxicity are well established [63]. Beyond the intolerance of AADs there may even be an increase in mortality with AADs [54, 55], possibly due to a pro-arrhythmic effect. This means AADs are not routinely used in heart failure.

ICDs are a non-pharmacological approach to treating ventricular arrhythmias. ICDs have
### Mechanisms Leading to Ventricular Arrhythmias in Heart Failure

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action potential prolongation</td>
<td>A hallmark of failing myocytes and intact ventricular preparations. Does not occur uniformly, leading to heterogeneity of electrical properties. Results in after-depolarisation-mediated triggered activity and functional re-entry resulting in ventricular arrhythmias.</td>
</tr>
<tr>
<td>Alterations in ion channels/currents</td>
<td>Ca(^{2+}): Increased diastolic leak of calcium due to impaired function of the calcium release channel, impaired reuptake in to SR by SERCA2a and upregulation of NCX. Results in triggered activity related to delayed after depolarisations leading to ventricular arrhythmias.</td>
</tr>
<tr>
<td></td>
<td>K(^{-}) currents: ↓ I(<em>{\text{in}}) ↓ I(</em>{\text{ir}}). Promotes early after depolarisations by either ↑ APD, or by ↓ repolarisation reserve</td>
</tr>
<tr>
<td></td>
<td>Na(^{+}): ↓ peak I(_{\text{sc}}) leads to ↑ APD which favours re-entry and early after depolarisations.</td>
</tr>
<tr>
<td>Connexins</td>
<td>Down regulation of Connexins (especially Connexin43,) results in: -conduction slowing, which predisposes to re-entrant arrhythmias -cellular uncoupling which enhances APD heterogeneity favouring re-entry</td>
</tr>
<tr>
<td>Ischaemia from coronary disease or supply demand mismatch in non-ischaemic cardiomyopathy</td>
<td>Ischaemic and infarcted myocardium have regional cellular and tissue remodelling in particular the borderzones of infarct leading to electrical heterogeneity -redistribution of connexins, -elaboration of cytokines -reduced cellular coupling -increased interstitial connective tissue</td>
</tr>
<tr>
<td>Altered neurohormonal signalling</td>
<td>Ventricular biopsy demonstrates increased density and heterogenous density of sympathetic nerves which is associated with ventricular arrhythmias Myocardial infarction destroys sympathetic nerves but this denervation leads to an exaggerated response to catecholamines elsewhere (shortening effective refractory periods) and eases VF inducibility This effect is not just seen in infarcts. Sympathetic denervation can be seen in MIBG scan of patients with non-ischaemic cardiomyopathy Mechanism unclear but may be related to the effect of angiotensin 2, and aldosterone on ion channels</td>
</tr>
<tr>
<td>Genetic predisposition</td>
<td>Population based studies demonstrate familial clustering of events Genetic abnormalities are linked to rare congenital diseases at high risk of SCD such as LQTS but not known in chronic heart failure.</td>
</tr>
</tbody>
</table>

**Table 1.6**: Summary of the various abnormalities observed in heart failure that predispose to ventricular arrhythmias. APD, action potential duration; Ca\(^{2+}\), calcium; I\(_{\text{Kf}}\), inward rectifier potassium current; I\(_{\text{Na}}\), sodium current; I\(_{\text{to}}\), transient outward potassium current; LQTS, long QT syndrome; MIBG, Meta-iodobenzylguanidine ; NCX, Na\(^{+}\)-Ca\(^{2+}\) exchanger; SCD, sudden cardiac death; SR, sarcoplasmic reticulum; VF, ventricular fibrillation.
<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion Criteria</th>
<th>Investigational therapy</th>
<th>Control</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class I anti-arrhythmic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAST [54, 55]</td>
<td>Post-MI ≥6 PVCs/hr, LVEF ≤40%</td>
<td>Flecaïnide, Encainide &amp; Moricizine</td>
<td>Placebo</td>
<td>Arrhythmic death increased in all treatment arms</td>
</tr>
<tr>
<td></td>
<td>Class II anti-arrhythmic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAPRICORN [56]</td>
<td>Post-MI LVEF ≤40%</td>
<td>Carvedilol</td>
<td>Placebo</td>
<td>Death &amp; VAs decreased in carvedilol arms</td>
</tr>
<tr>
<td></td>
<td>Class III anti-arrhythmic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANDROMEDA [57]</td>
<td>NYHA III-IV LVEF ≤35%</td>
<td>Dronedarone</td>
<td>Placebo</td>
<td>Increased mortality &amp; arrhythmias in treatment arm</td>
</tr>
<tr>
<td>CHF-STAT [53]</td>
<td>LVEF ≤40% ≥10 PVCs/hr</td>
<td>Amiodarone</td>
<td>Placebo</td>
<td>No effect</td>
</tr>
<tr>
<td>DIAMOND-MI [58]</td>
<td>Post-MI LVEF ≤35%</td>
<td>Dofetilide</td>
<td>Placebo</td>
<td>No effect on mortality</td>
</tr>
<tr>
<td>EMIAT [59]</td>
<td>Post-MI LVEF ≤40%</td>
<td>Amiodarone</td>
<td>Placebo</td>
<td>Amiodarone reduced arrhythmic death but not total mortality</td>
</tr>
<tr>
<td>MUSTT [60]</td>
<td>Post-MI LVEF ≤30% NSVT</td>
<td>ICD, Class I or class III agents</td>
<td>OMT</td>
<td>Improved survival with ICD no difference with antiarrhythmic therapy</td>
</tr>
<tr>
<td>SCD-HeFT [17]</td>
<td>NYHA II-III LVEF ≤35%</td>
<td>ICD Amiodarone</td>
<td>Placebo</td>
<td>Improved survival with ICD no effect of amiodarone</td>
</tr>
<tr>
<td>SWORD [61]</td>
<td>Post-MI LVEF &lt;40%</td>
<td>d-Sotalol</td>
<td>Placebo</td>
<td>Increased mortality in treatment arm</td>
</tr>
</tbody>
</table>

Table 1.7: Clinical trials of anti-arrhythmic drugs in patients with heart failure. ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NSVT, non-sustained VT; NYHA, New York Heart Association; OMT, optimal medical therapy; PVCs, premature ventricular complexes; SCD, sudden cardiac death; VAs, ventricular arrhythmias. Reproduced from Baher et al [62].
demonstrated the ability to prevent SCD in patients with heart failure and are indicated for certain groups of heart failure patients for primary and secondary prevention of ventricular arrhythmias [12, 16, 17]. ICDs do however also have drawbacks. There are short and long complications of device implantation, ICD shocks are associated with increased mortality [64], patients may receive inappropriate therapy from an ICD and receiving an ICD shock can have a negative psychological effect on patients [65]. An efficacious anti-arrhythmic therapy without the side effects of AADs or drawbacks of ICDs would be great asset to those treating patients with heart failure. A therapy which normalises the deranged Ca\(^{2+}\) handling known to exist in the failing heart could have just such an effect.

### 1.1.10 Abnormalities of Calcium Cycling in Chronic Heart Failure

The focus of this thesis is on the effect of restoring sarcoplasmic (endoplasmic) reticulum calcium ATPase 2a (SERCA2a) activity and the expected normalisation of Ca\(^{2+}\) cycling. While the abnormalities of Ca\(^{2+}\) cycling play a major role in the development of the heart failure phenotype, it must be placed in the context of the numerous other molecular abnormalities recognised to exist in the failing myocyte. These are summarised in Table 1.8. The role of Ca\(^{2+}\) in excitation-contraction-coupling (ECC) coupling is described in Figure 1.4. Multiple defects in Ca\(^{2+}\) handling are recognised to exist in the failing myocyte. These include prolonged Ca\(^{2+}\) transients indicating slow re-uptake of Ca\(^{2+}\) back in to the sarcoplasmic reticulum (SR) [67] and an elevated resting diastolic cytoplasmic Ca\(^{2+}\) level, both factors that contribute to the impaired ventricular relaxation frequently observed in patients with heart failure. Depletion of the SR Ca\(^{2+}\) store means that there is less Ca\(^{2+}\) available for contraction which contributes to systolic impairment. Furthermore, upregulation of sodium calcium exchange (NCX) in response to elevated cytoplasmic Ca\(^{2+}\) results in a leak of Ca\(^{2+}\)
<table>
<thead>
<tr>
<th>Functional changes</th>
<th>Cardiomyocyte Size and Shape</th>
<th>Surface Topology</th>
<th>Intercellular Communication</th>
<th>Extracellular Matrix</th>
<th>Impaired β-adrenoceptor signaling</th>
<th>Abnormalities of Ca(^{2+}) Handling</th>
<th>Abnormalities of Na(^{+}) Handling</th>
<th>Mitochondrial dysfunction</th>
<th>Increased Apoptosis</th>
<th>Abnormal Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertrophy characteristics dependent on aetiology of heart failure (HF)</td>
<td>Overall spatial disruption leading to delayed excitation-contraction coupling</td>
<td>Reduced electrical and functional coupling</td>
<td>Fibroblasts driven by neurohormonal activation leads to:</td>
<td>β-AR expression and density reduced (predominantly β1-AR is reduced)</td>
<td>Down regulation and reduced activity of SERCA2a</td>
<td>Elevated intracellular [Na(^+)]_. Possible mechanisms:</td>
<td>Reduced phosphocreatine:ATP ratio reflecting impaired mitochondrial function. Increased oxidative stress with increased ROS due to mitochondrial dysfunction. Effects of increased ROS:</td>
<td>Increased apoptosis due to a variety of factors including:</td>
<td>Activation of fetal gene expression. Results in physiology more akin to foetal ventricular myocardium:</td>
</tr>
<tr>
<td></td>
<td>• HF due to pressure overload: increase in width and length of cardiomyocytes</td>
<td>• Loss of Z grooves</td>
<td>• Connexin 43 redistribution away from intercalated discs</td>
<td>• Increased fibroblast number and metabolic activity</td>
<td>• β-AR: β2-AR reduced from around 4:1 in normal hearts to 1:1</td>
<td>• Overall reduction in PLB but relative increase in active (unphosphorylated) fraction thus inhibitory to SERCA2a</td>
<td>• Increased Na(^{+})/K(^{-}) ATPase gene expression or activity</td>
<td>• Increased SR Ca(^{2+}) leak via RyR2 oxidation</td>
<td>• Increased mitochondrial ROS causes mitochondrial dysfunction and ultimately mitochondrial rupture, releasing several pro-apoptotic factors</td>
<td>• Switch from FFA to carbohydrate metabolism</td>
</tr>
<tr>
<td></td>
<td>• HF due to volume overload: predominantly increase in length of cardiomyocytes</td>
<td>• Loss of t-tubule openings</td>
<td>• Connexin expression</td>
<td>• Increased production and deposition of extracellular matrix leading to spatial uncoupling of adjacent myocytes</td>
<td>• Elevated G,G, ratio. Along with the increased importance of β2-AR, which couple to G proteins, this contributes to the β-AR desensitisation and a negatively inotropic effect of β-AR stimulation in chronic heart failure</td>
<td>• Impaired Ca(^{2+}) re-uptake with prolonged Ca(^{2+}) transients with a smaller peak amplitude. Higher resting Ca(^{2+}) concentration in cytoplasm but reduced Ca(^{2+}) stores in the SR leading impaired contraction and relaxation</td>
<td>• Increased Na(^{+}) influx via the Na(^{+})/H(^{-}) exchanger (NHE)</td>
<td>• Impaired Na(^{+})/K(^{-}) ATPase and SERCA2a activity</td>
<td>• Abnormal Ca(^{2+}) homocostasis leads to impaired protein production in the endoplasmic reticulum.</td>
<td>• Changes in sarcolemmal ion channel expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Prolonged action potential duration</td>
<td></td>
<td>• Predisposition to apoptosis</td>
<td>• Increased Na(^{+}) influx via the late Na(^{+}) current</td>
<td>• Predisposition to apoptosis</td>
<td>• Abnormal Ca(^{2+}) sparks</td>
<td>• Alteration of myofilament myosin heavy chain isoforms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Hyperphosphorylation and increased oxidation of RyR2 causing Ca(^{2+}) leak and increased Ca(^{2+}) sparks</td>
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<td></td>
<td>• Increased importance of NCX for Ca(^{2+}) extrusion</td>
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</tbody>
</table>

Table 1.8: Summary of the major changes that occur in the failing ventricular cardiomyocyte. -AR indicates -adrenergic receptor; Ca\(^{2+}\), calcium; FFA, free fatty acid; H\(^{+}\), hydrogen ion; Na\(^{+}\), sodium; Na\(^{+}\)/K\(^{-}\) ATPase, sodium-potassium adenosine triphosphatase; NCX, Na\(^{+}\) Ca\(^{2+}\) exchanger; PLB, phospholamban; ROS, reactive oxygen species; RyR2, ryanodine receptor 2; SERCA2a, sarcoplasmic (endoplasmic) reticulum Ca\(^{2+}\) ATPase 2a; SR, sarcoplasmic reticulum; t-tubule, transverse tubule. Reproduced from Hayward et al [66].

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Figure 1.4: Schematic of a cardiomyocyte showing normal calcium (Ca$^{2+}$) handling during excitation-contraction coupling. (1) Normal Ca$^{2+}$ cycling begins with the cardiac action potential depolarizing the surface membrane and triggering a small Ca$^{2+}$ current into the cytoplasm through L-type Ca$^{2+}$ channels. (2) Triggering of a much larger influx of Ca$^{2+}$ from the sarcoplasmic reticulum (SR) store through the ryanodine receptor (RyR). (3) Calcium-induced calcium release triggers contraction through the binding of Ca$^{2+}$ to the troponin C component of the cardiac myofilaments. (4) During diastole, Ca$^{2+}$ is taken back up into the SR through the action of sarcoplasmic (endoplasmic) reticulum Ca$^{2+}$ ATPase 2a (SERCA2a) and extruded from the cell by the Na$^{+}$-Ca$^{2+}$ exchange (NCX). (5) SERCA2a function is regulated by phospholamban (PLN). Reproduced from Hayward et al [66].

from the cardiomyocyte which can lead to after depolarisations and promote arrhythmias.

1.1.11 SERCA2a in Chronic Heart Failure

The abnormalities in Ca$^{2+}$ handling described above have an impact on multiple aspects of the heart failure phenotype: systolic dysfunction, diastolic dysfunction and arrhythmias. SERCA2a, a pivotal molecule in intracellular Ca$^{2+}$ handling, has received the most attention in heart failure clinical trials, and is also the basis for this thesis. In numerous animal models of heart failure both SERCA2a mRNA and protein levels are reduced [68–70]. Studies in humans with end stage heart failure have also demonstrated consistent reductions in SERCA2a
mRNA [71, 72]. However, variable results have been found examining SERCA2a protein levels in human samples with some reporting reduced protein levels [73–75] while others report no change [76, 77]. This may be a result of the larger variability in measurement of protein concentration compared to mRNA and this is compounded by the small sample sizes available. Furthermore, even if SERCA2a protein levels were unchanged this does not equate to normal SERCA2a function as it is the relative SERCA2a:phospholamban (PLN) ratio which is important in determining the overall function of SERCA2a [78]. Irrespective of whether SERCA2a protein levels are reduced in human heart failure, the activity of SERCA2a has been shown consistently to be reduced in failing myocardium [73, 77, 79]. This association between reduced SERCA2a activity and heart failure does not prove causality. Support for causality can be derived from evidence that SERCA2a knock-out (KO) mice develop heart failure [80], that SERCA2a inhibition by targeting PLN leads to heart failure [81] and that restoring SERCA2a activity through gene therapy (discussed later) results in recovery from heart failure.

Istaroxime is a sodium-potassium adenosine triphosphatase (ATPase) inhibitor which also increases SERCA2a activity and as such is a pharmacological option for increasing SERCA2a activity. In a guinea pig model of heart failure intravenous istaroxime resulted in increased measures of contraction and relaxation. Furthermore, in muscle strips from failing human hearts istaroxime increased contraction and stimulated SERCA2a activity in SR microsomes [82]. Despite the signal from istaroxime for the benefits of increasing SERCA2a activity in heart failure there are, to date, no pharmacological agents that are able to activate SERCA2a with enough efficacy or specificity to consider practical for human therapeutics. One reason for this may be that SERCA2a protein levels are reduced to such an extent that there is little for these agents to target. Gene therapy is well placed to target this specific molecular target (as will be discussed later).
1.2 T Wave Alternans

1.2.1 Cardiac Alternans

An early description of cardiac alternans was provided in 1872 by Ludwig Traube [83]. He described a forty seven year old labourer who was in congestive cardiac failure a little under two months before the patient died. He commented, ”when one palpates the artery more firmly, distinct regular alternations in the height and depth of the pulse are noted” (displayed graphically in Figure 1.5). This was a description of pulsus alternans, one manifestation of mechanical alternans.

![Figure 1.5: Example of pulsus alternans. Reproduced from Classics of Cardiology Vol 2.](image)

When the electrocardiogram (ECG) emerged as a non-invasive means to measure the electrical activity of the heart, another manifestation of cardiac alternans could be identified, namely electrical alternans. This could be demonstrated in terms of alternation of the QRS complex or the ST-T wave. The latter is more commonly now referred to as T wave alternans (TWA). The presence of electrical alternans is associated with electrical instability and a tendency to ventricular arrhythmias [84, 85]. At a cellular level cardiac alternans can also manifest as mechanical alternans (represented as alternation in contraction amplitude) but also as action potential duration (APD) alternans and Ca\(^{2+}\) transient alternans or simply Ca\(^{2+}\) alternans (in which the amount of Ca\(^{2+}\) released from the SR alternates from larger to smaller amounts).

In its various manifestations, cardiac alternans occurring at rest portends a poor prognosis. Interestingly, a physiological property of normal ventricular myocardium is the emergence of
cardiac alternans at elevated heart rates [86]. The critical heart rate required to induce cardiac alternans depends on various environmental factors and the presence of underlying disease. Alternans will occur at lower heart rates in hypothermia [86], hypocalcaemia [87], ischaemia [88, 89], hypertrophy [90] and congestive heart failure [91, 92]. A higher heart rate is required to develop cardiac alternans in the presence of hypercalcaemia [87] digitalis [87] and Ca$^{2+}$ channel antagonists [93].

1.2.2 Pathophysiology of Cardiac Alternans

One can gain an insight into the underlying molecular mechanisms that lead to cardiac alternans from measuring intracellular Ca$^{2+}$ transients. A Ca$^{2+}$ transient is a rapid rise and decline in intracellular Ca$^{2+}$ during the cardiac action potential. The increase in Ca$^{2+}$ is comprised of a small amount of Ca$^{2+}$ that enters the cell through L-type Ca$^{2+}$ channels upon depolarisation of the cell membrane and a large amount of Ca$^{2+}$ released from the SR. Alternation of the Ca$^{2+}$ transient has been demonstrated in both isolated cardiac muscle preparations [94] and isolated perfused hearts [91, 92].

![Figure 1.6: Example of calcium Ca$^{2+}$ transient alternans. Large Ca$^{2+}$ transients in black, small in red. Reproduced from Diaz et al [95] with permission.](image-url)
One may hypothesise that beat-to-beat alternation of Ca\(^{2+}\) release from the SR leads to alternation of inotropic state and so mechanical alternans. Considering that mechanical, APD and Ca\(^{2+}\) alternans may occur simultaneously [94, 96, 97] it is challenging to determine which is the primary abnormality. Supporting the importance of Ca\(^{2+}\) in the generation of mechanical alternans, if Ca\(^{2+}\) release from the SR is blocked by ryanodine then mechanical alternans is abolished [94, 97]. Further support for the importance of the ryanodine receptor (RYR), and by proxy Ca\(^{2+}\) release, comes from a study in which tetracaine or acidosis were used to depress the activity of the RYR and this resulted in alternans [98]. Diaz et al [95] demonstrated in mechanically unloaded rat ventricular myocytes that Ca\(^{2+}\) alternans could be induced when the myocytes were depolarised with a fixed APD. This may suggest that Ca\(^{2+}\) alternans is the primary problem.

An alternative method to reduce the opening probability of RYRs is to use a small depolarising pulse to reduce the L-type Ca\(^{2+}\) trigger. This leads to more homogenous alternans than with tetracaine and allows measures of global SR Ca\(^{2+}\) content to be more interpretable. Using this approach, Diaz et al found that SR Ca\(^{2+}\) load was bigger before a large Ca\(^{2+}\) transient than before a small Ca\(^{2+}\) transient and there was a steep dependence of Ca\(^{2+}\) transient amplitude on SR Ca\(^{2+}\) content. Taken together, these data suggest that cardiac alternans is dependent on Ca\(^{2+}\) alternans which itself is a result of alternating Ca\(^{2+}\) content in the SR. Since Ca\(^{2+}\) load in the SR is intimately linked to SERCA2a activity, this represents a link between SERCA2a activity and the measurement of cardiac alternans (or more specifically for this thesis TWA). Furthermore, there is laboratory evidence that, SERCA2a plays a central role in the molecular mechanism of cardiac alternans [99].
1.2.3 Cardiac Alternans in Heart Failure

It is known in animal experiments that it is easier to induce alternans in animals with heart failure than control animals [91, 92]. As described above, cardiac alternans may primarily be propagated by properties of Ca\(^{2+}\) cycling. Significant abnormalities of Ca\(^{2+}\) cycling are recognised to exist in heart failure (as described in section 1.1.10) and this may explain why failing hearts are predisposed to develop alternans.

The clinical relevance of this, beyond anecdotal case histories, was demonstrated in a clinical study of 15 participants in 1915 showing that in patients with heart failure, the presence of continuous pulsus alternans was associated with a 1-year mortality of 53% [100]. Measuring mechanical alternans in man can be challenging, beyond identifying pulsus alternans which may be an end-stage form of cardiac alternans. It is not feasible to measure APD alternans in vivo in heart failure patients so the simplest way of assessing for cardiac alternans in patients with heart failure is by using the ECG and more specifically TWA.

1.2.4 T Wave Alternans as a Useful Clinical Tool to Measure Cardiac Alternans in Patients

TWA is the alternation in size or shape of the T wave as illustrated in Figure 1.7.

This can be readily assessed non-invasively in man. The presence of TWA has long been recognised to be associated with a poorer prognosis [101]. Generally this was described in case reports and anecdotal stories and once TWA was present to the extent that could easily be identified visually, this suggested advanced disease. More recently, Cohen et al sought to quantify TWA when it is present at magnitudes too small to easily identify visually, They
Figure 1.7: Example of T wave alternans (TWA). Alternating patterns are in an ABABABABAB pattern where all the A beats are similar to each other and all the B beats are similar to each other. TWA is the maximum difference between the A and B beats.

examined dogs and calculated a T wave alternans index (TWAI) based on 1,024 sequential ECG beats [102]. They then compared this TWAI with VF threshold during an electrophysiology study (EPS) under different conditions: hypothermia, tachycardia and ischaemia. As the VF threshold changed under the different test conditions, the TWAI changed in parallel. This was the first quantitative study of TWA and demonstrated that significant electrical alternans may be so subtle that it cannot be visually identified but can be measured with digital signal processing techniques. These data in animals led to a pilot clinical study in 19 patients undergoing an EPS. TWA was measured during atrial pacing at three different rates: 100 beats per minute (bpm), 120 bpm and 150 bpm. The patients with inducible arrhythmias on EPS could be identified by TWA magnitude with a sensitivity of 92% and specificity of 50% [84]. Taking these pilot data further, 83 patients referred for an EPS [103] were studied. TWA was quantified using a spectral method which later became the predominant method to analyse TWA and the methodology is described later (section 1.2.6). The primary end point for this study was inducibility of ventricular arrhythmias on EPS. Two independent risk factors for inducibility were identified:
TWA magnitude and impaired LV function. A positive correlation was described between TWA magnitude and the risk of inducible ventricular arrhythmias. Inducible ventricular arrhythmias during an EPS do not have clinical significance in current practice but in this study they also demonstrated that arrhythmia free survival was better for TWA negative than TWA positive patients. In this study an alternans ratio of 2.5 was best at discriminating between those with and without inducible arrhythmias (see Figure 1.8).

![Figure 1.8](image)

**Figure 1.8:** TWA ratios (defined as the peak at 0.5 cycles/beat in a power spectrum analysis of 128 consecutive ECG complexes divided by the standard deviation of spectral noise), in patients with and without inducible ventricular arrhythmias on electrophysiology study. Using a cut-point for TWA ratio of 2.5 best discriminated between those with and without inducible arrhythmia. Reproduced from rosenbaum et al [103].

Following on from these early clinical trials of TWA, numerous larger studies have been carried out using the spectral technique and other methodology. These will be discussed in detail after first considering, what is the mechanistic link between TWA and arrhythmias?
1.2.5 Mechanisms Linking TWA and Arrhythmogenesis

One hypothesis for the mechanism of re-entrant ventricular arrhythmias is the dispersion of refractoriness hypothesis. This proposes that while depolarisation of the myocardium is a synchronous process, with activation spreading from one region to its neighbouring region, repolarisation is an asynchronous process. The implication of this spatial variation in repolarisation is that areas of the myocardium can still be depolarised when the next wave of depolarisation arrives. This leads to fractionation of the depolarisation wave front and ultimately arrhythmias.

In an effort to investigate this hypothesis further, and how it applies to TWA, Smith et al [104] created a computer model of cardiac conduction in which subpopulations of sites within the myocardium exhibit prolonged refractory periods. In this model, different populations of myocytes are depolarised every other beat and TWA is observed. This led to the hypothesis that TWA is generated by underlying beat-to-beat alternation in conduction patterns. However, if this was the explanation then one would expect alternation in QRS complexes as well, but this is not observed in clinical practice.

Pastore et al [105] demonstrated that TWA is a projection to the surface ECG of repolarisation heterogeneity of individual cells, or clusters of cell. In this study, TWA was induced in Langendorff-perfused guinea pig hearts with fixed rate pacing at increasing heart rates. Using high resolution optical mapping, beat-to-beat alternation in the time course of repolarisation of individual cells was found to be the primary explanation for TWA. Discordant alternans (when action potentials in neighbouring cell regions alternate out of phase) was observed to produce gradients of repolarisation sufficient to cause unidirectional block and re-entrant ventricular arrhythmias (Figure 1.9). The magnitude of alternans on a cellular level was several orders of magnitude greater than that observed on the surface ECG, supporting the importance of subtle,
and visually undetectable, TWA.

Figure 1.9: Data from Langendorff-perfused guinea pig hearts with atrial pacing at increasing rates. At higher heart rates, TWA becomes apparent on the surface ECG (top panel). This is associated with increasing spatial heterogeneity of repolarisation and depolarisation. Reproduced from Pastore et al [105].

1.2.6 Methods of Measurement of TWA

Since the first clinical studies reported on the value of quantifying TWA using the spectral method, various other methods have been developed [106]. The two most utilised methods are the spectral method and the modified moving average (MMA) method.

Spectral method

The spectral method has been used in over 7,000 patients in prospective studies. In this method, consecutive ECG cycles are aligned by their QRS complexes. The amplitude of 128 points in the ST-T wave segments (identified relative to the JT interval) are measured. Beat-to-beat amplitude fluctuations at each of these points along the ST-T wave are analysed using the Fast Fourier Transform (FFT) to create a power spectra for each point. These spectra are then averaged resulting in a single composite spectrum. The magnitude of TWA is expressed as the alternans
ratio which is equal to the alternans power at 0.5 cycles/beat divided by the standard deviation of spectral noise [107] (Figure 1.10).

![Figure 1.10: Schematic representation of the spectral method for analysing TWA. Reproduced from Verrier et al [107]. See text for details.](image)

During testing, there is a requirement to elevate the heart rate to approximately 105 to 110 bpm and to maintain this for at least two minutes. This can be performed with graded exercise, atrial pacing or chronotropic drugs. The importance of heart rate during the analysis of TWA will be discussed later. Special high-resolution electrodes are required for the spectral technique in order to minimise noise, and analysis is performed using standard precordial and orthogonal leads.

A TWA level \(>1.9\mu V\) maintained for at least two minutes is considered a positive test. As a result of a high incidence of indeterminate results (20-40% of cases) classifications of either "abnormal due to patient factors" (e.g. excessive ectopy or inability to achieve and maintain an adequate heart rate) or "technically indeterminate" (e.g. electrode noise) were created. Individuals with indeterminate results due to patient factors are at greater risk than those with
positive results. As a result of these issues it is common that TWA results from spectral analysis are classified as negative or non-negative (combination of positive and abnormal due to patient factors).

The spectral method has significant limitations in patients with heart failure. Firstly, spectral methods are intolerant of irregularity and as such AF is an exclusion criterion. Ventricular pacing is also an exclusion criterion. There is evidence that during chronic right ventricular pacing, there is action potential prolongation near the site of abnormal activation and significant dispersion of ventricular gradients of repolarisation in both apical-basal and right-left directions [108]. The precise nature of this effect may depend on exactly where in the right ventricle the lead sits. While it is technically possible to analyse TWA in patients with right ventricular pacing, these data make it uncertain whether such results can be compared to those in sinus rhythm. The exclusion of patients in AF and those with ventricular pacing excludes a number of heart failure patients from analysis (to be discussed in more detail later). Furthermore, the requirement to exercise to a heart rate of 105-110 bpm and maintain this can be a significant limitation of the technique in heart failure. The problems of the spectral technique in patients with heart failure was highlighted by a study that investigated 1003 patients hospitalised with heart failure. 648 patients returned for TWA testing using the spectral technique one month after discharge. 318 were ineligible to undertake the test due to AF, pacemaker dependency or the inability to exercise. The remaining 330 were tested with the following outcomes: positive, 30%; negative, 24%; indeterminate, 46% [109]. This suggests that almost half of heart failure patients may be ineligible to undergo the test and of those who do, almost half record an indeterminate result.
Modified Moving Average method

The MMA method has been used in over 5,000 patients. Development of the MMA method by Verrier and Nearing was in response to the limitations of the spectral technique which were preventing the wider application of TWA as a measure of risk in the clinical arena. They set out to develop a technique which would provide interpretable results without controlling heart rate [110]. They developed the MMA method, previously called median beat analysis. First, arrhythmias and segments of ECG with noise are removed. Then alternate beats are classified as A for even beats and B for odd beats. This results in a stream of A beats and a stream of B beats. Each stream then undergoes a process of averaging (specifically a modified moving average in which each subsequent beat contributes an eighth to the moving average. This limits the effect of any one individual beat on the overall average). To measure T-wave alternans, the maximum absolute value of the difference between the A and B modified moving average computed templates is determined within the ST segment and T-wave region, from the J point to the end of the T wave and this is performed every 15 seconds [110] (Figure 1.11). The amount that each beat contributes to the ongoing average is called the update factor. If this is set high then aberrant beats may contribute too much to the average and lead to an erroneous average result, if set too low then true surges in TWA may be missed. The recommended update factor of one-eighth was better than one-sixteenth or one-thirtysecond in published data [111].

The algorithm was tested with simulated ECGs and it could reliably detect simulated TWA. The algorithm was then tested in an animal model. Coronary artery occlusion in dogs was carried out during atrial pacing at 150 bpm (to exclude HR as a variable). Predictive power of TWA in identifying risk of ischaemia-induced VF was exceptionally high with sensitivity and specificity both being 100%. However, this experiment was not directly comparable to TWA measured through the surface ECG because in this study the recording lead was inside the left
ventricle.

This work led to the first trial assessing the use of the MMA method as a measure of TWA in man which will be described later. Advantages of this technique are that there is no requirement for special electrodes and there is no need to exercise. The MMA algorithm of TWA analysis can be performed during an exercise test but can also be performed on an ambulatory electrocardiogram (AECG). An advantage of analyses based on AECGs is that sympathetic surges occurring during day-to-day activities can be recorded.

![Schematic representation of the spectral method for TWA analysis.](image_url)

**Figure 1.11:** Schematic representation of the spectral method for TWA analysis. Reproduced from Verrier et al 2011 [107]. See text for details.

### 1.2.7 Evidence of Prognostic Value of TWA in Clinical Trials

Clinical trials using measures of TWA started by assessing if TWA can predict inducibility of ventricular arrhythmias on EPS. This led to larger trials with more clinically relevant endpoints such as all-cause mortality or SCD. The ultimate goal was to identify people who would benefit
from implantation of an ICD. Although spectral and MMA methodologies are different, they both ultimately both use a cut-point value for TWA to dichotomise populations in to TWA positive and TWA negative.

**Prognostic evidence for the spectral method of TWA analysis**

The first clinical study using the spectral technique is described above, briefly, Rosenbaum et al [103] demonstrated that measurement of TWA using the spectral method during atrial pacing was equivalent to an EPS at predicting arrhythmia free survival [103]. Gold et al [112] also studied a population referred for EPS and found that TWA predicted the primary endpoint of SCD, sustained VT, VF or appropriate ICD therapy with a relative risk of 10.9, which was better than EPS. EPS testing is not used in contemporary practice for risk stratification. Numerous trials have since gone beyond comparison with EPS and demonstrated the TWA could predict clinically relevant end points such as all-cause death or SCD (Table 1.9).

The studies summarised in Table 1.9 recruited a range of patient groups. From a heart failure perspective, the largest four studies are Chow et al (n=514, 2006) [113], Bloomfield et al (n=549, 2006) [114], Chow et al (n=768, 2007) [115] and Salerno-Uriate et al (n=446, 2007) [116]. Comparison between these studies is difficult because the end points were different. All-cause mortality appeared to be 2.24-2.27 times greater in those with a positive versus negative TWA result. A composite outcome of all-cause mortality or non-fatal sustained VT was 6.5 times more likely to occur in TWA positive versus negative patients. While this makes TWA appear promising as a tool to identify high risk patients, the ultimate goal is to identify high risk individuals and perform a therapeutic intervention, such as implantation of an ICD. The alternans before cardioverter defibrillator (ABCD) trial tested the ability of TWA to guide prophylactic ICD therapy. 566 patients with coronary artery disease, an LVEF ≤40% and NSVT were enrolled. All patients underwent TWA and analysis using the spectral method and an
Cardiomyopathy

Chow et al. 2006
514 patients with ischemic cardiomyopathy, LVEF <35%, no previous sustained ventricular arrhythmia and positive or indeterminate TWA test results
26%–29%
2.24 (1.34–3.75) for all-cause mortality; 2.29 (1.00–5.46) for arrhythmic mortality; NS for nonarrhythmic mortality

Salerno-Uriarte et al. (ALPHA) 2007
446 patients with DCM, LVEF <40%
29.5%
4.0 (1.4–11.4) for cardiac death and life-threatening arrhythmias at 18 months

Chow et al. 2007
768 patients with ischemic cardiomyopathy, LVEF <35%, no previous sustained ventricular arrhythmia
26%–29%
2.27 (1.22–4.24) for all-cause mortality in patients without an ICD; 2.42 (1.07–5.41) for all-cause mortality or appropriate ICD discharge in patients with an ICD.

Chan et al. 2008
768 patients with ischemic cardiomyopathy, LVEF <35%, no previous sustained ventricular arrhythmia
26%–29%
2.19 (1.1–4.34) for all-cause mortality and appropriate ICD shocks at 1 yr; 3.36 (1.28–8.83) at 2 yrs

Constantini et al. (ABCD) 2009
566 patients with ischemic cardiomyopathy, LVEF <40%, and NSVT
28 ± 8%
2.1 for SCD or appropriate ICD discharge at 1 yr

Depressed LVEF

Rashba et al. 2004
144 patients with CAD and LVEF <40%
28 ± 7%
2.2 (1.1–4.7) for death, sustained ventricular arrhythmia, or appropriate ICD discharge at 17 ± 13 months; NS in patients with LVEF <30%; NS in patients with LVEF >30%

Bloomfield et al. 2006
549 patients with LVEF <40%, no history of sustained ventricular arrhythmias
25%
6.5 (2.4–18.1) for all-cause mortality or nonfatal sustained ventricular arrhythmia at 2 yrs

Post-MI

Ikeda et al. 2000
102 post-MI patients
20%–40%
16.6 (2.2–127.8) for arrhythmic events

Ikeda et al. 2002
850 post-MI patients
51 ± 13%
5.9 (1.6–21.4) for SCD or resuscitated VF at 25 ± 13 months; 82% were monitored at 2–10 weeks after MI

Bloomfield et al. 2004
177 MADIT-II like post-MI patients with LVEF <30
23 ± 6%
4.8 for all-cause mortality at 2 yrs

Ikeda et al. 2006
1,041 post-MI patients with LVEF >40%
55 ± 10%
23.5 monitored at 48 ± 66 days for SCD or life-threatening arrhythmia at 32 ± 14 months

Exner et al. (REFINE) 2007
322 post-MI patients with LVEF <50%
40% within 1 week and 47% at 8 weeks after MI
2.75 (1.08–7.02) monitored at 10–14 weeks after MI for cardiovascular death or resuscitated cardiac arrest (primary endpoint) at 47 months

Referred for electrophysiological study

Gold et al. 2000
313 patients
44 ± 18%
10.9 for SCD, sustained VT, VF, or appropriate ICD discharge at 400 days

Rashba et al. 2002
251 patients with CAD and LVEF
27 ± 8%
2.2 (1.1–4.7) for arrhythmic events (arrhythmic death, VT, aborted VF) at 499 ± 395 days; NS for TWA during atrial pacing at 100–120 beats/min

Table 1.9: Predictive trials of the spectral method of TWA analysis recruiting more than 100 people. CAD, coronary artery disease; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NS, not statistically significant; REFINE, risk estimation following infarction non-invasive evaluation; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia. Adapted from Verrier et al [107] with permission.
EPS. ICD implantation was mandated if either test were positive. The event rate (appropriate ICD discharge or sudden death at one year) in patients where both tests were negative was significantly lower than in those who had both tests positive (2% vs 12%; p=0.017). TWA as a predictive tool for the end point: 9% positive predictive value (PPV) and 95% negative predictive value (NPV) which was comparable to EPS. This is a poor PPV and once again a comparison is being made with EPS which is no longer routinely used to assess arrhythmic risk. A number of studies have failed to demonstrate any ability of TWA to predict SCD, ventricular arrhythmias or ICD therapies (Table 1.10). The two largest neutral studies were the Microvolt T Wave Alternans Testing for Risk Stratification of Post-Myocardial Infarction Patients (MASTER) trial [117] and the SCD-HeFT TWA sub-study [118]. The MASTER trial enrolled 566 patients and the SCD-HeFT trial enrolled 490 patients. In both trials the patients had heart failure with a mean LVEF in the severely impaired range. Both studies used the spectral method to quantify TWA and in neither study could TWA predict SCD or appropriate ICD therapy. One confounding factor in these studies is the cessation of β- blockers before measuring TWA. This has been reported to diminish the predictive strength of TWA by nearly 4 fold [119]. However, other neutral studies have not mandated the cessation of β-blockers [120]. This essentially means that TWA using the spectral method can identify patients with poor

<table>
<thead>
<tr>
<th>First Author Patient Population</th>
<th>Mean LVEF</th>
<th>Hazard Ratios (95% CI) for TWA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold et al. (SCD-HeFT TWA substudy) 2008 490 patients with congestive heart failure</td>
<td>24 ± 7%</td>
<td>NS for SCD, sustained VT/VF, or appropriate ICD discharge at 2.5 yrs</td>
</tr>
<tr>
<td>Chow et al. (MASTER) 2008 575 post-MI patients with LVEF &lt;30%</td>
<td>24 ± 5%</td>
<td>2.04 (1.10–3.78) for total mortality at 2.1 ± 0.9 yrs; NS for SCD or appropriate ICD discharge</td>
</tr>
<tr>
<td>Huikuri et al. (CARISMA) 2009 312 post-MI patients</td>
<td>31 ± 6%</td>
<td>NS for TWA monitored at 6 weeks after MI for VF or symptomatic, sustained VT at 2 yrs</td>
</tr>
</tbody>
</table>

Table 1.10: Non-predictive trials of the spectral method of TWA analysis recruiting more than 100 people. LVEF, left ventricular ejection fraction; MI, myocardial infarction; SCD, sudden cardiac death; NS, not statistically significant; TWA, t wave alternans; VF, ventricular fibrillation. Adapted from Verrier et al [107] with permission.
prognosis (all-cause mortality) but not identify those in whom an ICD will improve outcomes.

**Prognostic evidence for the MMA method of TWA analysis**

The first clinical study employing the MMA method to analyse TWA was a retrospective analysis of AECG data available from participants of the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) trial. A nested case-control study was performed, identifying patients who suffered a cardiac arrest secondary to a ventricular arrhythmia during follow-up. Fifteen cases were identified and they were matched with 29 controls. TWA was reported as the maximum TWA value at three separate time points: at maximum heart rate, at 0800 am and at maximum ST segment deviation. TWA was higher in cases than controls at 8am and at peak heart rate in lead V5 and tended to be higher in lead V1 in cases compared to controls. A cut-point to define patients as TWA positive or negative was created as the 75% percentile of the whole group. This cut-point value varied depending on the time of measurement and lead analysed. The 75th percentile cut-points of TWA used at maximum heart rate in leads V1 and V5 were respectively 46.6µV and 53.0µV and at 0800am these figures were 45.3µV and 42.5µV. A 4 to 7-fold higher risk of life-threatening arrhythmias was predicted by a TWA magnitude above the 75th percentile at maximum heart rate in leads V1 (hazard ratio (HR) 4.2, 95% confidence interval (CI): 1.1-16.3, P = 0.04) and V5 (HR 7.9, 95% CI: 1.9-33.1, P = 0.005). Using a cut-point of the 75th percentile for TWA at 8:00 A.M. also predicted risk of life threatening arrhythmias in leads V1 (HR = 5.0, 95% HR: 1.9-20.5, P = 0.02) and V5 (HR = 4.2, 95% CI: 1.1-16.3, P = 0.04).

Numerous other clinical studies have since published using the MMA method(Table 1.11). From a heart failure perspective, another retrospective study analysed AECG data from patients in the Eplerenone PostAcute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study [121] and found that elevated levels of TWA (using 47µV as a cut-off)
Routine exercise testing

Nieminen et al. (FINCAVAS) 2007
1,037 consecutive patients referred for routine exercise testing; 58 ± 13 yrs (patients included in Leino et al. [66])
Mostly preserved
6.0 (2.8–12.8) for CV death, 7.4 (2.8–19.4) for SCD at 44 ± 7 months for 65 µV TWA cutpoint; NPV for CV death = 97.6; PPV = 12.6; NPV for SCD = 98.6; PPV = 8.0

Minkkinen et al. (FINCAVAS) 2009
2,119 consecutive patients referred for routine exercise testing; 57 ± 13 yrs
Mostly preserved
4.6 (2.2–9.9) for CV death, 4.4 (1.5–12.7) for SCD at 47 months for 65 µV cutpoint; NPV for CV death = 97.4; PPV = 10.2

Leino et al. (FINCAVAS) 2011
3,598 consecutive patients referred for routine exercise testing; 56 ± 13 yrs
Mostly preserved
1.55 (1.150–2.108, p < 0.004) for CV death; 1.58 (1.041–2.412; p < 0.033) for SCD at 55 months per 20 µV TWA in lead V5

Ambulatory ECG monitoring

Verrier et al. (ATRAMI) 2003
Acute post-MI; case: control analysis (15 cases: 29 control subjects) from 1,284 ATRAMI patients, monitored at 15 ± 10 days post-MI; 60–62 yrs
Moderately depressed (42 ± 3%)
7.9 (1.9–33.1) for cardiac arrest or arrhythmic death at 21 months for a priori 75th percentile cutpoint (47 µV); patients were monitored at 15 ± 10 days post-MI

Stein et al. (EPHESUS) 2008
Acute post-MI, LVEF <40%, and heart failure; case: control analysis (46 cases: 92 control subjects) from 6,632 EPHESUS patients, monitored at 2–10 days post-MI; 68 ± 11 yrs
Depressed (34 ± 5%)
5.5 (2.2–13.8) for SCD at 16.4 months for 47-µV cutpoint; patients were monitored at 2–10 days post-MI; For SCD

Sakaki et al. 2009
295 consecutive cardiomyopathy patients with ischemic or nonischemic left ventricular dysfunction; 66 ± 16 yrs
Depressed (34 ± 6%)
17.1 (6.3–46.6) for CV death, 22.6 (2.6–193.7) for witnessed SCD at 1 yr for 65-µV cutpoint; NPV for CV death = 97%; PPV = 37%

Stein et al. (CHS) 2010
General population patients age >65 yrs; case: control analysis (49 cases: 98 control subjects) from 1,649 CHS patients
Not tested, assumed preserved
4.8 (1.48–15.81) for SCD at 14 yrs

Hou et al. 2011
219 consecutive acute post-MI patients; 55 yrs
>35% in 201; <35% in 18
17.78 (3.75-84.31) for SCD within 16 months for 47-µ-V cutpoint; patients were monitored at 1–15 days post-MI; NPV = 99%; PPV = 17%

Table 1.11: Clinical trials using the MMA method to analyse TWA. CV, cardiovascular; REFINE, risk estimation following infarction non-invasive evaluation; SCD, sudden cardiac death; TWA, T wave alternans. Adapted from Verrier et al [107] with permission.

predicted the occurrence of SCD and cardiovascular mortality during 20±6 month follow-up.

The first prospective study using the MMA method in patients with heart failure studied 295 patients with LV dysfunction and analysed TWA on AECG in leads V1 and V5 [122]. A cut-point of 65 µV was used to discriminate between TWA positive and negative patients. Patients who were TWA positive were 17.1 times more likely to experience cardiovascular death than patients who were TWA negative. A cut-point value of 65 µV was based on a Cox regression for outcomes in the Finnish Cardiovascular Study (FINCAVAS) trial. This trial used exercise testing not AECG and it is unclear why Sakaki et al did not use a cut-point of 47 µV that had been used in other studies using AECG.
In the second prospective study of TWA using the MMA method, Yu et al. [123] examined 227 patients in the stable period at 1 to 13 days after an acute MI using AECG monitors (examining leads V2 and V5). The primary end point was SCD or resuscitated cardiac arrest over a follow-up period of 16 ± 7 months. Multivariate Cox regression analysis demonstrated that, after adjustment for age, sex, LVEF, culprit artery and revascularisation therapy, a positive TWA (≥ 47 µV) resulted in a hazard ratio of 15.1 (95% CI, 2.9-78.8; P=0.003). They also found that the greater the number of episodes of TWA ≥ 47 µV the higher the risk. In this study the sensitivity and specificity of TWA for identifying those who experience SCD was 80% and 82% respectively. The NPV was 99% but once again, as with other TWA studies, there was a poor PPV of 17%.

There are no MMA-based studies published that have attempted to assess whether TWA can identify those who would benefit from an ICD. The Risk Estimation Following Infarction Noninvasive Evaluation (REFINE ICD) study (ClinicalTrials.gov Identifier: NCT00673842) is currently recruiting an estimated 1000 patients to assess whether primary prevention ICD implantation in those with an LVEF of 36%-50%, secondary to ischaemic cardiomyopathy, and abnormal TWA (measured with the MMA method) and heart rate turbulence will improve mortality. The estimated date for this study to complete is December 2021.

### 1.2.8 Utility of TWA as Tool to Measure the Effectiveness of Anti-arrhythmic Therapies

Aside from attempts to use TWA as a marker to identify patients at risk of SCD, there is evidence that TWA can serve as an outcome measure for the efficacy of AADs. A number of clinical studies have reported that the magnitude of TWA is reduced in response to β-blockade. Klingensheben et al. [124] demonstrated the infusions of metoprolol reduced TWA by 35% in
patients with documented or suspected VT during EPS. Rashba et al [125] infused esmolol in patients with inducible sustained VT at EPS and TWA was significantly reduced. Oral β-blockers have also been shown to reduce TWA. Murata et al [126] reported a reduction in positive TWA test results after three months of oral β-blockers.

TWA has also detected changes associated with the infusion of sodium channel blockers in animal studies. Two studies reported induction of TWA and spontaneous VF in dogs given high dose intracoronary flecainide [127, 128]. Given the high dose of flecainide delivered through the intracoronary route in these studies, it is uncertain whether these results have any relevance to man.

Finally, Groh et al [129] found that patients with an ICD with ischaemic or non-ischaemic cardiomyopathy who were prescribed amiodarone had fewer TWA positive results (11%) compared to those not taking amiodarone (64%).

1.2.9 Limitations of TWA Analysis

The most clinically relevant limitation of TWA analysis it that it cannot predict who would benefit from an ICD. A more fundamental limitation of the spectral technique in patients with heart failure is the number of patients who are ineligible for testing or derive an indeterminate results when tested [109].

The limitations of the spectral technique in patients with heart failure is the main reason I focused on the MMA method of TWA analysis. The MMA method also has limitations which includes exclusion of groups of heart failure patients (those in AF and those with a high burden of ventricular pacing such as those with a biventricular pacemaker). Furthermore, the MMA method has failed to add prognostic value. This may be due to the methodology of analysing one peak TWA in one lead over a 24 hour period instead of taking in to account other TWA
values (a TWA value is generated every 15 seconds in each ECG so there is the potential for 5,760 TWA values in each lead and a total of 69,120 values of TWA if 12 leads are used) (Figure 1.12). I will address the following limitations in data use from the MMA method in this thesis:

**TWA result is based only on one point in time (the peak result)**

The MMA method of TWA analysis has the potential to generate a TWA value every 15 seconds in each lead recorded. Over a 24 hour period of AECG this equates to 5,760 potential data points per lead. Almost exclusively, in MMA analysis of TWA only a single data point is used (the peak value over 24 hours) to classify a patient as TWA positive or negative. A study by Yu et al described earlier [123] recruited 227 consecutive patients who were treated for an acute MI and showed, for a primary end point of SCD or resuscitated cardiac arrest, after adjustment for age, sex, LVEF, culprit artery and revascularisation therapy, a positive TWA (≥47µV) resulted in a hazard ratio of 15.1 (95% CI, 2.9-78.8; P=0.0031). This is one of the few studies to investigate the value of using more than one TWA value. If five or more episodes of a TWA level ≥47µV was used to dichotomise the population in to TWA positive or negative, the HR for the primary outcome increased from 15.1 (using one value) to 18.2 (95% CI, 4.2-82.6) (Figure 1.13). The PPV increased from 17% if using one TWA value to 33% when using the presence of 5 or more episodes of TWA being ≥47µV.

This is one example of the potential added value in using more than one TWA value. However, even this study only used a relatively small amount of data and still focused on the high values of TWA ≥47µV. The vast amount of data captured on AECG MMA-based TWA analysis falls below this peak level and no analysis of the value of these non-peak data points has been performed.
Figure 1.12: Typical data points derived during a 24 hour ambulatory ECG with 12 leads. A T wave alternans (TWA) value is generated every 15 seconds in each lead. Typically only one of these data points (out of potentially 69,120 data points) is used to classify an individual as TWA positive or negative.
Figure 1.13: The effect of detecting TWA values $\geq 47 \mu V$ never, at least once or $\geq 5$ times on the primary end point of sudden cardiac death or resuscitated cardiac arrest. Reproduced from Yu et al [123] with permission.

Only one ECG lead is normally examined

TWA is a regional phenomenon and it has been shown in animal models of ischaemia that alternans occurs in the ischaemic area of myocardium and then projects to the precordium where it can be detected on the ECG [130]. Martinez et al [131] showed how regional the effect could be during measurement of TWA during angioplasty of the right and left coronary arteries. Similar to ischaemia, one could hypothesise that, dependent on the location of pathology (whether that is ischaemia or not) in the myocardium, then TWA could potentially present differently in different ECG leads. Using limb leads, compared to precordial leads, for
TWA analysis is often limited by noise. There is evidence to support the importance of lead selection in the analysis of TWA from analysis of exercise test ECG data from the FINCAVAS study [132]. 3598 patients with successful ETTs were enrolled and the peak TWA in each of the precordial leads was determined. The findings from this study were that maximum TWA when including all precordial leads was predictive of all-cause mortality and cardiovascular mortality but not for SCD. Using single ECG leads produced different results. Maximum TWA in lead V5 performed the best as this was predictive of SCD, cardiovascular mortality and all-cause mortality. Data from lead V3 only predicted SCD and data from leads V1,2,4,6 when used as single leads was not significant for mortality at all. This is an exercise test study though and due to noise etc. from exercise test this may not be generalisable to AECG data. Other trials using AECGs have investigated the use of different ECG leads. These studies include Sakaki et al [122] who demonstrated that in patients with heart failure TWA >65µV in either V1 or V5 predicted cardiac mortality and SCD. Maeda et al [133] showed that TWA >65µV in either V1 or V5 was able to predict ventricular arrhythmias but not all-cause mortality. In EPHESUS [121] the prognostic power of TWA in V1 was nearly the same as in V3. However, the ATRAMI [134] researchers found on their AECG analysis that V5 was superior to V1. It is still not understood though whether a single lead or a combination of leads provide better prognostic power.

**Heart rate and T wave height are not accounted for in the MMA algorithm**

TWA can develop in normal hearts at elevated heart rates > 170bpm in guinea pigs and > 200 bpm in dogs. The heart rate at which TWA occurs can be dependent on factors such as heart failure, ischaemia or the presence of autonomic neurotransmitters or drugs. The importance of heart rate in the analysis of TWA using the spectral method is recognised because the spectral technique has been shown to be heart rate dependent [135]. The heart rate at which significant
TWA develops is dependent on the individual subject. Patients at high risk of SCD exhibit TWA at lower heart rates than those at low risk [135] (Figure 1.14). If too elevated a heart rate was used then normal subjects may develop apparently significant levels of TWA resulting in false positives. The underlying mechanism for the dependence of TWA on heart rate may be due to limitations in Ca$^{2+}$ cycling. The Ca$^{2+}$ cycling hypothesis would predict that at resting state the same amount of Ca$^{2+}$ released from the SR on each beat is taken back up to the SR. Anything that impairs the ability to load the SR (largely a function of SERCA2a function) [95] or release from the SR [136] can lead to Ca$^{2+}$ alternans and then TWA. The ability to load or release from the SR may be limited at higher heart rates.

Figure 1.14: An example of two patients in whom TWA is measured using the spectral method at increasing heart rate. This demonstrates that TWA magnitude is dependent on heart rate and that the heart rate required to generate significant TWA is lower in an individual at risk of ventricular tachycardia (VT) than a control individual. The specific point at which the heart rate reaches the threshold to develop TWA for each patient is denoted by the arrows. Reproduced from Kaufman et al [135](permission not required).

The threshold heart rate at which TWA begins to increase depends on the individual. The examples illustrated in Figure 1.14 show that this starts at heart rates above 120bpm in the control subject and at 100bpm in the VT patient. It is plausible that over the course of a 24 hour AECG record, the heart rate, rarely reaches in to these ranges so the MMA method may be less dependent on heart rate but this has not been investigated.
Another parameter which is not adjusted for in either the MMA or spectral method is the amplitude of the T wave being analysed. The suggestion that there may be an association between the magnitude of TWA and the amplitude of the T-wave (independent of the risk of sudden death and malignant arrhythmias) comes from data from patients with intraventricular conduction delay. These patients have higher values for TWA, [113, 137, 138], and it has been proposed that this may be due to the larger T-waves observed in this population. There have been no studies to investigate the effect of T wave height on the magnitude of TWA using the MMA method.

**Exclusion criteria are commonly present in patients with heart failure**

The presence of AF or ventricular pacing are generally exclusion criteria for TWA analysis. AF and ventricular pacing (particularly in the form of biventricular pacing) are common in heart failure. This was demonstrated in the study by Jackson et al [109] described earlier. In this study of 648 patients who returned for TWA analysis after a heart failure hospitalisation, 242 (37%) were ineligible due to AF and 33 (5%) ineligible due to constant ventricular pacing. The irregularity of AF is not suited to spectral techniques and in those studies that have included AF, there was a large number of indeterminate results. The methodology of the MMA method may make it less susceptible to irregular intervals and more able to deal with AF. Ventricular pacing has also only been investigated in small studies using the spectral method and no studies have analysed the effect of ventricular pacing on TWA analysis using the MMA method.
1.3 Gene Therapy

1.3.1 What is Gene Therapy?

"Gene therapy is a technology by which genes or small DNA or RNA molecules are delivered to human cells, tissues or organs to correct a genetic defect, or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases" [139]. This can be in the form of interfering RNA to block the production of deleterious proteins but is more commonly described in terms of increasing the production of a beneficial protein. Commonly this is performed by engineering a vector in to which a therapeutic gene is inserted. The vector, which is commonly a virus, binds to the cell membrane of the target cell, enters the cytoplasm through a process of endocytosis before delivering the therapeutic gene to the cell nucleus. This gene can then either integrate in to the host genome or persist in the nucleus in a concatemeric episomal form. The therapeutic gene is then transcribed and the therapeutic protein synthesised (Figure1.15).

1.3.2 Vector Choice

The majority of gene therapy studies use a vector to increase the efficiency of transduction of the therapeutic gene in to the target cell. Injecting naked DNA has been performed with some success in gene therapy studies but naked DNA is rapidly broken down, furthermore, without a viral vector to selectively transduce the target organ, naked DNA must be injected directly in to the target organ. The majority of vectors are viruses which increases transduction as viruses are well equipped to deliver DNA to cells and because viruses can have a degree of tropism for specific organs, they can be delivered remote from the target organ. A summary of some advantages and disadvantages of various vectors is displayed in Table 1.12. Adenoviruses have been extensively used as a vector in gene therapy studies in cardiovascular disease. However, a
major limitation of adenoviruses is the immune reaction they can generate in the host. This can limit tolerability and safety in clinical trials. A vector more commonly used in clinical trials of heart failure is the adeno-associated virus (AAV). AAVs are derived from the parvovirus [140] and are not known to cause any human disease. Thirteen different AAV serotypes of AAV have been identified (AAV1-AAV13) with differential tissue tropisms [141]. AAV serotypes 1, 6, 8 and 9 transduce skeletal and cardiac muscle efficiently. Wild-type AAVs integrate their DNA into the host genome, commonly in a site-specific location on chromosome 19. This location does not generally lead to any adverse effects but since insertion can also occur at sites of DNA damage, the possibility of insertional mutagenesis remains. The AAV vector used in clinical trials is a recombinant adeno-associated virus (rAAV), not wild type, and this rAAV
<table>
<thead>
<tr>
<th>Vectors</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Viral</td>
<td>Low cost, non-pathogenic and non-immunogenic.</td>
<td>Low transduction efficiency and lack of cardiotoxic.</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Easy to produce and all major cardiac cell types efficiently transduced.</td>
<td>Immune and inflammatory reactions, short term gene expression and moderate transduction of all organs at high doses.</td>
</tr>
<tr>
<td>AAV</td>
<td>Non-pathogenic, minimal, immunogenicity long term gene expression and cardiac tropism.</td>
<td>Presence of neutralising antibodies. Short phase expression window not currently available.</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Long term gene expression.</td>
<td>Inability to transduce non-dividing cells such as cardiac myocytes.</td>
</tr>
</tbody>
</table>

Table 1.12: Advantages and disadvantages of vectors available to deliver genetic material. AAV, adeno-associated virus. Reproduced from Hayward et al [66].

is not known to integrate in to the host genome but instead persists in the cell nucleus in a concatameric episomal form making the risk of insertional mutagenesis theoretically very low. This lack of integration in to the host genome is a limitation if the target organ contains cells that are rapidly dividing because any new cells will not contain the transgene. Persistence of the transgene is more likely in non-dividing cells like cardiomyocytes [142]. There is some evidence that the transgene delivered by an AAV can persist for more than 8 years in a dog model [143]. The main limitation of using AAVs as a vector is the existence of neutralising antibodies (nAbs) which will be discussed later.

### 1.3.3 Delivery Methods

A number of methods are available to deliver gene therapy products to the heart. These include antegrade intracoronary infusion, which can be performed with or without coronary artery balloon occlusion to reduce immediate washout, a closed loop recirculation method, retrograde delivery by infusion through the coronary sinus and direct injection in to the myocardium or in to the pericardial space. There are advantages and disadvantages for each method [66] (Table 1.13).

From a clinical perspective, only two of the methods have been adopted in to trials: antegrade
<table>
<thead>
<tr>
<th>Delivery Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antegrade Intracoronary infusion without coronary artery occlusion*</td>
<td>Homogenous delivery to whole myocardium and tolerated in patients with heart failure</td>
<td>Limited in patients with significant coronary artery disease</td>
</tr>
<tr>
<td>Antegrade Intracoronary infusion with coronary artery occlusion</td>
<td>Homogenous delivery to whole myocardium and allows flow of vector to occur without dilution</td>
<td>Limited in patients with significant coronary artery disease</td>
</tr>
<tr>
<td>Closed loop recirculation method Vector is infused into a coronary artery, removed from the circulation from the coronary sinus, oxygenated extracorporeally and redelivered down the coronary artery</td>
<td>Vector has a longer exposure time to myocardium and has been shown to improve transduction in an animal model.</td>
<td>Complicated system and requires anticoagulation</td>
</tr>
<tr>
<td>Retrograde infusion through coronary sinus</td>
<td>High transduction efficiency in large animal models. Still feasible in the presence of significant coronary artery disease.</td>
<td>High coronary pressure can result in myocardial oedema or haemorrhage</td>
</tr>
<tr>
<td>Direct myocardial injection*</td>
<td>Avoids first pass of liver, the effect of neutralising antibodies and the inflammatory response</td>
<td>Limited vector delivery due to restricted area of injection. Myocardial injury from injection</td>
</tr>
<tr>
<td>Peripheral Intravenous Infusion</td>
<td>Simplest, most convenient delivery method</td>
<td>Dilutional effect and no vector with high enough cardiotropism to be clinically viable currently</td>
</tr>
<tr>
<td>Pericardial Injection</td>
<td>Feasible and safe when guided by imaging modalities and could potentially allow a high concentration of vector to be in contact with a large area of myocardium for prolonged time</td>
<td>Vectors in this space preferentially transduce the pericardial cells with transduction in the myocardial layers of only superficial extent. Deeper penetration requires cardio toxic proteolytics</td>
</tr>
</tbody>
</table>

Table 1.13: Advantages and disadvantages of delivery methods available for gene therapy. * denotes techniques used in human trials. Reproduced from Hayward et al [66].
intracoronary infusion without coronary artery occlusion (SERCA2a gene therapy and adenyl cyclase (AC) 6 programs) and direct intramyocardial injection (stromal cell-derived factor 1 (SDF-1) gene therapy program). The technique used in the largest number of patients to date is an intracoronary infusion without balloon occlusion. A number of factors affect the efficiency of transduction when using such a technique. The cardiotropism of the viral vector is one factor, but there is also the dose of vector used, the dwelling time of the gene therapy product in the coronary artery, the coronary flow and the perfusion pressure. Furthermore, permeability agents, which are not commonly used in clinical trials, can also improve transduction efficiency Figure 1.16.

**Figure 1.16:** Factors that affect transduction efficiency during intracoronary delivery of gene therapy products.

The use of intracoronary infusion to deliver the gene therapy product in clinical trials is commonly accompanied by intravenous glyceryl trinitrate (GTN). This approach has been shown to increase gene (SERCA2a) transduction by more than two fold in animal studies
when compared to intracoronary infusion without GTN [144]. The mechanism for how GTN improves transduction is unknown but may relate to changes in vascular permeability, coronary vasodilatation, or by increasing myocardial perfusion through a reduction in left ventricular end diastolic pressure (LVEDP). This effect being mediated by coronary vasodilation seems less likely to be the key mechanism since intracoronary GTN did not increase SERCA2a expression [144]. The clinical trials using direct intramyocardial injection use a percutaneous approach and deliver the injections in to the endocardium as guided by echocardiography.

1.3.4 Molecular Targets

The underlying molecular derangements that are recognised in the failing myocyte are described further in section 1.1.10. Many of these abnormalities occur regardless of the cause of heart failure and include alterations in gene expression and protein expression which ultimately lead to deterioration of the failing heart. There are many potential processes that could be targeted by gene therapy but there are three important systems that have progressed to the stage of clinical trials these are Ca\(^{2+}\) handling, the \(\beta\)-adrenergic system and a stem cell based repair process mediated in part through the action of SDF-1. Targets within these systems will be discussed further.

**Calcium cycling as a target for gene therapy in heart failure**

The abnormalities of Ca\(^{2+}\) cycling recognised to occur in heart failure are discussed further in section 1.1.10. A summary of the underlying abnormalities in Ca\(^{2+}\) handling that occur in heart failure can be represented by comparison of Ca\(^{2+}\) transients in heart failure subjects with those of controls. In heart failure, Ca\(^{2+}\) transients occur more slowly with lower peak amplitude. This finding suggests that the quantity of Ca\(^{2+}\) stored in the SR is reduced and there is impaired
re-uptake of Ca\(^{2+}\) back in to the SR [67]. Both these features could be explained, at least in part, by impaired SERCA2a function which is known to occur in heart failure. The effect of Ca\(^{2+}\) being removed from the cytoplasm more slowly is for there to be an elevated resting diastolic cytoplasmic Ca\(^{2+}\) level, contributing to the impaired ventricular relaxation frequently seen in patients with heart failure. Depletion of the SR Ca\(^{2+}\) store means there is less Ca\(^{2+}\) available for contraction which contributes to systolic impairment. Furthermore, in response to elevated levels of cytoplasmic Ca\(^{2+}\) there is upregulation of NCX as an alternative means of normalising the cytoplasmic Ca\(^{2+}\) content. This results in Ca\(^{2+}\) leak from the cardiomyocyte which can promote after depolarisations and arrhythmias. In principle, one may therefore consider that a therapy that normalises the Ca\(^{2+}\) transient in heart failure could improve systolic and diastolic function and reduce the risk of ventricular arrhythmias. SERCA2a, is a key molecule involved in intracellular Ca\(^{2+}\) handling and is a prime candidate for impacting on the above abnormalities. As a result SERCA2a as a target has received the most attention in heart failure clinical trials and it is SERCA2a that forms the basis for this thesis. As a result, the focus for reviewing progress in gene therapy in heart failure will be on those data informing on the value of SERCA2a but other targets will be discussed to illustrate the progress that is being made in other areas of gene therapy in heart failure.

**The β-adrenergic system as a target for gene therapy in heart failure**

\(β\)-1 and \(β\)-2 adrenoreceptors (ARs) are both present in human cardiomyocytes with \(β\)-1 ARs accounting for around 75% of \(β\)-ARs. \(β\)-1 ARs and \(β\)-2 ARs have separate signalling pathways and they respond to activation differently suggesting they regulate different aspects of catecholaminergic stimulation. The interaction between \(β\)-ARs and G-proteins is regulated by kinases that reduce the activity of the \(β\)-ARs. In heart failure there is evidence that \(β\)-1 ARs are down-regulated and there is upregulation of \(β\)-ARs kinase, and increased inhibitory G protein
function. This array of abnormalities leads to desensitisation of the $\beta$-ARs and decreased signalling through their pathway. Several gene therapy studies have been performed in an attempt to correct these abnormalities.

**Stromal Cell-Derived Factor-1 as a target for gene therapy in heart failure**

There is a stem cell based repair process which attempts to repair tissue following injury. SDF-1 is part of this system by its action to inhibit cell death and recruit stem cells. Studies have been performed to try and treat heart failure by re-establishing SDF-1 expression locally to the site of damage in ischaemic cardiomyopathy. This approach is different from most of the other forms of gene therapy in which a segment of DNA is introduced which codes for a protein that itself is hoped will improve cardiac function by acting on all cardiomyocytes that are failing (which may be cardiomyocytes remote from any form of damage such as an MI). Contrasting with this approach, the introduction of SDF-1 is specifically to damaged areas of the heart that have sustained a MI and the aim is to recruit stem cells that can repair damaged areas of heart.

**1.3.5 Laboratory Evidence for Calcium Cycling as a Target for Gene Therapy in Heart Failure: targeting SERCA2a**

SERCA2a is the main focus of gene therapy research aimed at normalising $\text{Ca}^{2+}$ cycling. There are three established approaches to increase the activity of SERCA2a: upregulate the expression of SERCA2a, down regulate or inhibit the action of PLN (the endogenous inhibitor of SERCA2a) or by affecting post-translational modification of SERCA2a.
Upregulation of SERCA2a expression

SERCA2a gene therapy has demonstrated the ability to improve cardiac contractility in a variety of experimental models of heart failure [145–147]. In addition to improving contractility, SERCA2a gene therapy has demonstrated multiple other beneficial effects including reduced ventricular arrhythmias and improved coronary flow mediated through activation of endothelial nitric oxide synthase [148–151]. An interesting feature of heart failure is the reactivation of the foetal gene program which results in further deterioration in ventricular function. SERCA2a gene therapy reverses this effect with partial normalisation of the transcriptome [152, 153]. A summary of the laboratory data illustrating the efficacy of SERCA2a gene therapy in a variety of physiological parameters is presented in Table 1.14.

Inhibition of phospholamban as a mechanism to increase SERCA2a activity

An alternative approach to improve SERCA2a activity is to target PLN, the endogenous inhibitor of SERCA2a. SERCA2a activity is inhibited by PLN, when PLN is in a dephosphorylated state. Conversely, when PLN is phosphorylated, primarily through the actions of protein kinase A and calmodulin kinase II, its inhibition of SERCA2a is reduced (Figure 1.17).

There is experimental evidence for the importance of PLN in the pathogenesis of heart failure. For example, elevated levels of PLN in a rabbit model resulted in heart failure [160]. The SERCA2a:PLN ratio is reduced in patients with advanced heart failure, with a relative increase in the dephosphorylated PLN fraction. Cross-breeding PLN KO mice with two different models of heart failure either stopped or rescued the mice from the heart failure phenotype [161, 162]. AAV-mediated overexpression of a mutant (inactive) form of PLN prevented deterioration in a hamster model of cardiomyopathy and in post-MI rats [163, 164].
<table>
<thead>
<tr>
<th>Physiological Parameter</th>
<th>Model</th>
<th>Effect of SERCA2a Gene Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular-Ca(^{2+}) Transient Alteration</td>
<td>Neonatal rat cardiomyocytes (non-failing)</td>
<td>Increased amplitude of Ca(^{2+}) transients and faster relaxation kinetics [154].</td>
</tr>
<tr>
<td></td>
<td>Rat cardiomyocytes treated with PMA (reduces SERCA2a expression)</td>
<td>Shortens Ca(^{2+}) transients [155].</td>
</tr>
<tr>
<td></td>
<td>Isolated failing human cardiomyocytes</td>
<td>Normalised Ca(^{2+}) transients [156].</td>
</tr>
<tr>
<td>Contractility</td>
<td>Neonatal rat cardiomyocytes (non-failing)</td>
<td>Enhanced contraction measured by shortening of myocytes [154].</td>
</tr>
<tr>
<td></td>
<td>Rabbit myocytes (non-failing).</td>
<td>Reduced time to peak contraction and 50% relaxation [157]</td>
</tr>
<tr>
<td></td>
<td>Isolated failing human cardiomyocytes</td>
<td>Faster contraction velocity and enhanced relaxation velocity of myocytes [156]</td>
</tr>
<tr>
<td></td>
<td>In vivo rat model of heart failure (aortic banding)</td>
<td>Improved rate of change of LV systolic pressure and isovolumic relaxation [147]</td>
</tr>
<tr>
<td></td>
<td>In vivo porcine model of heart failure (mitral regurgitation)</td>
<td>Increased rate of change of systolic LV pressure [158]</td>
</tr>
<tr>
<td>LV Remodelling</td>
<td>In vivo rat model of heart failure (aortic banding)</td>
<td>Normalised LV volumes [149]</td>
</tr>
<tr>
<td></td>
<td>In vivo Porcine model of heart failure (mitral regurgitation)</td>
<td>Reduced LV size [158].</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>Rat in vivo heart failure model (post-myocardial infarction)</td>
<td>Reduced in vivo ventricular arrhythmias, reduced susceptibility to arrhythmias during programmed stimulation ex vivo, reduced Ca(^{2+}) leak [146].</td>
</tr>
<tr>
<td></td>
<td>Guinea pig model of heart failure (aortic banding)</td>
<td>Reduced cardiac alternans &amp; susceptibility to inducible ventricular arrhythmias [159]</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>In vivo Porcine model of heart failure (mitral regurgitation)</td>
<td>BNP stabilised in treatment group (increased in placebo) [158]</td>
</tr>
<tr>
<td>Cardiac Microstructure</td>
<td>Rat heart failure model (post-myocardial infarction)</td>
<td>Restoration of the sarcolemmal and transverse tubule microarchitecture</td>
</tr>
<tr>
<td>Fetal Gene Expression</td>
<td>Rat model of heart failure (aortic banding)</td>
<td>Partial normalisation of the transcriptome [152]</td>
</tr>
<tr>
<td></td>
<td>Rat model of heart failure (post-myocardial infarction)</td>
<td>Partial normalisation of microRNA signature [153]</td>
</tr>
</tbody>
</table>

**Table 1.14:** A summary of different physiological parameters that have been enhanced by SERCA2a gene therapy in animal models of heart failure. LV, left ventricular; PMA, phorbol 12-myristate 13-acetate. Reproduced from Hayward et al [66].
Factors which increase SERCA2a activity
- Sumoylation of SERCA2a
- Phosphorylation of PLN
- CaMkII activity
- PKA activity
- I-1 activity
- Beta adrenergic stimulation

Factors which inhibit SERCA2a activity
- Nitric oxide inhibits SERCA2a activity via thiol oxidation and nitrification of tyrosine residues
- Reactive oxygen species
- Unphosphorylated PLN
- PP1 activity
- PKA activity
- CaMkII activity
- I-1 activity

Figure 1.17: Illustrating the regulation of SERCA2a by phospholamban (PLN). When PLN is unphosphorylated it inhibits SERCA2a activity (as displayed on the left). When PLN is phosphorylated it forms a pentamer and the inhibition of SERCA2a is removed. Factors which increase the activity of SERCA2a are displayed. Ca$^{2+}$, calcium; CaMkII, Ca$^{2+}$/calmodulin-dependent kinase II; I-1, inhibitor 1; PKA, protein kinase A; PP1, protein phosphatase 1. Reproduced from Hayward et al [66].

Data from human cardiomyocytes has produced similar results. Reducing levels of PLN improves contraction and relaxation velocities to an extent that is similar to the benefit seen with SERCA2a gene therapy [165]. As an alternative approach to gene therapy targeting PLN, RNA interference therapy has been used in a rat model of heart failure to suppress PLN expression. An rAAV-RNA interference product suppressed PLN expression and SERCA2a activity increased as a result. This achieved an improvement in systolic and diastolic ventricular function [166]. These animal data may not easily be transferred to studies in man as demonstrated by the naturally occurring mutation of PLN in the human population that results in severe heart failure [167].

As described above, the inhibitory effect of PLN on SERCA2a activity is reduced when PLN is in its phosphorylated state and protein phosphatase 1 (PP1) dephosphorylates PLN thereby increasing the inhibitory action of PLN on SERCA2a. PP1 in is itself inhibited by protein phosphatase inhibitor 1 (I-1). Murine models in which either I-1 is over expressed in an active
form or PP1 is inhibited have resulted in improved cardiac function [168–170].

**Post-translational modification to increase SERCA2a activity**

Small ubiquitin-like modifiers (SUMOs) are a group of proteins which change the function of other proteins. This function is mediated through post-translational modification, termed sumoylation. Sumoylation of SERCA2a increases its stability and activity but small ubiquitin-like modifier type 1 (SUMO1) levels are reduced in heart failure. Gene therapy using AAV9-mediated SUMO1 transduction in animal models of heart failure markedly improves cardiac function which is comparable to SERCA2a gene therapy [171].

**1.3.6 Laboratory Evidence for the β-adrenergic system as a Target for Gene Therapy in Heart Failure**

**Upregulation of β-receptors**

Genetically modified mice that over express (30-fold) human β1-ARs develop a severe cardiomyopathy [172]. In contrast, overexpression of β2-ARs is associated with increased basal myocardial AC activity and increased contractility [173]. Furthermore, overexpressing β2-ARs in mice after MI resulted in preservation of LV function [174]. The advantageous effect of human β2-AR on cardiac performance has also been demonstrated in larger animals such as rabbits [175, 176]. This differential effect of the β-ARs may be partly explained by studies which have demonstrated that β1-AR signaling leads to cell death whereas β2-AR stimulation leads to cell survival [177, 178]. These results appear optimistic as a potential future therapy for heart failure but there are other data that conflict with the findings from these studies. In particular it should be acknowledged that transgenic mice expressing very high levels (>200 fold) of human β2-ARs developed fibrotic cardiomyopathy and heart failure [179].
Inhibition of G-protein-Coupled Receptor Kinase

A group of G protein-coupled receptor kinases (GRK) mediates the desensitisation of β-ARs by functional uncoupling of the β-AR and its related G-protein. In the heart, GRK2 is the most abundant GRK and is involved in the development of heart failure [180]. Interventions to reduce the activity of GRK2, either by ablation or with an inhibiting peptide (βARKct), have been associated with improved cardiac function, beneficial cardiac remodeling and improved survival in animal models of heart failure [181, 182]. In a porcine model of heart failure, delivering AAV6-mediated βARKct therapy two weeks after an MI lead to preservation of cardiac function [183].

Activation of Adenyl Cyclase (AC)

AC is important for β-AR signaling by catalysing the formation of cyclic adenosine monophosphate (cAMP). There are 9 isoforms of AC (AC1 to AC9) with AC5 and AC6 being the most abundant isoforms in the heart. Overexpression of AC6 in transgenic mice resulted in enhanced cardiac function in response to adrenergic stimulation. The effect of AC6 overexpression in cardiomyopathic transgenic mice was to improve LV function, reverse dysfunctional β-AR signaling and reduce mortality [184, 185]. These effects have been borne out in larger animal models with transduction of an adenovirus encoding AC6 in a pig model of heart failure leading to improved LV function and remodeling [186].

1.3.7 Laboratory Evidence for SDF-1 as a Target for Gene Therapy in Heart Failure

JVS-100 (previously called ACRX-100) is a non-viral SDF-1 encoding plasmid (a version of naked DNA gene therapy without a vector). Injection of JVS-100 into pigs with heart failure
following MI demonstrated accelerated healing in the infarct zone, increased vessel density and reduced left ventricular end systolic volume (LVESV).

1.3.8 Clinical Evidence for SERCA2a Gene Therapy in Heart Failure

The first clinical trial of gene therapy in heart failure was the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) study [187–189]. An initial open label phase 1 study recruiting nine participants demonstrated sufficient safety data to conduct a phase 2 study (the CUPID trial). 39 patients with advanced heart failure were randomised to receive a 10 minute intracoronary infusion of either AAV1-mediated SERCA2a gene delivery (AAV1.SERCA2a) or placebo, using intravenous GTN as an adjuvant therapy during the infusion. Inclusion criteria included the requirement for an LVEF $\leq 35\%$, NYHA classification of 3 or 4, a $\dot{V}O_2 \leq 20\text{ml/Kg/min}$, for an ICD to have been implanted and for the absence of nAbs to AAV1. All patients must also have been on optimal medical therapy prior to enrolment. These 39 patients were randomised to receive an intracoronary infusion of placebo (n=14) or one of three doses of AAV1.SERCA2a: low dose ($6 \times 10^{11}$ DNase-resistant particles (DRP))(n=8), mid dose ($3 \times 10^{12}$ DRP)(n=8) and high dose ($1 \times 10^{13}$ DRP) (n=9).

Prior to the CUPID study there was no data to inform on the potential impact of AAV1.SERCA2a on clinical outcomes. In this context, the end point selection made by the trialists was based on the demonstration of concordant changes across multiple end points. This approach of using concordant changes in multiple end points as opposed to a single primary end point in such an early heart failure studies using cellular therapy had also been suggested by the Food and Drug Administration [190]. The end points for the CUPID trial are not typical of heart failure trials and are better illustrated in Figure 1.18. The factors that combine to make up each of the three potential primary end-point success criteria are four efficacy domains and clinical
outcomes and the primary outcome was assessment of these outcomes at six months. There was also an additional analysis which investigated the time-to-recurrent events in the presence of terminal events (essentially a joint frailty model approach to analysis of recurrent events). The joint frailty model will be further discussed in the general methods chapter.

### Pre-defined endpoints of the CUPID trial

<table>
<thead>
<tr>
<th>Efficacy Domains</th>
<th>Clinical Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Symptomatic (NYHA, MLWHFQ)</td>
<td>-Time to event (death, LVAD, transplantation)</td>
</tr>
<tr>
<td>-Functional (6MWD, VO₂)</td>
<td>-Kaplan Meier AND duration of cardiovascular hospitalisations</td>
</tr>
<tr>
<td>-Biomarker (NT proBNP)</td>
<td>-Time to multiple clinical events (Joint Frailty Model)</td>
</tr>
<tr>
<td>-LV function/remodelling (LVESV, LVEF)</td>
<td></td>
</tr>
</tbody>
</table>

### Primary End-point Success Criteria

- **Group level analysis (based on efficacy domains)**
  - Improvement in ≥2 out of 4 efficacy domains at P<0.2 but all parameters must also be numerically superior in treatment arm

  **OR**

- **Individual level analysis (based on efficacy domains)**
  - Each patient’s baseline values were compared to 6 months and a numeric score calculated (+1 for improvement, -1 worsened, 0 no change)
  - Improvement at P<0.2

  **OR**

- **Outcome endpoint**
  - Time to event better than placebo (P<0.2) OR if Kaplan Meier in favour of AAV1/SERCA2a but P>0.2 then also needs reduction in mean duration of cardiovascular hospitalisations (P<0.2)

### Additional Analysis

- Time to multiple recurrent clinical events (including worsening HF, MI, HF hospitalisations, CV death, LVAD or transplant) taking in to account terminal event (the joint frailty model)

---

**Figure 1.18:** CUPID trial predefined outcomes. CV, cardiovascular; HF, heart failure; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; MLWHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association.

The AAV1.SERCA2a high-dose group versus placebo met the pre-specified criteria for success at six months (and this was later confirmed at one year). Furthermore, comparing the high dose group with placebo, there was a significant increase in time-to-recurrent events (MI, heart failure hospitalisations or worsening heart failure) in the presence of terminal events (death or the requirement for a left ventricular assist device (LVAD) or cardiac transplantation) at 12 months (HR 0.12; P=0.003). Importantly, there were also no signals of harm from the
At three years the effect on recurrent events persisted with a significant reduction in the high dose group versus placebo (HR 0.18; P=0.048). These data should be interpreted with caution as the only beneficial results were when the high dose group (n=9) was compared to the placebo group (n=14). These are small numbers of patients and of some concern, no dose response was observed, there was no difference between placebo and either low or mid doses of AAV1.SERCA2a. (Figure 1.19). The safety and potential efficacy demonstrated was, however, enough to pursue a larger clinical trial which lead to the multi-national Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease-2 (CUPID-2) trial.

**Figure 1.19**: Results from the CUPID trial: effect of AAV1.SERCA2a on recurrent cardiovascular events. Only the high dose group demonstrated a significantly better outcome than placebo. Figure adapted from data made available to the United States Securities and Exchange Commission.

The CUPID-2 trial was a phase 2, double-blind, placebo-controlled, randomised study evaluating the safety and efficacy of intracoronary administration of AAV1.SERCA2a in subjects with heart failure. The study design was very similar to the CUPID trial. Patients who
had chronic heart failure and a LVEF of $\leq 35\%$ with symptoms despite optimal medical therapy were recruited. Patients were also required to have either been admitted with decompensated heart failure in the previous 6 months or have a raised NT-proBNP (>1200ng/L in sinus rhythm or >1600ng/L in atrial fibrillation). Patients who met these criteria were pre-screened for the presence of nAbs to AAV1. If the nAbs were present at a titre of $>1:2$ then the patient excluded from the trial. Two hundred and fifty patients were recruited across 67 sites around the world. Patients were randomised 1:1 to receive a single ten minute intracoronary infusion of placebo or the high dose of AAV1.SERCA2a ($1 \times 10^{13}$DRP). GTN was co-administered intravenously during the intracoronary infusion.

The primary end point of the trial was time-to-recurrent heart failure related hospitalisations in the presence of terminal events (all-cause death, heart transplantation, LVAD implantation) analysed using the joint frailty model. The secondary efficacy endpoint is the time-to-terminal event (all-cause death, heart transplantation, LVAD implantation). Our site was one of the recruitment sites for this study and this provided data for this thesis that comprise Chapters 7 and 8. During the writing of this thesis the main trial published neutral results and this will be discussed in more detail in Chapter 7.

The neutral results of the CUPID-2 trial resulted in the termination of two other studies that were recruiting at the time, both using the same gene therapy product. The SERCA2a Gene Therapy in LVAD Patients (SERCA-LVAD) trial (ClinicalTrials.gov id: NCT00534703) was recruiting patients with advanced heart failure who had received an LVAD. Patients were to receive an intracoronary infusion of either AAV1.SERCA2a or placebo. There were three unique aspects of this trial; first is the opportunity to investigate whether mechanical unloading could potentiate the functional response to transduction. Secondly, by taking tissue biopsies correlation between changes in clinical outcome could be made with the expression of
SERCA2a. Thirdly, both nAbs positive and negative patients were to be enrolled to investigate the effect of antibody status on gene expression efficacy and safety. The other trial that was terminated was the AAV1.SERCA2a GENe Therapy Trial in Heart Failure (AGENT-HF) trial (ClinicalTrials.gov id: NCT01966887). The primary objective of this study was to investigate the impact of AAV1.SERCA2a on cardiac remodelling in patients with severe heart failure. Cardiac remodelling was assessed as a change in LVESV (measured with a 256-slice CT-scan) 6 months after treatment.

1.3.9 Clinical Evidence for Targeting the $\beta$-adrenergic System

The first clinical trial of gene therapy targeting the $\beta$-adrenergic system in heart failure began enrolment in May 2010 (ClinicalTrials.gov, NCT00787059). This is a randomized, double blinded, placebo controlled study to evaluate the safety and clinical effectiveness of increasing doses of human adenovirus-5 encoding human AC6 (Ad5.hAC6) in patients with stable but severe heart failure. Vector delivery is via intracoronary injection with adjuvant intracoronary nitroprusside, to increase gene transfer efficiency. Enrolment is complete and the estimated study completion date is October 2017. The primary outcome is a combined endpoint of exercise treadmill time and LV function by echocardiography.

1.3.10 Clinical Evidence for Targeting Stromal Cell-Derived Factor-1

JVS-100 is a non-viral, naked DNA plasmid encoding human SDF-1. An initial open label dose escalation study was performed to evaluate the safety of JVS-100 delivered by endomyocardial injection to patients with ischaemic heart failure. The injections were delivered under echocardiography guidance to sites within the infarct border zone. This study demonstrated safety and some signals for efficacy. This led to a phase 2 study, the Stromal Cell-Derived
Factor-1 Plasmid Treatment for Patients with Heart Failure (STOP-HF) study, which recruited 93 patients with ischaemic cardiomyopathy. The study failed to meet its primary end point of improved composite of change in 6MWD, and Minnesota Living with Heart Failure Questionnaire (MLWHFQ) at 4 months. The study demonstrated safety and a subgroup analysis suggested there may be benefit in those with more severely impaired LV function [191].

1.3.11 Limitations of Gene Therapy in Clinical Studies of Heart Failure

While the concept of gene therapy is appealing, one should note that despite the first clinical trial of gene therapy taking place over 25 years ago, to date, only one gene therapy product is approved for use in Europe or the United States (Glybera as a treatment for lipoprotein lipase deficiency). This reflects the challenges of gene therapy and in particular translation into man.

Limitations of vectors

Two broad categories in to which many of the limitations of current vectors can be placed are safety and efficacy. Safety of gene therapy studies is always paramount but there are two well-known examples of when serious harm came to patients in gene therapy studies. The first example was a study of eleven children who were receiving gene therapy to treat severe combined immune deficiency. Nine of the children were cured of their disease but the retroviral vector employed, caused insertional mutagenesis resulting in leukaemia in the recipients. The second example is of Jesse Gelsinger who was 18 years old when he died and was the first person known to have died in a clinical trial of gene therapy. He received gene therapy using an adenoviral vector to treat ornithine transcarbamylase deficiency. He died four days after receiving the gene therapy product from what is assumed to be a massive immune response to the adenoviral vector. These are extreme examples, but such examples have resulted in a
more cautious approach to gene therapy studies and the requirement for more detailed safety approvals.

A specific limitation to the efficacy of viral vectors is the presence of nAbs in the host. The prevalence of nAbs in the study population varies depending on the viral vector used and the geographic region investigated. The presence of pre-existing antibodies has considerable implications for gene therapy because nAbs can significantly impede cell transduction and so patients are excluded from receiving gene therapy products if they have nAbs to the viral vector. The prevalence of nAbs against AAV1 (the vector used in the CUPID) trial will be discussed later. There is also a high prevalence of nAbs against adenoviruses. Approximately 97% of the population has nAbs against type C adenoviruses, which include the commonly used adenovirus 2 and adenovirus 5.

**Limitations of delivery methods**

The options for delivery method for clinical trials in heart failure is partly determined by the limited cardiac tropism of current vectors. Essentially, this means that antegrade intracoronary infusion, retrograde (via the coronary sinus) infusion or direct intramyocardial injection are the only viable delivery methods available currently. These techniques are associated with an element of risk in heart failure populations. An ideal vector would exhibit such tropism for the heart that it could be delivered through a peripheral infusion. To improve cardiac tropism, work is ongoing to modify the AAV capsids using artificial peptide libraries.

**Limitations of outcome measures**

The goal of treating heart failure is generally to either improve quality of life or survival. As such, key end points in trials of gene therapy for heart failure will always be survival, heart failure hospitalisations, quality of life scores and markers of functional capacity. It is also
important from a scientific and mechanistic point of view to determine the efficiency of each step of the gene transfer pathway, in particular to measure the quantity of transgene in the target cell and the amount of the therapeutic protein expressed. This is particularly challenging in clinical trials of SERCA2a gene therapy since there is no blood test or imaging modality that can be used as a marker. Cardiac tissue can be analysed but this requires either an invasive cardiac biopsy, which has associated risks, or for patients to undergo cardiac transplantation, implantation of an LVAD or to die. Even under these circumstances, the nature of the samples derived, such as those reported in the long term follow-up of the CUPID trial [192], may only be sufficient to detect the presence of vector DNA and not quantify the efficiency of transgene expression. This limitation has several important consequences, one of which is if clinical trials demonstrate no benefit of SERCA2a gene therapy, as was ultimately the case for the CUPID-2 trial, it will be impossible to determine at which stage of the process the failure occurred, for example if no transgene can be detected in the target organ then the failure could be the delivery method or vector.

Financial limitations

A final significant limitation of clinical gene therapy trials in heart failure relates to costs. Finances play a major role in the development of any new therapy but in particular for therapies like gene therapy. Prior to any first-in-man study, new products must undergo toxicology and dose finding studies, audits of manufacturing practices, stability testing and regulatory submissions. The costs for such development are great and if the subsequent trial reports neutral results it can have catastrophic impact on a company thus halting progress of that particular product.
1.4 Hypotheses and Rationale

1.4.1 Hypotheses

- SERCA2a gene therapy will act as an anti-arrhythmic in patients with advanced heart failure as evidenced by a reduction in T wave alternans (a surrogate marker of arrhythmia risk) and a reduction in ventricular arrhythmias (as detected by a reduction in ICD therapies).

- Refinement of the MMA method for TWA analysis on AECG will better characterise an individual and may lead to improved clinical utility of this test.

1.4.2 Rationale for the Hypothesised Effect of SERCA2a Gene Therapy on Ventricular Arrhythmias and TWA

There is evidence in an ischaemia-reperfusion model that SERCA2a gene therapy reduces ventricular arrhythmias [193]. In a rat model of heart failure SERCA2a gene therapy reduces both spontaneous and catecholamine induced ventricular arrhythmias [146]. SERCA2a gene therapy normalised Ca\(^{2+}\) transients and remodelled the SR with a reduction in the total SR Ca\(^{2+}\) leak. SERCA2a plays a central role in the molecular mechanism of cardiac alternans in the normal heart [99] and in a guinea pig model of heart failure SERCA2a gene therapy significantly reduced Ca\(^{2+}\) alternans [159].

SERCA2a gene therapy has never been assessed for anti-arrhythmic properties in man but if the physiological effects in man are similar to those described in animal models then I hypothesise that there will be a reduction in ventricular arrhythmias and cardiac alternans (detected as TWA in this work).
1.5 **Aims**

- To refine the methodology for analysing TWA using the MMA method on AECGs to address the limitations described in section 1.2.9.

- To set up and recruit to, the first gene therapy trial for patients with heart failure in the UK.

- To determine if AAV1.SERCA2a is effective at reducing ICD therapies in the CUPID-2 arrhythmia sub-study, or at reducing TWA in CUPID-2 participants recruited to our site.

1.6 **Description and Structure of Thesis**

Chapters 3, 4 and 5 will describe methodological refinements to the analysis of TWA using the MMA method.

Chapter 6 will describe the applicability of measuring TWA in patients with AF or biventricular pacing (commonly criteria that exclude patients from TWA analysis and commonly present in heart failure.)

Chapters 7 and 8 are concerned with SERCA2a gene therapy. Chapter 7 discusses the requirements to set-up and recruit to the CUPID-2 trial and Chapter 8 describes the burden of ventricular arrhythmias in patients with heart failure and the effect of AAV1.SERCA2a on ICD therapies in the CUPID-2 arrhythmia sub-study.

1.7 **Change in direction of thesis**

The title of my PhD is SERCA2a Gene Therapy as an Anti-arrhythmic in patients with Advanced Heart Failure. The primary study that was planned at the start of my PhD to address
this title never came to fruition. The intention was to perform a local, prospective, randomised, double blind clinical trial investigating the effect of SERCA2a gene therapy (the same product used in the CUPID clinical trial program) in patients with heart failure who were at high risk for ventricular arrhythmias. The gene therapy product was to be delivered as a single intracoronary infusion. This study was primarily designed to assess whether SERCA2a gene therapy acts as an antiarrhythmic in patients with heart failure and outcomes included incidence of appropriate shocks/ATP, burden of non-sustained VT and magnitude of TWA. TWA values used would be the traditional peak TWA value and any other parameters that appeared useful from my other TWA studies in the thesis. This study did not come to fruition largely due to funding issues but also when the CUPID-2 trial published neutral results this study was no longer pursued. Imperial College regulations do not allow the title of a thesis to change. As an alternative to the planned prospective local study, I wrote a proposal to the company running the CUPID-2 trial requesting the ICD data from all patients enrolled in the CUPID-2 program. In this proposal I included the rationale for the hypothesis that SERCA2a gene therapy may be an antiarrhythmic in heart failure and I formulated the statistical design for the study. The proposal was approved by the steering committee. After completion of the CUPID-2 study I was given the data from the ICD interrogations and I performed all the analyses. This ultimately became the arrhythmia sub-study of the CUPID-2 trial. Furthermore, given the absence of what was to be the main data chapter of this thesis, I developed the TWA chapters further than had been anticipated. This further creates a disconnect between the thesis title and thesis content since TWA is not mentioned in the title. The final thesis is ultimately made up of two parts; the development of the TWA methodology for heart failure and the effect of SERCA2a gene therapy in heart failure. These two parts would have been linked by the described study had it come to fruition but instead they read as two relatively separate bodies of work.
Chapter 2

General Methods

2.1 Patient Recruitment and Ethical Approval

The patients recruited for various components of this thesis were identified from a variety of sources. Each component also had individual ethical and research and development approval.

2.1.1 The CUPID-2 trial

Potential participants for the CUPID-2 study were identified from a number of sources. At our own institute, individuals attending the heart failure outpatient clinic were screened by the clinical team for potential eligibility. Patients were also identified at a number of district general hospitals through scrutiny of lists of heart failure hospitalisations, lists of patients with an elevated natriuretic peptide and from heart failure clinics. Finally, a number of patients approached us directly after media reports were released about the initiation of the trial. The full approval process required to conduct the study are further described in Chapter 7. The CUPID-2 trial had two consent forms approved for use and a detailed patient information leaflet. The first consent form was for a blood sample to be tested for the presence of nAbs. The second consent form was only relevant to those with a negative nAbs blood test who met all other
eligibility criteria and was for full enrolment in the study. A section of the full consent form dealt with the optional component of agreeing to biopsy samples being taken in the event of death, transplantation or LVAD insertion. This optional section did not affect the eligibility of a patient to enrol in the study. A sub-study of the main CUPID-2 trial was a study to investigate the effect of AAV1.SERCA2a on ICD therapy in those with an ICD. This received approval during the conduct of the main trial and the TWA sub-study of the main CUPID-2 trial also received separate approvals and a separate ethical approval was given. Local research and development office approval was also gained.

2.1.2 Heart Failure Patients Recruited for TWA Studies

All heart failure patients recruited to the prospective TWA studies were enrolled from clinics at the Royal Brompton Hospital and Harefield Hospital. Patients were either recruited from the heart failure clinics at each site or from the pacemaker clinic for those with an ICD. Ethical approval was granted by the research ethics committee (REC) Oxford. Local research and development office approval was also gained.

2.1.3 TWA during Head up Tilt

The tilt table test (TTT) results retrospectively analysed in the TWA and head-up tilt (HUT) study were performed at the Royal Brompton Hospital between 2012 and 2015. They were clinically indicated tests. The data from the TTTs and clinical information were pseudonymised prior to analysis. Ethical approval to use these data and carry out this study was obtained from REC.
2.1.4 AAV1 Neutralising Antibody Prevalence

Prior to commencing recruitment to the CUPID-2 trial a cohort of blood samples from heart failure patients were analysed to determine the prevalence of nAbs in a UK population. These blood samples were derived from the Biobank at the Royal Brompton Hospital. These blood samples were provided by patients who consented to their blood being stored and used for research purposes. This was covered by local ethical approval of the Biobank. Patients who were screened as clinically eligible for the CUPID-2 trial had nAbs levels sent as part of their screening process which was covered by the screening ethical approval of the CUPID-2 study.

2.2 The CUPID-2 Trial Design

2.2.1 Study Overview

The CUPID-2 trial was designed to evaluate the safety and efficacy of AAV1.SERCA2a versus placebo added to an optimal heart failure regimen in the treatment of subjects with moderate to advanced NYHA class III/IV symptoms of systolic heart failure. Efficacy was to be determined by delaying and/or reducing the frequency of heart failure hospitalisations. The target population is those with moderate to severe heart failure who have a high risk for recurrent heart failure hospitalisation, in particular those with either elevated natriuretic peptides and/or a recent (within 6 months of screening) heart failure hospitalisation.

2.2.2 Study Design

This was a phase 2b, multi-national, multi-centre double-blind, placebo-controlled, randomised study of a single intracoronary infusion of $1 \times 10^{13}$ DRP versus placebo. Subjects were
randomised 1:1 and followed up as per the schematic of the study design displayed in Figure 2.1.

**Figure 2.1:** Schematic depicting the time line for the CUPID-2 trial. DRP, DNAse resistant particles.

All subjects were reviewed at screening, day 0 for investigational medicinal product (IMP) infusion, and at months 1, 3, 6, 9 and 12 for safety and efficacy assessments. After the 12 month active observation period, or if discontinued from the study prior to the end of the 12 month active observation period, all subjects entered quarterly long-term follow-up (months 15, 18, 21, 24, etc.) until the primary analysis data cut-off (when all subjects had a minimum of 12 months follow-up and a pre-specified number of clinical events occurred). Subjects continued to be followed up even after the primary analysis data cut-off until they had a minimum of 24 months follow-up.

Prior to enrolment patients were required to provide written informed consent. In the UK two consent processes took place. The first was a consent form to screen for nAbs to the viral vector as it was anticipated this would exclude approximately half of patients. If nAbs were absent
then the second consent process was for the full trial. Included in the full consent process was the option to allow, in the event of death, heart transplantation or insertion of an LVAD, cardiac biopsy samples to be taken to test for the presence of AAV1.SERCA2a DNA. The Primary Analysis Data Cutoff was based on a minimum length of time and cumulative total number of clinical events (at least 180 adjudicated heart failure hospitalisations must have occurred).

A third-party was commissioned to implement the randomisation process and deliver the IMP. Both the study drug and placebo were formulated and supplied in identical glass vials with rubber stoppers and crimp seals and provided in identical IMP packs. The allocation to AAV1.SERCA2a or placebo was be randomly assigned and stratified by country and the ability of the participant to walk between 150 to 425 meters in the 6MWD.

2.2.3 Study Population

A summary of the main inclusion and exclusion criteria for the CUPID-2 study are displayed in Tables 2.1 and 2.2 respectively.

### Inclusion Criteria for the CUPID-2 trial

<table>
<thead>
<tr>
<th>Inclusion Criteria for the CUPID-2 trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 to 80 years of age</td>
</tr>
<tr>
<td>Chronic systolic heart failure due to ischaemic or non-ischaemic cardiomyopathy</td>
</tr>
<tr>
<td>LVEF less than or equal to 35% during the 60 days before administration of IMP</td>
</tr>
<tr>
<td>NYHA functional class II, III or IV (UK criteria excluded NYHA II)</td>
</tr>
<tr>
<td>Optimal heart failure therapy</td>
</tr>
<tr>
<td>Absence of neutralising antibodies to the AAV1 vector (titre &lt;1:2 or equivocal) within 90 days of screening</td>
</tr>
</tbody>
</table>

The presence of at least one of the following (added early in the study to increase events rates):
- (i) Hospitalisation for heart failure within 6 months of screening
- (ii) NT proBNP >1200pg/ml (if in AF then NT proBNP>1600pg/ml)

**Table 2.1:** Inclusion criteria for the CUPID-2 trial. AAV1, adenoassociated virus serotype 1; IMP, investigational medicinal product; LVEF, left ventricular ejection fraction, NT proBNP, N terminal pro-B type natriuretic peptide; NYHA; New York Heart Association.
Exclusion criteria for the CUPID-2 trial

IV inotropes, vasodilators or diuretics within 30 days before screening
Restrictive CM, obstructive CM, acute myocarditis, pericardial disease, amyloidosis, infiltrative CM, uncorrected thyroid disease or discrete LV aneurysm
Cardiac Surgery, PCI or valvuloplasty within 30 days before screening
MI within 90 days before screening
Prior heart transplant or implanted LVAD
Likely need for an immediate heart transplant or LVAD
Prior CABG not ideal but can be considered case by case
Liver function tests greater than 3 times upper limit of normal
Current or imminent need for haemodialysis or GFR less than or equal to 20ml/min/1.73m²
Bleeding diathesis or platelets less than 50
Haemoglobin less than 9
Diagnosis of, or treatment for any cancer other than BCC within the past 5 years

<table>
<thead>
<tr>
<th>Exclusion criteria for the CUPID-2 trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV inotropes, vasodilators or diuretics within 30 days before screening</td>
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<td>Prior CABG not ideal but can be considered case by case</td>
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<tr>
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<tr>
<td>Current or imminent need for haemodialysis or GFR less than or equal to 20ml/min/1.73m²</td>
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<tr>
<td>Bleeding diathesis or platelets less than 50</td>
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<tr>
<td>Haemoglobin less than 9</td>
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<tr>
<td>Diagnosis of, or treatment for any cancer other than BCC within the past 5 years</td>
</tr>
</tbody>
</table>

Table 2.2: Exclusion criteria for the CUPID-2 trial. BCC, basal cell carcinoma; CABG, coronary artery bypass graft; CM, cardiomyopathy; GFR, glomerular filtration rate; IV, intravenous; LV, left ventricle; LVAD, left ventricular assist device; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Early in the recruitment period an additional requirement was made of the inclusion criteria, that patients have at least one of the following: a hospitalisation for heart failure within 6 months of screening, or an elevated level of natriuretic peptide (NT-proBNP >1200pg/ml (if in AF then NT-proBNP>1600pg/ml). This was to increase the event rate in the study. Furthermore, some centres were unable to recruit patients who were NYHA II dependent on the local ethical approvals.

2.2.4 Investigational Medicinal Product

The IMP is a rAAV vector consisting of a single-stranded cDNA encoding the human SERCA2a flanked by inverted terminal repeats derived from AAV serotype 2 and the capsid from AAV serotype 1 (Figure 2.2). The SERCA2a protein is the only protein expressed after AAV1.SERCA2a treatment, and is a fully human, intracellular, endoplasmic protein that is naturally expressed in cardiomyocytes.

The AAV1.SERCA2a product is contained in a buffer containing sodium chloride, L-histidine,
Figure 2.2: The AAV1.SERCA2a vector incorporates inverted terminal repeats (ITRs) from AAV2, the cytomegalovirus immediate early enhancer/promoter (CMVie), a hybrid intron, the human SERCA2a cDNA (huSERCA2a) and a bovine growth hormone polyadenylation signal (BGHpA). Reproduced from Greenberg et al [194]).

Magnesium chloride, polysorbate 20 and Water for Injection. The buffer without AAV1.SERCA2a serves as the matching placebo. Both AAV1.SERCA2a and placebo are clear, colorless solutions provided in clear glass vials. Storage requirements for the IMP are displayed in Table 2.3.

<table>
<thead>
<tr>
<th>IMP Condition</th>
<th>Storage Temperature, Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unopened, frozen vials</td>
<td>Frozen at ≤-60°C, indefinitely</td>
</tr>
<tr>
<td></td>
<td>Frozen at -20°C ± 5°C for up to 3 months</td>
</tr>
<tr>
<td></td>
<td>Refrigerated at 2-8°C for up to 3 months</td>
</tr>
<tr>
<td>Unopened, thawed vials</td>
<td>Refrigerated at 2-8°C for up to 3 months</td>
</tr>
<tr>
<td>Diluted product</td>
<td>Refrigerated at 2-8°C for up to 24 hours</td>
</tr>
<tr>
<td></td>
<td>Ambient for up to 1 hour</td>
</tr>
</tbody>
</table>

Table 2.3: Storage of investigational medicinal product (IMP).

AAV1 was used because it has been shown to be effective in transducing cardiomyocytes and does not bind heparin sulfate, allowing passage freely through the interstitial space resulting in a more homogeneous spread through the myocardium [195]. The AAV2 inverted terminal repeats necessary for formation of non-integrated episomal concatamers in the transduced cell, were selected due to their established safety profile.

2.2.5 Infusion of Investigational Medicinal Product

The IMP was infused as a 10 minute intracoronary infusion with concomitant intravenous GTN infusion titrated to the maximum tolerated dose. The distribution of the infusion was dependent on the patient’s coronary anatomy but for normal coronaries the infusion was split
to give two thirds down the left coronary artery and one third down the right coronary artery. The maximum tolerated dose of intravenous GTN was infused prior to intracoronary infusion of the IMP.

2.2.6 Assessments Made at Baseline and During Follow-up

Investigations required at each visit are summarised in Table 2.4.

Medical History
A complete medical history, including co-morbid conditions, NYHA classification and medications, was taken during screening. A more focused medical history, including interim illnesses, number of days hospitalised, changes to medication and adverse events was taken at each follow-up visit.

Examination
A routine physical examination was performed at each visit and observations recorded including blood pressure, heart rate and temperature.

Laboratory Measurements
Blood samples were analysed at a core lab and tests included full blood count, renal and liver profiles and NT-proBNP and enzyme-linked ImmunoSPOT (ELISpot) assay to assess for any possible anti-AAV1 capsid cellular immune response.

Quality of Life
The Kansas City Cardiomyopathy Questionnaire (KCCQ) was completed at time points listed in Table 2.4.

Six-minute Walk Distance
A 6MWD was performed at time points listed in Table 2.4. The same person conducted the test.
<table>
<thead>
<tr>
<th>Data Collected</th>
<th>Pre-screen</th>
<th>Screen</th>
<th>12-Month Active Observation Period</th>
<th>Long-Term FU</th>
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<tr>
<td></td>
<td>Day 0</td>
<td>M1</td>
<td>M3</td>
<td>M6</td>
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<td>Recruitment criteria</td>
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<td>NYHA classification</td>
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<td>Medications</td>
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<td>IMP administration</td>
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<td>ICD interrogation</td>
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<tr>
<td>LVEF</td>
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</table>

**Table 2.4:** Schedule of Events for the CUPID-2 trial. ICD, implantable cardioverter defibrillator; IMP, investigational medicinal product; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; nAb, neutralising antibody.
at each visit. An exploratory analysis of 6MWD was planned on the subset of subjects walking
150 to 425 meters at baseline. Randomisation was stratified by the ability of a subject to walk
this distance at baseline and in those unable, 6MWD was not part of the follow-up assessments.

*Left Ventricular Ejection Fraction*

LVEF was determined at screening and could be done so with any of the following modalities:
echocardiography, computed tomography, cardiac magnetic resonance imaging or X-ray
ventriculography.

*ICD Interrogation*

If present, an ICD check was be performed at the time points listed in Table 2.4. The full details
of the data collected is available in Figure 8.1.

*Electrocardiogram*

Twelve-lead ECGs were performed and used to measure intervals and to assess axis, rhythm,
conduction abnormalities and evidence for MI.

*Resource Utilisation and Health-Related Quality of Life*

Resource utilisation and health-related quality of life data was collected throughout. Health-related quality of life was measured using the EQ-5D-5L and the SF36v2 Health Survey (SF-36).

### 2.2.7 Primary Endpoint

The primary efficacy endpoint is the time-to-recurrent heart failure hospitalisations in the
presence of terminal events (all-cause death, heart transplant, LVAD implantation). Sensitivity
analyses for the primary endpoint include: the time-to-first clinical event (including all-cause
death, heart transplant and LVAD implantation and hospitalisation), and the average rate and
duration of hospitalisations.
It is known that the risk of all-cause mortality in patients with advanced heart failure is correlated with recurrent heart failure hospitalisations [6,196]. In addition, as many heart failure patients can experience recurrent hospitalisations, each increasing the probability of another hospitalisation, as well as death, LVAD placement and/or heart transplantation, it is important to evaluate the multiple hospitalisations over time, rather than just the first hospitalisation. As death, LVAD implant and/or heart transplant are important clinical events (otherwise referred to in this study as terminal events), they must be accounted for in the efficacy evaluation. The joint frailty was used in an attempt to gain an unbiased assessment of the impact of AAV1.SERCA2a on recurrent heart failure hospitalisations taking into account the competing risk of these terminal events.

### 2.2.8 Secondary Endpoint

The secondary endpoint is the time-to-terminal event (including all-cause death, heart transplant and LVAD implantation) based on the joint frailty model and performed simultaneously with the primary endpoint analysis. The sensitivity analysis for this secondary endpoint includes, time-to-all-cause death analysis using a statistical approach based on the Kaplan-Meier model and log rank test.

### 2.2.9 Exploratory Endpoints

Exploratory analyses include NYHA class, change from baseline in 6MWD and quality of life.

### 2.2.10 Safety

*Definitions*

Adverse event (AE): An AE is any untoward medical occurrence in a patient or clinical trial
subject administered an IMP and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of the IMP.

Adverse reaction (AR): An AR is defined as all untoward and unintended responses to an IMP related to any dose administered. The definition implies a reasonable possibility of a causal relationship between the event and the IMP.

Serious Adverse Reaction (SAR): An AR that meets the criteria for serious

Suspected unexpected serious adverse reaction (SUSAR): A serious adverse reaction, the nature or severity of which is not consistent with the investigators brochure.

Safety was assessed based on the incidence of adverse events, all-cause mortality and laboratory evaluations of AAV1.SERCA2a versus placebo groups.

Adverse events possibly related to AAV1.SERCA2a in previous studies have in general been mild and self-limiting. It is difficult to determine if AAV1.SERCA2a is the direct cause of these events or whether these events are part of the underlying heart failure condition. The study required careful monitoring of adverse events by an independent Data Monitoring Committee, the treating investigator and by the Medical Monitor and a Steering Committee.

2.2.11 Statistics

Analysis of the data as three separate populations was planned. The full analysis set was the intention to treat population which consists of all randomised subjects. The modified intention to treat population consists of all randomised subjects who received study medication. Finally, the per-protocol analysis was based on those subjects who had no major protocol violations.

Patients who are hospitalised with heart failure are at increased risk of being admitted again, as
such recurrent heart failure hospitalisations within a patient may not be independent. Patients with a high rate of heart failure hospitalisations may also have an increased risk of terminal events (implantation of LVAD, heart transplantation, or death), and therefore the risks of recurrent heart failure hospitalisations and terminal events are likely to be correlated and may need to be jointly estimated to avoid any substantial bias. The unbiased assessment of the impact of therapy on recurrent clinical events (hospitalisations) can be confounded by the competing risk of terminal events. To address this challenge, the joint frailty model, a semi-parametric analysis that accounts for recurrent clinical events, unequal follow-up times between treatment groups, and terminal events as a competing risk, was used for the study primary analysis.

2.3 Measurements

2.3.1 TWA analysis-commercial software

Ambulatory ECG

All AECG data was recorded prospectively on a GE SEER 12, 12-lead AECG monitor. This records ECG data at a scanning rate of 1,024 Hz. The 12-leads were derived from red dot electrodes placed in the standard positions for a 12-lead ECG (Figure 2.3). Prior to placement of the electrode, care was taken to prepare the skin. This was performed with shaving if required, light abrasion and alcohol wipes. Patients were provided with a diary to document any symptoms during the recording period and spare electrodes and educated on how to reattach an electrode if one should fall off. The patients were instructed on how to remove the monitor after 24 hours and they could return the monitor either through the post with an envelope supplied and postage paid for or they could return it in person. On receipt of the monitor, the memory card was removed and data uploaded to the commercial GE software MARS for analysis. Prior
to any TWA analyses, all AECG monitors were analysed from a standard clinical perspective to ensure, in particular, that there were no clinically relevant tachy or bradyarrhythrias and to rule out any occult AF.

Figure 2.3: Illustration of lead placement for a 12-lead ambulatory ECG.

**Modified Moving Average Algorithm: GE software**

Analysis of TWA from AECG data was performed using the MARS (GE) platform which employs the MMA method developed by Verrier and Nearing and described in detail in section 1.2.6. This algorithm analyses three leads of the ECG at a time and potentially results in a TWA every 15 seconds. The analysis has a noise rejection method but after the algorithm has completed it is necessary to confirm the peak TWA in the lead of interest manually. This involves reviewing the ECG data for noise and then reviewing that the template data is appropriate (Figure 2.4). If either the ECG contains excessive noise or the templates do not align then this TWA value is rejected and the next lowest value is interrogated. This process continues until a visually verified TWA is confirmed and this represents the TWA value for that recording. Consistent with other published data, an update factor of 8 was employed for all
studies (relevance of update factor described earlier).

Figure 2.4: Example of data overreading for the GE MMA algorithm of TWA analysis. ECG traces (top panel) used by the algorithm for a particular TWA value need to be reviewed for the presence of noise. Bottom left panel shows the templates generated by the algorithm from the ECG traces in the top panel, equivalent to the averaged beats A and beats B in the algorithm, the alignment of the templates must be reviewed. The bottom right panel shows all the TWA results generated by the algorithm in lead V6 over a four hour window.

2.3.2 Echocardiography

Transthoracic echocardiography is routinely used in clinical and research practice. The primary purpose of echocardiography in these studies was as an assessment of the severity of systolic heart failure (the LVEF). Echocardiography was performed with the subjects in a left lateral decubitus position. A Philips iE33 machine with a S5-1 transducer was used for all studies. While the primary purpose of the scan was to record a measure of LVEF, all scans fulfilled the requirements of the British Society of Echocardiography for the minimum dataset to be acquired for a resting echocardiogram. LV dimensions were acquired in the parasternal long axis views. LVEF was calculated from biplane apical echocardiographic views using Simpsons method.
2.3.3 New York Heart Association Classification

The NYHA classification is used to classify patients based on the severity of their symptoms (Table 2.5). The NYHA classification is commonly used as an inclusion criterion in clinical trials of heart failure and has important prognostic implications.

<table>
<thead>
<tr>
<th>Class 1</th>
<th>No heart failure symptoms and no limitation to ordinary physical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 2</td>
<td>Mild symptoms of heart failure which slightly limit ordinary physical activity</td>
</tr>
<tr>
<td>Class 3</td>
<td>Marked limitation of activity due to symptoms of heart failure</td>
</tr>
<tr>
<td>Class 4</td>
<td>Severe limitation of activity. Symptomatic at rest</td>
</tr>
</tbody>
</table>

Table 2.5: New York Heart Association Classification of Heart Failure.

2.3.4 Six-Minute Walk Distance

The 6MWD was performed according to the American Thoracic Society guidelines of March 2002 using a 30m corridor with a turnaround point marked with a cone. A set of instructions are read out to the patient explaining that they are to walk up and down the corridor and the object is to walk as far as possible for six minutes. The 6MWD has prognostic importance in heart failure and can be used as an outcome measure in clinical trials of heart failure.

2.3.5 Questionnaires

Several questionnaires are available to allow the quantification of patient symptoms. While some of these questionnaires provide prognostic information they are often used in research trials as an outcome measure to quantify if the patients’ symptoms have improved.

KCCQ

The KCCQ is a 23-item, self-administered questionnaire that quantifies physical function, symptoms (frequency, severity and recent change), social function, self-efficacy and knowledge,
and quality of life. An overall score can be calculated from these domains. Scores are transformed to a range of 0-100, in which higher scores reflect better health status.

**EQ-5D-5L**

The EQ-5D-5L is a preference-based measure of health-related quality of life which consists of five items measuring mobility, self-care, usual activities, pain/discomfort and anxiety/depression using five possible responses varying from no problems to extreme problems. The EQ-5D-5L also includes a Visual Analogue Scale. This questionnaire can be used to generate estimates of cost-effectiveness in terms of quality-adjusted life-years.

**SF-36**

The SF-36 consists of 36 questions designed to measure functional health and well being from the point of view of the respondent. The questions posed encompass eight health domains (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health) and psychometrically-based physical component summary and mental component summary scores.

**Minnesota Living with Heart Failure Questionnaire**

The MLWHFQ is a self-administered questionnaire that has reliability and validity in patients with heart failure [197]. It assesses multiple dimensions of quality of life (physical, social and psychological) over the previous month. Higher scores suggest increased symptoms and reduced quality of life.
2.3.6 Natriuretic Peptides Assay

Natriuretic peptides are released by the ventricle when the ventricle is subjected to stretch. BNP is initially synthesised as a preprohormone (preproBNP) which is converted to proBNP which in turn is cleaved to produce BNP and NT-proBNP. The CUPID-2 trial used an NT-proBNP cut-off for eligibility. At our site NT-proBNP is not performed, instead BNP is measured by immunoassay on the Beckman Access 2 Immunoassay analyser (Beckman Coulter, UK). There is no calculation that can directly convert a BNP to an NT-proBNP so in some patients eligibility could not be confirmed until the results from the core lab were available.

2.4 Statistical Analyses

Statistical analyses were performed using SPSS version 20 (IBM, Illinois, USA). The full details of the statistical techniques used will be provided in the relevant chapter.
Chapter 3

Assessment of Non-peak Values of T Wave Alternans using the Modified Moving Average Method

3.1 Introduction

As described earlier (1.2.6), typical analysis of TWA using the MMA method on ambulatory monitoring involves identifying the peak TWA value in one ECG lead over a 24h hour period. Using this value, an individual is classified as TWA positive or negative. Applying this approach, a large amount of data are not utilised: the MMA method of analysis generates a TWA value every 15 seconds resulting in the potential for 5760 TWA values in a single ECG lead in 24 hours. A practical challenge of utilising more of these data points is that each TWA value must be confirmed manually. This involves ensuring the ECG signal is not noisy and visually confirming that the templates created by the algorithm appear to display true TWA. This process can be time consuming.

The current method of TWA analysis has failed to identify patients who would benefit from the implantation of an ICD, above and beyond conventional markers such as LVEF and QRS
duration, and as such is not used in clinical decision making. It is perhaps too simplistic to dichotomise a population into high versus low risk based on a single TWA value in a 24 hour period when so much more data are available.

A small number of studies have used more than one TWA value over 24 hours. These limited data suggest that this approach could improve the accuracy for identification of high risk patients. Yu et al [123] recorded ambulatory ECG data in the early recovery period after an acute MI. The primary end point was SCD or resuscitated cardiac arrest. Multivariate Cox regression analysis demonstrated that, after adjustment for other factors such as age, sex and LVEF, a positive TWA ($\geq 47 \mu V$) was associated with a hazard ratio of 15.1 (95% CI, 2.9-78.8; $P=0.0031$). This hazard ratio increased to 18.2 (95% CI, 4.2-82.6) if 5 or more episodes of TWA $\geq 47 \mu V$ were recorded see Figure 1.13. This supports the idea that additional TWA data points may improve the ability of TWA analysis to identify patients at high risk of ventricular arrhythmias. Although Yu et al extended their analysis of TWA beyond analysis of a single data point, they still only examined the highest levels of TWA. There is no data on low or mid-range levels of TWA. For example, a question that has not been addressed is whether a patient with a single peak TWA $\geq 47 \mu V$ and low level of TWA the rest of the time is at a higher risk of ventricular arrhythmias than a patient who has a constant background level of moderately high TWA but never has high peaks.

The premise of using non-peak TWA values may result in the use of low magnitude levels of TWA. There is data to suggest that levels of TWA as low as $20 \mu V$ can be visually confirmed using the MMA method. Values lower than this are more difficult to confirm as it is necessary to evaluate the separation between the two ECG templates created by the MMA algorithm to confirm that the algorithm is measuring a true difference in T wave magnitude. If this difference is very small it may not be possible to do so reliably (Figure 3.1). One can, however, review the
quality of the ECG signal that is analysed by the algorithm and reject ECG signals with noise.

---

**Figure 3.1**: An illustration to demonstrate the challenge of visually confirming low-level TWA (right) compared to standard values greater than 20µV (left).

In this chapter I sought to determine whether examination of more than just the peak TWA value in 24 hours affected the interpretation of TWA. I planned to interrogate all magnitudes of TWA and due to concerns about the validity of TWA values less than 20µV I first undertook to assess whether the algorithm can detect physiological perturbations in this low-level range.

### 3.2 Aims

Part 1 (reliability of low levels of TWA): To assess if low-level TWA (that may not be confirmed visually) can detect perturbations in cardiac physiology by determining if the HUT manoeuvre, known to provoke sympathetic activation, leads to an expected increase in TWA within the
low-level range.

Part 2 (utility of non-peak TWA variables in the identification of individuals at high risk of ventricular arrhythmias): To assess which non-peak TWA variables are reproducible and able to discriminate between healthy individuals and those with heart failure. To assess if these variables can discriminate, within a heart failure population, those at highest arrhythmia risk more accurately than the traditional peak TWA.

### 3.3 Part 1 (reliability of low levels of TWA)

#### 3.3.1 Methods

**Study Population**

I retrospectively analysed data from clinically indicated TTTs performed between 2012 and 2015. Patients were excluded if they were in AF or there was excessive noise on the ECG. Patients were also excluded if they were known to have cardiovascular disease or were taking relevant cardiac medications (anti-hypertensive or anti-arrhythmic agents) as these factors are known to affect TWA.

**Tilt Table Test Protocol**

The Italian protocol of tilt table testing was employed as described previously [198] (Figure 3.2). Before starting the test, all patients were acclimatised to their environment with a 15 minute period of supine rest while connected to the monitoring equipment. Patients were monitored using the Nexfin system (BMEYE, Netherlands) providing continuous monitoring of six lead ECG (acquired at 1000Hz) and continuous non-invasive arterial blood pressure (acquired at 200 Hz).

**T Wave Alternans Measurement**
An in-house algorithm was programmed in MATLAB R2012a (Mathworks, United States) to replicate the MMA method described elsewhere [110]. To validate the in-house algorithm, 100 simulated ECGs were created with TWA values from 1-100µV. The algorithm correctly identified the TWA with a correlation coefficient of 1.0. ECG data from lead II collected during the TTT was analysed. A 50Hz notch filter and band pass filters were applied. The MMA algorithm was utilised and manual over reading was performed to exclude noise and erroneous results. The mean TWA value for the 5 minutes before HUT was assigned as pre-tilt TWA and the mean TWA value in the 5 minutes after HUT was assigned post-tilt TWA. Additional parameters were recorded for these two 5 minute periods: mean heart rate (HR), mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP) and the heart rate variability parameter low frequency:high frequency ratio (LF:HF). LF:HF was derived using autoregressive spectral estimation with a model order of 16.

**Statistics**

Continuous variables are displayed as mean and standard deviation (SD) and comparison pre and post HUT is made using paired Students t-tests. Categorical data are displayed as count and percentage. Correlation (r) was assessed using Pearsons test. A P value of <0.05 was used as the threshold of statistical significance.
National Health Service (UK) Management Permission for ethical use of anonymised patient data for research was obtained.

3.3.2 Results

Subject Characteristics

1067 patients underwent TTT during the study period but the following patients were excluded: 31 were in AF, 84 lost consciousness within 5 minutes of HUT and there was excessive noise in either the pre or post-tilt ECG of 397 patients. From the remaining 555 patients, 212 had a prior history of cardiovascular disease or were taking cardiovascular medication that could affect TWA. The remaining 343 patients made up the study population. The mean age of the population was 29.2 (16.4) years and 208 (61%) were women.

Effect of HUT

HUT resulted in a significant increase in TWA from \( 28.7 \mu V \) (SD 13.5) to \( 33.4 \mu V \) (SD 18.2), \( P<0.001 \). There was also a significant increase in LF:HF (consistent with sympathetic activation), HR and DBP and a reduction in SBP (Figure 3.3). There was no significant correlation between the change in TWA and the change in any of the other parameters measured.

3.3.3 Conclusions

There is evidence from ambulatory monitoring that TWA is elevated during periods of the day associated with heightened sympathetic activity [134]. Further indirect support that TWA is influenced by sympathetic activation comes from studies which have demonstrated elevated TWA during stressful scenarios [199] and a reduction in TWA with medication that target the sympathetic nervous system [200].
**Figure 3.3:** Comparison of parameters before and after head-up-tilt (n=343). Mean values are displayed and error bars represent standard deviations. TWA, T wave alternans; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LF:HF, low frequency:high frequency ratio. *** represents a statistically significant change in a parameter on tilting to a significance level of $P<0.001$ calculated with a Student’s paired t-test.

HUT is a manoeuvre that is well documented to be associated with sympathetic activation [201, 202] and consistent with this I have demonstrated an increase in LF:HF in this study population. Associated with this I have demonstrated, for the first time, that TWA magnitude increases during HUT. This change could potentially be explained by factors other than sympathetic activation. Firstly, there is a significant increase in heart rate during HUT. The relationship between heart rate and TWA is not fully understood but under certain situations TWA can be influenced by heart rate. I found no correlation between change in heart rate and change in TWA suggesting that in this study the change in TWA on HUT was independent of heart rate. A second potential confounder is the possibility that the magnitude or morphology of the T wave may change on HUT due to changes in the position of the heart relative to the ECG electrodes independent of any sympathetic activation. I did not investigate whether there
was a change in T wave magnitude on HUT, in part because if a change was observed it would not be possible to determine if this change was due to body positioning or due to sympathetic activation. The effect of T wave magnitude on TWA will be discussed in more detail later. The magnitude of TWA measured in this study was low and often could not reliably be confirmed by eye, yet the algorithm measured changes that would be consistent with sympathetic activation during HUT. These findings support the use of low values of TWA for Part 2 of this chapter.

3.4 Part 2 (utility of non-peak TWA values in the identification of individuals at high risk of ventricular arrhythmias)

3.4.1 Methods

Study Population

Healthy volunteers were recruited and patients with heart failure were recruited from outpatient heart failure clinics and from the pacemaker clinic (to identify those with an ICD). Heart failure patients were required to have an LVEF ≤ 35% and be aged 18-80 years. Patients had ischaemic or dilated cardiomyopathy with other causes of heart failure being excluded. Patients were also excluded if they had a biventricular pacemaker because TWA has not been validated in paced rhythms.

T Wave Alternans Measurement

Participants wore a 12 lead AECG monitor for 24 hours (GE SEER 12) and TWA analysis was performed using the MARS (GE) platform as described in the main methods Chapter 2. To
assess for reproducibility this was repeated on a separate day when the participant expected daily activities to be similar with a maximum interval of two weeks. This second monitoring period was performed in all healthy volunteers and in those heart failure patients who were willing. This second day of data collection was not a mandatory component so as to avoid limiting recruitment to only those who were willing to travel twice to the hospital. All TWA data analysis was performed in a blinded fashion and patient data pseudonymised.

Non-peak values assessed

The peak TWA value was assessed in the traditional manner as described in the general methods Chapter 2. In addition, a number of other exploratory parameters were measured. All TWA values \( \geq 20 \mu V \) in the 24 hour period were visually inspected and erroneous data points excluded. The resultant TWA values then formed a data series for each participant. This data series was described with different variables: peak TWA over the 24hr period, number of TWA values \( \geq 47 \mu V \), number of TWA values \( \geq 20 \mu V \), sum of these TWA values, median TWA and IQR.

Two other TWA data series were created for each participant by examining every TWA value in two 1 hour periods. Nocturnal ECG recordings (4am to 5am) are less prone to noise which leads to more efficient analysis. A waking period (7am to 8am) was chosen as this is a time when a surge in sympathetic activity occurs and this can unmask proarrhythmia. An early study of TWA using the MMA method also examined values at 8am [134] which would make my results potentially comparable, however, each patient was asked to record the time they woke and if this was significantly earlier or later than 8am then a more appropriate "waking" hour was chosen for that patient. These two data series (nocturnal and waking) were then summarised using the following variables: peak TWA, sum of all TWA values and the median TWA.

A summary of the non-peak parameters that were derived is illustrated in Table 3.1.
Every TWA value ≥20µV over the 24 hour period was visually confirmed. This created a data series which was described with the following variables:

- Peak TWA (µV)
- Number of episodes when TWA ≥47µV
- Number of episodes when TWA ≥20µV
- Sum of TWA values (µV)
- Median TWA (µV)
- TWA interquartile range (µV)

Every TWA value in each one hour window were interrogated. For TWA values <20µV the templates created by the MMA method were difficult to confirm visually but noise on the ECG was excluded. This created a data series which was described with the following variables:

- Peak TWA (µV)
- Sum of TWA (µV)
- Median TWA (µV)

<table>
<thead>
<tr>
<th>Table 3.1: Description of the non-peak TWA values examined.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values that were reproducible and able to distinguish between healthy subjects and those with heart failure were assessed for their ability to distinguish between two cohorts within the heart failure population. These two cohorts were patients with an ICD who had received an appropriate shock within the last 4 months and patients with an ICD who had not received an appropriate shock for over 12 months. The rationale for this is that those who recently received a shock are more likely to be considered as high risk for arrhythmias than those who have not.</td>
</tr>
</tbody>
</table>

**Statistics**

Group data are described as median and IQR. Populations are compared using a Mann-Whitney U test. Reproducibility is assessed using coefficient of variation. Ethical approval for this study was granted by the UK National Research Ethics Service.

### 3.4.2 Results

Characteristics of the heart failure patients are displayed in table 3.2. The probability of recording a TWA of different magnitudes during the nocturnal hour (4am to 5am) in the healthy subjects compared to heart failure subjects is presented in figure 3.4.

The probability of recording a TWA magnitude of zero in this nocturnal hour appeared to be a parameter that could distinguish well between heart failure and healthy subjects and as such
Age (years) 64.5 (59.8-74.0)  
Gender (male) 66%  
Ejection Fraction (%) 30.5 (26.0-33.0)  
Ischaemic 70%  
DCM 30%  
Diabetes 40%  
Prescribed beta-blocker 84%  
Prescribed ACEi or ARB 90%  
Prescribed MRA 60%  
ICD 64%  

Table 3.2: Characteristics of the 50 heart failure patients studied. Continuous variables described as median (IQR). ACEi, angiotensin converting enzyme inhibitor; ARB, angiotension II receptor blocker; DCM, dilated cardiomyopathy; ICD, implanted cardioverter defibrillator (dual chamber or single chamber devices, no biventricular devices); MRA, mineralocorticoid receptor antagonist.

this parameter was used in addition to those pre-specified in the methods.

Figure 3.4: Probability density distribution for all TWA values during the nocturnal hour (4-5am). The probability of recording different TWA values during a one hour nocturnal period are presented as medians and interquartile range. Two populations are displayed: healthy subjects and heart failure patients.

A comparison of heart failure patients and healthy subjects for all measured TWA parameters and the coefficient of variation for each parameter is displayed in Table 3.3. These data are
non-parametric so described as median and interquartile range, however, the results for one variable, the number of TWA values $\geq 47\mu V$, are not well described in this way. In particular, the result for 17 out of 18 healthy subjects is zero and for the heart failure patients most results are either zero or one which leads to both groups having a median value of zero but there being a statistically significant difference between the two groups. To aid in the description of this variable it is also represented as mean and standard deviation.

### Table 3.3: Utility of T wave alternans (TWA) nocturnal values and values $\geq 20\mu V$ at distinguishing between healthy subjects and those with heart failure. Values displayed are medians and IQR and statistical comparisons are made using a Mann-Whitney U test except for * for which the data presented are mean (standard deviation) and the comparison is made with a Student’s t-test. CV=within subject coefficient of variation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy (n=18)</th>
<th>Heart Failure (n=50)</th>
<th>P</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All values $\geq 20\mu V$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak TWA ($\mu V$)</td>
<td>33 (26-40)</td>
<td>45 (31-50)</td>
<td>0.009</td>
<td>10</td>
</tr>
<tr>
<td>No. of values $\geq 47\mu V$</td>
<td>0 (0-0)</td>
<td>0 (0-2)</td>
<td>0.004</td>
<td>18</td>
</tr>
<tr>
<td>*No. of values $\geq 47\mu V$</td>
<td>0.06 (0.24)</td>
<td>1.14 (1.69)</td>
<td>0.009</td>
<td>18</td>
</tr>
<tr>
<td>No. of values $\geq 20 \mu V$</td>
<td>21 (11-30)</td>
<td>49 (28-76)</td>
<td>&lt;0.001</td>
<td>52</td>
</tr>
<tr>
<td>Sum of TWA ($\mu V$)</td>
<td>602 (321-777)</td>
<td>771 (654-1534)</td>
<td>0.001</td>
<td>54</td>
</tr>
<tr>
<td>Median TWA ($\mu V$)</td>
<td>24 (21-25)</td>
<td>24 (21-24)</td>
<td>0.398</td>
<td>11</td>
</tr>
<tr>
<td>TWA IQR ($\mu V$)</td>
<td>8 (7-9)</td>
<td>8 (7-9)</td>
<td>0.34</td>
<td>34</td>
</tr>
</tbody>
</table>

| TWA 4-5am                                      |                |                      |      |        |
| Prob TWA=0                                     | 0.86 (0.81-0.88)| 0.32 (0.30-0.38)    | <0.001| 6      |
| Peak TWA ($\mu V$)                            | 10 (9-11)      | 15 (14-16)          | <0.001| 19.5   |
| Sum of TWA ($\mu V$)                          | 75 (65-79)     | 367 (289-467)       | <0.001| 33     |
| Median TWA ($\mu V$)                          | 0 (0-0)        | 1 (1-2)             | <0.001| x      |

| TWA 7-8am                                      |                |                      |      |        |
| Prob TWA=0                                     | 0.82 (0.79-0.84)| 0.31 (0.29-0.37)    | <0.001| 9      |
| Peak TWA ($\mu V$)                            | 11 (7-14)      | 17 (15-20)         | <0.001| 16     |
| Sum of TWA ($\mu V$)                          | 86 (74-91)     | 416 (326-525)       | <0.001| 42     |
| Median TWA ($\mu V$)                          | 0 (0-0)        | 1 (1-2)            | <0.001| x      |

Parameters displayed in Table 3.3 that were reproducible (coefficient of variation $\leq 10\%$) and able to distinguish between health and heart failure were used to compare heart failure patients with an ICD who have had a recent shock to those with an ICD who have not had a recent
shock (Table 3.4). These parameters were the traditional peak TWA over 24 hours, probability of recording a TWA of zero in the nocturnal hour (4am to 5am) and the probability of recording a TWA of zero in the waking hour (7am-8am). The only one of these three parameters that could distinguish between the two heart failure populations was the traditional peak TWA over 24 hours.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ICD no shock (n=21)</th>
<th>ICD with shock (n=7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak TWA</td>
<td>49 (36-51)</td>
<td>81 (51-86)</td>
<td>0.002</td>
</tr>
<tr>
<td>Prob TWA=0 (4-5am)</td>
<td>0.32 (0.31-0.35)</td>
<td>0.25 (0.17-0.34)</td>
<td>0.126</td>
</tr>
<tr>
<td>Prob TWA=0 (7-8am)</td>
<td>0.31 (0.29-0.35)</td>
<td>0.24 (0.16-0.32)</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 3.4: Ability of different T wave alternans (TWA) parameters to distinguish between heart failure patients with or without a recent appropriate shock from their implantable cardioverter defibrillator (ICD). Statistical comparisons made using a Mann-Whitney U test.

**Limitations**

The most significant limitation of this study is that I retrospectively used the presence of a recent appropriate ICD shock as a surrogate of being high risk for future arrhythmias compared to a heart failure population with an ICD who had not had a recent shock. There is evidence that recent ICD therapy does predict future ICD therapy. A better study design would have been to prospectively recruit patients due to undergo ICD implantation, record these TWA parameters prior to implantation and follow the patient using appropriate ICD therapy as an endpoint. However, I felt that the event rate in this study would have been low, requiring a large number of participants making this study design unfeasible.

### 3.4.3 Conclusions

There are non-peak values of TWA that are reproducible and able to distinguish between normal subjects and those with heart failure. However, these parameters were not able to identify,
within a heart failure population who have an ICD, those who had recently had a shock versus those who have not. The traditional peak TWA was still the best discriminator between those with a recent shock and those without a recent shock.

3.5 Discussion

Traditional measurement of the peak TWA measurement in a 24 hour period on ambulatory monitoring has the capacity to dichotomise patient populations into those at high risk of ventricular arrhythmias and those at lower risk. This approach has, however, failed in attempts to apply a TWA cut-off value in individuals that can reliably select those patients who would benefit from implantation of an ICD and as such is not used as a clinical tool. I hypothesised that using more than just the single peak TWA value in a 24 hour period would provide more information about an individual and help identify which patients are at greatest risk.

My approach required interrogation of low-level TWA which has not been widely published. As such, I first demonstrated that low-level TWA did appear to measure physiological perturbations created by HUT. All TWA values measured using the MMA method must be visually confirmed which is time consuming and so I limited my analysis to three approaches: 1. to assess all TWA values $\geq 20\mu V$ in the 24 hour period ($20\mu V$ is the lowest value reported in the literature as reliably confirmed visually), 2. to analyse all TWA values in a nocturnal one hour period (4am to 5am) when the ECG is least noisy and 3. to analyse all TWA values in a waking hour (7am to 8am) during which there is a sympathetic surge that may unmask high levels of TWA [134].

A comparison between healthy subjects and those with severe heart failure is not of particular clinical utility but the parameters I measured are all novel and as such this exaggerated comparison was made in order to eliminate any variables that could not distinguish between these very different populations. When considering all values $\geq 20\mu V$ it is not surprising that
the heart failure population have significantly higher peak TWA and have more TWA values \( \geq 20\mu V \) and \( \geq 47\mu V \). Interestingly, the median TWA value in the 24 hour period was not significantly different between the two populations. This may suggest that both populations have a similar background low-level magnitude of TWA but the heart failure patients have surges of high level TWA and it has been noted in animal studies that ventricular arrhythmias are preceded by surges in TWA. The most reproducible parameter measured in the \( \geq 20\mu V \) range, was the traditional peak TWA and this parameter was able to distinguish within the heart failure population those at higher arrhythmia risk.

Examining the nocturnal hour and the waking hour, the most reproducible parameter was the probability of recording a TWA value of zero. This parameter also provided a good distinction between healthy subjects and those with heart failure. During each of these hours, healthy subjects display a TWA of zero over 80% of the time, while in heart failure patients it’s just over 30%. However, this parameter was not able to discriminate between those heart failure patients at higher risk and those at lower risk.

Overall, to extract the parameters I analysed was time consuming and did not appear to add value above and beyond the use of the traditional peak TWA. These findings lend support the notion that it is the vulnerability of the heart to develop surges of high levels of TWA that is more important that the magnitude of background levels of TWA.
Chapter 4

The Importance of Lead Selection in the Interpretation of T Wave Alternans Using the Modified Moving Average Method

4.1 Introduction

There is no universal recommendation as to which lead of the ECG should be examined when interpreting TWA. In previous TWA studies using the MMA method lead V5 with an abnormality threshold of $\geq 47\mu V$ is the most commonly quoted [121, 123, 134]. Lead selection may be crucial since myocardial pathology can be regional and therefore project to different leads on the ECG, as observed in myocardial ischaemia. This may reduce diagnostic sensitivity and result in type 2 errors, with a TWA positive patient classified as TWA negative if the wrong lead is interrogated. The application of 12-lead AECG monitors could overcome this limitation by allowing TWA to be recorded from multiple anatomical regions of the heart which could improve the ability of the technique to identify those at high risk of ventricular arrhythmias.
4.2 Aims

To investigate whether lead selection, using 12-lead AECGs, significantly affects the interpretation of TWA using the MMA method.

To investigate, using a simulation study, how the location within the ventricle of action potential duration alternans affects the location and magnitude of TWA on the surface ECG.

4.3 Methods

This study was made up of two parts: a clinical study in which 12-lead AECG monitors from patients with heart failure were examined and a simulation study in which simulated TWA was created.

Part 1 (clinical study)

The patient population recruited were the same heart failure patients recruited for study in Chapter 3. In brief they were stable adult patients with a confirmed diagnosis of chronic heart failure who were in sinus rhythm and had an LVEF $\leq$ 35%. They were monitored using 24 hour 12-lead AECG monitors (GE SEER 12). The underlying aetiology was either ischaemic cardiomyopathy or dilated cardiomyopathy with exclusion of patients who had other underlying aetiologies. Peak TWA over the 24 hour period was determined using the MMA method (MARS, GE) in each of the 12 ECG leads. Patients were classified as TWA positive if the peak TWA value in any of the 12 leads was $\geq 47 \mu V$. The remaining patients were classified as TWA negative. The sensitivity for identifying TWA positive patients was calculated for each of the individual 12 ECG leads and for different combinations of leads. Statistical comparison of the TWA positive and negative cohorts was performed using Fishers exact test for categorical
variables and Mann-Whitney U test for continuous variables. Ethical approval for this study was granted by the UK National Research Ethics Service.

**Part 2 (simulation study)**

To investigate potential explanations for any regional variation discovered in the clinical study, a simulation study was performed. TWA has been shown to originate from underlying action potential duration alternans (APDA) [105] and this can be a regional phenomenon. I simulated APDA in different regions of the heart and inspected the resultant 12-lead ECG for the magnitude of TWA in each lead (Figure 4.1). I carried out the simulation study using ECGsim which is an open source program that models the transmembrane action potential at 1500 nodes in the heart [203]. Action potential parameters (including depolarisation time and repolarisation time) can be altered and the effect of these changes observed on the simulated 12-lead ECG. In order to model APDA I simulated two consecutive beats. The first using default settings and the second by shortening repolarisation time by 4ms. The selection of 4ms as the magnitude of APDA to simulate was in part to allow direct comparison with the only other study that has simulated APDA in this manner [204]. In addition, this magnitude of APDA has been demonstrated in a clinical study in which monophasic action potentials were recorded during pacing in 53 patients with LV dysfunction (LVEF 28±8%) and 18 control subjects. The beat to beat difference in APD was 3±4 ms in those with preserved LV and 8±14ms in those with LV dysfunction [205]. This process of simulating APDA was carried out globally across the whole myocardium and then in different locations within the left ventricular myocardium (anterior, apical, posterior and lateral) and in the right ventricle (RV). I then measured TWA in each lead of the ECG as the maximum difference in amplitude between the two T waves (a method that would provide results analogous to the MMA method).
Figure 4.1: Methodology for simulating action potential duration alternans using ECGsim and measuring the resultant T wave alternans. A single node is selected on the surface of the heart which can be within the left ventricle or right ventricle. The example displayed in panel A illustrates a node representing the anterior surface of the left ventricle. The action potential at this node is represented in panel B. The white action potential line in panel B represents default settings and the red line shows a shortened version of the action potential. The effect that this change in action potential duration has on the 12-lead ECG can be seen by comparing the corresponding white and red complexes in panel C. T wave alternans for each lead is calculated as the maximum difference between the amplitude of the white and red T wave complexes (illustrated for lead V3). APD; action potential duration, TWA; T wave alternans

4.4 Results

Part 1 (clinical study)

Fifty patients with heart failure were enrolled. Thirty-six were classified as TWA positive and 14 as TWA negative. Patient characteristics are displayed in Table 4.1
Table 4.1: Characteristics of TWA positive and TWA negative patients. Continuous variables are presented as median (IQR) and categorical variables are presented as count (percentage). Statistical comparisons made between TWA positive and negative patients with a Mann-Whitney U test for continuous variables and a chi square test for categorical variables. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ICD, implantable cardioverter defibrillator; MRA, mineralocorticoid receptor antagonist.

<table>
<thead>
<tr>
<th></th>
<th>Complete cohort (n=50)</th>
<th>TWA positive (n=36)</th>
<th>TWA negative (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 (54-71)</td>
<td>60 (54.5-70)</td>
<td>61 (54-74)</td>
<td>0.810</td>
</tr>
<tr>
<td>Max TWA in any lead</td>
<td>51 (46-63)</td>
<td>55 (51-65)</td>
<td>43 (38-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>28 (25-30)</td>
<td>26.5 (24-3)</td>
<td>29.5 (26-30)</td>
<td>0.285</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>124 (120-131)</td>
<td>125.5 (120-134)</td>
<td>122 (120-125)</td>
<td>0.243</td>
</tr>
<tr>
<td>Ischaemic aetiology</td>
<td>30 (60.0)</td>
<td>23 (63.9)</td>
<td>7 (50.0)</td>
<td>0.391</td>
</tr>
<tr>
<td>Male</td>
<td>36 (72.0)</td>
<td>27 (75.0)</td>
<td>9 (64.3)</td>
<td>0.470</td>
</tr>
<tr>
<td>Presence of ICD</td>
<td>16 (32.0)</td>
<td>12 (33.3)</td>
<td>4 (28.6)</td>
<td>0.771</td>
</tr>
<tr>
<td>Beta-blocker use</td>
<td>35 (70.0)</td>
<td>25 (69.4)</td>
<td>10 (71.4)</td>
<td>0.911</td>
</tr>
<tr>
<td>ACEi/ARB use</td>
<td>45 (90.0)</td>
<td>32 (88.9)</td>
<td>13 (92.9)</td>
<td>0.745</td>
</tr>
<tr>
<td>MRA use</td>
<td>31 (62.0)</td>
<td>22 (61.1)</td>
<td>9 (64.3)</td>
<td>0.852</td>
</tr>
</tbody>
</table>

The utilisation of β-blockers in this population is relatively low at 70%. Of those patients not prescribed a β-blocker, one fifth were prescribed ivabradine. While the National Heart Failure Audit would suggest that 86% of patients admitted with heart failure are discharged on a β-blocker, there is evidence that prescriptions for patients in outpatient clinics may be as low as 70% [206]. The use of β-blockers is important when interpreting the results of TWA analyses as described above (particularly using the spectral method) so the low use of β-blockers could be of some concern. However, the TWA positive and negative groups were balanced in terms of β-blocker use so the effect should be minimised.

The largest TWA value across all 12 leads was found in V5 in 32% of patients. The remaining 68% displayed the peak TWA across a different precordial lead (V1-4) or limb lead (II, III)(Figure 4.2).

The peak TWA for each of the 12 leads is displayed for individual patients in Figure 4.3. This illustrates that, of the TWA positive patients, 39% were TWA positive in only one lead, 14% in two leads and 22% in 3 leads. Leads with a tendency to display high TWA values were V5,
II, V3 and V2. There is a drop-off in TWA magnitude in leads adjacent to those that register a TWA positive value. This is exemplified in the 16 TWA positive patients for whom the peak TWA value was found in lead V5. In these patients, if an adjacent lead was examined 13 would be reclassified as TWA negative.

**Figure 4.3:** All T wave alternans (TWA) results for all patients for all leads and patients grouped based on TWA status (TWA positive versus TWA negative). Each horizontal line represents a patient and the intensity of black/white along the horizontal line represents the magnitude of peak TWA in each ECG lead on the x-axis.

The sensitivity to correctly identify a patient as TWA positive for each of the 12 leads and for
combinations of leads is displayed in Figure 4.4. The highest sensitivity for a single lead is 71% when V5 is used. The addition of lead II to V5 increases the sensitivity to 88% and if the combination of V5, lead II and V2 is used the sensitivity increases to 97%.

![Sensitivity of Single Leads](image1.png)

![Sensitivity of Lead Combinations](image2.png)

**Figure 4.4:** The sensitivity of each ECG lead and combinations of ECG leads for identifying TWA positive patients

**Part 2 (simulation study)**

The magnitude of TWA observed in each lead of a simulated 12-lead ECG for each anatomical location of 4ms simulated APDA is displayed in Figure 4.5. The lead that displayed the maximum TWA was dependent upon the location of the APDA. During global and anterior APDA the peak TWA was observed in lead V3. During apical APDA the peak TWA was in
lead V4 while lateral APDA resulted in a peak TWA being observed in V6. During posterior and RV APDA the peak TWA was seen in V2. Despite the same magnitude of APDA being simulated in each region, there was a large variation in the magnitude of TWA recorded from 16.5µV (during lateral APDA) to 44.1µV (during anterior APDA). In addition, TWA magnitude dropped off rapidly in the leads adjacent to the highest recorded TWA.

**Figure 4.5:** Results of TWA simulation study. Action potential duration alternans (APDA) of 4ms is simulated in different regions of the left ventricle and in the right ventricle. The magnitude of TWA generated on the simulated ECG for each lead is represented. With the same amount of APDA the different regions of myocardium display different peak TWA in different ECG leads.

**Limitations**

The clinical findings from this study are derived from one cohort of heart failure patients and they may not be generalizable to other populations. Furthermore, outcome data was not available for this population to suggest that those with high TWA levels in leads other than V5 are at high risk of events. However, I consider these findings relevant to investigators conducting prospective studies measuring TWA that will have outcome data. If multiple leads are examined in these studies then the diagnostic importance of non-V5 leads can be better established and the accuracy of TWA to identify individuals at high risk of ventricular arrhythmias may be improved. For my simulation study I employed an APDA magnitude of 4ms consistent with a previous simulation study [204] but the absolute magnitude of the resultant TWA seen on
the simulated ECG may not be comparable to the levels of TWA seen in our clinical study. However, it is the relative effect of changing the location of the APDA on the different leads of the ECG that is the key finding from the simulation study.

### 4.5 Discussion

The main finding of this study is that the sensitivity of identifying TWA positive patients using the MMA method on 12-lead AECG monitoring in this cohort is increased from 71% when examining one ECG lead to 97% if three leads (specifically V2, V5 and II) are examined. This may be due to regional variation in underlying pathology.

The importance of lead selection in the analysis of TWA using the MMA method was illustrated in the first description of this method [134]. In a retrospective study of 44 patients with a previous myocardial infarction, data from modified leads V1 and V5 from 24 hour ambulatory monitors were analysed. Lead V5 was superior to lead V1 at predicting cardiac arrest or arrhythmic death. Patients with a peak TWA equal to or greater than 47µV in lead V5 were at high risk of ventricular arrhythmias and sudden death. This 47µV cut-off was tested in a retrospective study using modified leads V1 and V3 (n=138) [121] and the 47µV cut-off was predictive for lead V3 but the optimal cut-off for V1 was 43µV. The 47µV cut-off was further tested in a prospective study (n=219) [123] which designated the highest TWA value found in lead V2 or V5 as the peak TWA value and demonstrated that a value $\geq 47\mu V$ was predictive for SCD. While these three studies provide valuable and corroborating data, the findings are limited since 12-lead ECG monitoring was not available.

In addition to measuring TWA on ambulatory monitoring it can also be performed on exercise testing and further evidence to support the importance of lead V5 comes from a study that
recruited 3598 patients undergoing a clinically indicated exercise test [207]. The ability to use the maximum TWA in each precordial lead, along with combinations of precordial leads, to predict events was determined using Cox regression analysis. Maximum TWA in lead V5 was the most predictive for risk of cardiovascular mortality and SCD increasing by 55% and 58% respectively for each 20µV increase in TWA. When using combinations of leads the prediction of SCD was lost. This study was able to interrogate all the precordial leads but was limited since the limb leads were not examined (due to the greater problem of noise in limb leads during exercise). Furthermore, the findings of an exercise study may not directly translate to ambulatory monitoring.

My aim was to use the MMA method to examine all 12 ECG leads from ambulatory monitors and assess whether this provided additional information compared to the more traditional assessment of one or two precordial leads. The most important finding from my clinical study was that a third of patients would have been classified as TWA negative if lead V5 was interrogated alone but had high levels of TWA in other leads. For the purpose of this study I classified these patients as TWA positive because they had a TWA ≥47µV in at least one ECG lead. There are no established cut-points in leads other than V5 and 47µV may not prove to be a suitable discriminator between high and low risk patients. However, even with this caveat, the magnitude of TWA in non-V5 leads for these patients was high with a median peak TWA of 61µV (IQR 52-64µV). In the absence of outcome data, I cannot confirm whether such levels of TWA in these leads place an individual at high risk of arrhythmias. This would require studies to investigate cut-points in each of the 12-leads of the ECG, but in the interim one may be concerned if these patients were classified as TWA negative in a clinical study.

The importance of lead selection is possibly due to regional variation in the underlying pathology. In canines cardiac alternans has been shown to occur in regions of ischaemia
and linearly projected to the precordium [130]. In patients with cardiomyopathy, cardiac
alternans is not uniformly distributed within the heart. Instead there can be areas of alternating
and non-alternating myocardium adjacent to each other [208]. This heterogeneity in cardiac
alternans may be due to regional heterogeneity in restitution kinetics [209] which itself is
as a consequence of abnormalities in intracellular calcium handling in diseased segments of
myocardium [210].

My simulation study demonstrates the effect of regional APDA on the magnitude of TWA
observed in each of the 12-leads of the ECG. The lead displaying the peak TWA level varied
depending on where the underlying APDA was simulated. Furthermore, despite the same
magnitude of APDA being simulated in each region of the heart, the maximum TWA varied
from 16.5\(\mu\)V to 44.1\(\mu\)V. If this simulation holds true in patients then there may regions of the
heart with a large burden of pro-arrhythmic APDA which may never register as a high TWA
value on the surface ECG. The effect of this may be to classify a high risk patient as TWA
negative.

My findings may have implications for future clinical studies examining TWA. I have illustrated
two potential sources of false negative results: if only one ECG lead is examined then high levels
of TWA may be present in other leads but not identified and patients categorised inappropriately
as TWA negative. There also may be patients with cardiac alternans in regions of the heart that
project poorly to the surface ECG.
Chapter 5

The Effect of Heart Rate and T Wave Amplitude on the interpretation of T Wave Alternans Using the Modified Moving Average Method

5.1 Introduction

The relationship between heart rate and TWA has not been fully elucidated. Animal studies of normal guinea pig hearts have demonstrated that cardiac alternans may be dependent on heart rate. Alternation of repolarisation and depolarisation became evident when hearts were paced at approximately 275 bpm and increased in magnitude with increasing heart rate up to around 325 bpm when the magnitude of alternans plateaued or declined with further increases in heart rate [105]. Furthermore, a requirement for the spectral technique of analyzing TWA is that the heart rate be elevated to within a narrow range (105-110 beats per minute) [107]. T wave alternans values derived using the spectral method can not be compared between subjects if they are recorded at significantly different heart rates. Heart rate is not taken in to account when analysing TWA using the MMA method and there is no data to guide whether TWA is dependent on heart rate in the range of heart rates recorded during ambulatory monitoring.
Another parameter which is not adjusted for in either the MMA or spectral method is the amplitude of the T wave being analysed. The suggestion that there may be an association between the magnitude of TWA and the amplitude of the T-wave (independent of the risk of sudden death and malignant arrhythmias) comes from data from patients with intraventricular conduction delay. These patients have higher values for TWA, [113, 137, 138], and it has been proposed that this may be due to the larger T-waves observed in this population.

If the magnitude of TWA is dependent on heart rate or T wave amplitude [211, 212] this could have significant implications for the interpretation of TWA as one may have to index the TWA value based on these other parameters as has previously been suggested [213]. The potential implication of this could be the reclassification of patients from TWA positive to negative and vice versa depending on the heart rate and amplitude of the T wave recorded at the time the peak TWA was recorded.

### 5.2 Aim

To determine if there is an association between TWA magnitude and heart rate or T wave amplitude using the MMA method on ambulatory monitoring.

### 5.3 Methods

Fifteen healthy volunteers wore a 12-Lead AECG monitor (GE SEER 12) for 24hrs. Participants were asked to avoid vigorous exercise during the monitoring period as this could produce heart rates in a range greater than that expected from a heart failure population. TWA was analysed using the MMA method (GE MARS TWA analysis software) as described in the general methods Chapter 2. In order to perform correlation analysis between heart rate and...
TWA, a number of data points recording simultaneous heart rate and TWA were required. To this end, each TWA value \( \geq 20 \mu V \) in lead V5 was manually checked to eliminate noise and the heart rate was documented for each valid TWA value. A cut-off of \( \geq 20 \mu V \) was chosen as the minimum TWA magnitude that has been reported to be reliably confirmed visually [107]. In order to perform correlation analysis between T wave amplitude and TWA, a range of T wave amplitudes with corresponding TWA values was required. This was achieved by interrogating different ECG leads in the same participant: the peak TWA value over the 24hr period was determined in leads I, aVF and V5 and the corresponding T wave amplitude measured by printing out the relevant 15 second ECG strip, manually measuring the amplitude of all T waves in the strip and calculating the average. Correlation between TWA magnitude and each of heart rate and T wave amplitude was assessed using Kendalls tau-beta.

Ethical approval for this study was granted by the UK National Research Ethics Service.

5.4 Results

The range of heart rates recorded was 53-97 bpm. There was no significant correlation between heart rate and TWA magnitude (Kendalls tau-b = -0.018, 95% CI= -0.087 to 0.052), Figure 5.1. There was a weakly positive correlation between T-wave amplitude and TWA magnitude (figure 5.2, Kendalls tau-b = 0.234).

5.5 Discussion

The principal findings of this study are that in healthy subjects there was no evidence for a correlation between heart rate and TWA within the range of heart rates recorded on ambulatory monitoring and there is a weak correlation between T wave amplitude and TWA magnitude.
Figure 5.1: The association between heart rate and magnitude of T wave alternans. (Kendalls tau-b = -0.018). Only TWA values of 20µV or more were interrogated.

Figure 5.2: The association between T wave amplitude and magnitude of T wave alternans (Kendalls tau-b = 0.234).

These findings suggest that adjusting TWA analysis to account for differences in heart rate or T wave amplitude would not significantly affect the interpretation of TWA.

Animal studies have demonstrated that the magnitude of TWA is dependent on heart rate [105]. This effect of heart rate on TWA may be due to the effect of heart rate on calcium cycling [214]. The effect of heart rate on TWA in man is less clear. In the first clinical study using atrial pacing
to measure TWA with the spectral technique, a subgroup of 10 patients were examined and there was no effect of heart rate (in the range 95-150 bpm) on TWA [103]. Kavesh et al specifically designed their study to investigate the effect of heart rate on TWA [215]. Forty-five patients due to undergo electrophysiology study were recruited. TWA was measured using the spectral technique while in sinus rhythm, with atrial pacing at 100 bpm and with atrial pacing at 120 bpm. TWA increased significantly with increasing heart rate. Furthermore, the sensitivity of TWA to identify patients who would exhibit inducible VT during the EP study increased as heart rate increased (4%, 42% and 65% at 77, 100 and 120 bpm respectively). Conversely, increasing heart rate reduced the specificity to identify inducible VT. This study supported aiming for a target heart rate of between 100-120 bpm when using the spectral technique. It is important to note, however, that the use of inducible VT as an outcome measure would not be considered of strong clinical relevance in contemporary practice. Further support for the importance of heart rate on TWA analysis using the spectral technique comes from a study designed to investigate whether the heart rate at which a patient becomes TWA positive (onset heart rate) was important. Kitamura et al enrolled 104 patients with DCM and measured TWA using the spectral technique during controlled bicycle exercise testing [216]. The development of TWA positivity at a heart rate less than or equal to 100 bpm was an independent predictor of arrhythmic events.

While these data are available for the spectral method no similar data are available for the MMA method and heart rate is not considered when using the MMA method. My findings cannot be generalised to all populations and all heart rates but do suggest that in normal individuals in the range of heart rates observed (55-98 bpm) that TWA magnitude is not dependent on heart rate. One could hypothesise that since patients with heart failure demonstrate abnormalities of calcium cycling, that TWA in this population may be more dependent on heart failure but I did not specifically study this relationship.
No studies have been published that specifically examine if TWA magnitude is influenced by the amplitude of the T wave. Surrogate data from patients with large T waves due to intraventricular conduction delay suggests that large T waves are associated with larger values for TWA independent of the risk of sudden death and malignant arrhythmias [113, 137, 138]. However, this cannot be extrapolated to suggest that TWA is dependent upon T wave amplitude. The findings from my study suggest that there is a weakly positive correlation in healthy individuals between T wave amplitude and TWA but it is doubtful that this has any clinical relevance.

In summary my findings suggest that TWA measured using the MMA method does not need to be indexed for heart rate or T wave amplitude.
Chapter 6

Measuring T Wave Alternans in Patients with Heart Failure: the effect of atrial fibrillation and biventricular pacing

6.1 Introduction

The presence of AF or ventricular pacing are both exclusion criteria in the majority of clinical studies of TWA. This significantly limits the applicability of TWA to patients with heart failure as the prevalence of AF reported in heart failure studies is between 13-27% [51,217–220] while the presence of cardiac resynchronization therapy (CRT) devices, and therefore a high degree of ventricular pacing, is a limitation in those with severe LV systolic dysfunction.

The exclusion of patients in AF from the early TWA studies using the spectral method was based on the absence of a gradual rise in heart rate with exercise and doubts about the validity of measuring irregular R-R, and hence Q-T, intervals using spectral techniques [221]. In those few trials that did include patients with AF, it was found to be a significant cause of indeterminate results [222]. The use of the MMA method for analysing TWA has not been investigated in participants with AF. Theoretically, the MMA method may be more robust than spectral
techniques when analysing irregular intervals.

The exclusion of those with ventricular pacing is also based on limited data. There are small studies which have investigated the effect of ventricular pacing on TWA using spectral analysis techniques but these studies have produced conflicting data. There are no data on the use of the MMA method in those with ventricular pacing.

6.2 Aims

To determine if the presence of AF or CRT ventricular pacing significantly affects the magnitude of TWA measured using the MMA method.

6.3 Methods

DCCV study

To investigate the effect of AF on the magnitude of TWA, the DC cardioversion (DCCV) study was performed: patients in AF who were due to undergo elective DCCV were recruited. Eligible patients who consented wore a 12 lead ambulatory monitor (GE SEER 12) for 24 hours while in AF within four weeks of, and as close as possible to, the date of their DCCV. This monitoring period was repeated after the DCCV while the patient was in sinus rhythm. TWA magnitude while in AF was compared to that recorded while in sinus rhythm. Patients were excluded if they had a permanent pacemaker implanted or if the DCCV was not successful at restoring sinus rhythm. Patients were also excluded if the follow-up monitor exhibited more than 10% AF and only TWA values while in sinus rhythm were recorded in the follow-up monitor.
CRT study

To investigate the effect of CRT on TWA magnitude, the CRT study was performed: patients who were in sinus rhythm due to undergo a de novo, elective implantation of a CRT device were recruited. Eligible patients who consented wore a 12 lead ambulatory monitor (GE SEER 12) for 24 hours within a four week period before their procedure to acquire an unpaced TWA value. This was performed as close to the date of their procedure as possible. On discharge from hospital, patients in whom the procedure was successful wore another monitor to record a paced value of TWA. Patients were excluded if the pacemaker procedure was unsuccessful at implantation of a left and right ventricular lead, or if the patient was receiving less that 80% biventricular pacing at the time of the post-implant AECG monitor. All ambulatory monitors were analysed using a commercial MMA algorithm as previously described. The TWA value assigned to each analysis was the traditional peak TWA over 24 hours. Basic demographics were collected for each participant. Statistical comparison of TWA magnitude before and after each intervention was performed using Wilcoxon signed rank test.

Ethical approval for this study was granted by the UK National Research Ethics Service.

6.4 Results

Fifteen patients were recruited to the AF study and eight to the CRT study. In four patients DCCV was unsuccessful at restoring sinus rhythm and these patients were excluded from the AF study leaving eleven patients with paired TWA data while in AF and sinus rhythm. In the CRT study there was failure to implant an LV lead in one patient who was excluded leaving seven patients with pre and post CRT pacing TWA data. These seven patients all received at least 80% biventricular pacing after the procedure. Median time from first AECG to procedure
was 12 days. Ejection fraction was available in all patients except two patients undergoing DCCV in whom LV function was reported as normal and so they were assigned an LVEF of 55%. Data are summarized as median and IQR.

<table>
<thead>
<tr>
<th></th>
<th>DCCV study (n=7)</th>
<th>CRT study (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 (55-74)</td>
<td>74 (64-79)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>55</td>
<td>57</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>50 (45-55)</td>
<td>29 (25-31)</td>
</tr>
</tbody>
</table>

Table 6.1: Demographics of the patients in the DC cardioversion (DCCV) and cardiac resynchronisation (CRT) studies. LVEF, left ventricular ejection fraction.

Basic demographics are displayed in Table 6.1. In the DCCV study, peak TWA magnitude was significantly lower in sinus rhythm compared to AF [34(30-35) vs 39(34-42) P=0.02] (Table 6.2). In the CRT study there was no statistically significant difference in TWA magnitude comparing sinus rhythm with biventricular pacing [42(34-49) vs 46(41-49) P=0.08]. There was a trend for a higher peak TWA when paced compared to sinus rhythm (Table 6.2).

<table>
<thead>
<tr>
<th></th>
<th>Pre-intervention TWA</th>
<th>Post-intervention TWA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCCV (n=7)</td>
<td>39 (34-42)</td>
<td>34 (30-35)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRT (n=11)</td>
<td>42 (34-49)</td>
<td>46 (41-49)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 6.2: The effect of DC cardioversion (DCCV) and cardiac resynchronisation therapy (CRT) on T wave alternans (TWA). Data displayed are median (IQR) and comparisons between pre and post-intervention are made using a Mann-Whitney U test.

6.5 Limitations

Patient recruitment was difficult which has a significant impact on the reliability of any conclusions that can be drawn from such a small population. A number of factors were responsible for limiting patient recruitment. Patients were excluded from the CRT study if they were in AF or if the procedure was an upgrade from RV pacing to CRT. A limited number of DCCVs were performed during the study period and some were unsuccessful at restoring sinus rhythm. In addition, patients who were familiar with wearing AECG monitors were reluctant to
be fitted twice. Another potential limitation is that after DCCV, medication was altered in four patients: two had their digoxin stopped, one had their beta-blocker dose reduced while one had both a reduction in beta-blocker dose and digoxin discontinued. However, all patients received all their medication on the morning of the DCCV so the effect of changes in medication should be minimal as the second monitoring period would have started on a day when all medications were taken.

6.6 Discussion

DCCV study

The presence of AF has generally been an exclusion criterion in the analysis of TWA [221]. In those few trials that did include patients with AF, it was found to be a significant cause of indeterminate results [222]. There have been some preliminary studies that have used ventricular pacing to control heart rate and impose regular R-R intervals in patients with AF [222, 223], but these studies included small numbers of patients and further validation is needed. My findings suggest that TWA magnitude, as measured with the MMA method, is significantly greater while in AF compared to sinus rhythm. These data must be interpreted with caution due to the small number of participants. If these findings are borne out by larger studies the explanation could be that the MMA algorithm is intolerant to irregular QT intervals and results in sinus rhythm cannot be compared to those in AF or that the higher peak TWA I have demonstrated while in AF reflects a detrimental effect of AF on the ventricle.

Ling et al [224] studied the effect of pacing rat ventricular myocytes in an irregular vs regular pattern at the same average rate. Those paced in an irregular pattern displayed abnormalities that are observed in failing myocytes: reduction in peak calcium transient, reduced expression
of SERCA and reduced phosphorylation of PLN. Translating these findings to man, they also observed reduced SERCA protein expression and a reduced phosphorylation of PLN in the left ventricular myocardium of heart failure patients with AF compared to those in sinus rhythm. These findings support the hypothesis that the irregularity in ventricular activation produced when in AF, independent of the rate, is detrimental to ventricular function. The higher level of TWA I observed in AF compared to sinus rhythm could reflect the negative effect that AF is exerting on ventricular function but my study is underpowered and was not designed to explain the mechanism of any differences observed in TWA between sinus rhythm and AF.

**CRT study**

A small number of clinical studies have attempted to assess TWA during ventricular pacing. These studies have all used the spectral method of TWA analysis and correlated TWA values during ventricular pacing with the traditional method of spectral TWA analysis in which the ventricular rate is increased to, and maintained at, 105-120 bpm by either atrial pacing or exercise. Shalaby et al [225], reported 80% concordance between short term atrial vs ventricular pacing. In three further studies, TWA characteristics during ventricular pacing were assessed in patients receiving an ICD and/or CRT [226–228]. This allowed the comparison of RV, LV and BiV pacing. The concordance of TWA test results between the different pacing modes varied between the three studies. Ehrlich et al found 80% concordance between RV and RA pacing while Kraaier et al [226] found 67% concordance between RV and RA pacing and 50% concordance between exercise and RV pacing.

There are no published data reporting the effect of ventricular pacing on TWA when analysed using the MMA method. My data suggests that while there is a trend for higher levels of TWA with biventricular pacing compared to sinus rhythm, this difference did not reach statistical significance. To increase the sample size and statistical power of the CRT study, I considered
recruiting an alternative population. This population was patients who already have a CRT
device in place. TWA could be assessed with the pacemaker set to firstly maximise biventricular
pacing and then to minimise biventricular pacing. However, data suggests that after just one
week of ventricular pacing it may take up to four weeks without pacing for T wave morphology
to normalise. This is due to a phenomenon known as cardiac memory in which the abnormal
ventricular activation sequence observed during ventricular pacing persists even when the heart
is not paced [229]. Subjecting a heart failure patient to four weeks without biventricular pacing
has ethical implications that would likely be prohibitive to such a study.

Summary

My data would suggest that TWA values recorded in AF may not be comparable to those in sinus
rhythm. Also, there was a trend for higher values of TWA to be recorded during biventricular
pacing than with a patient’s intrinsic rhythm, though this did not reach significance. These
data must be interpreted with great caution given the small populations studied. Recruitment
challenges were discussed above and this has resulted in an underpowered study. These data
can best be considered hypothesis generating. If, however, AF does have such an impact
on TWA magnitude then the continued exclusion of patients with AF from studies using
TWA may be appropriate. Alternatively, patients with AF could be recruited but their TWA
data interpreted independently from those in sinus rhythm. This approach could establish a
different TWA cut-off that represents a high risk individual for those in AF compared to sinus
rhythm. Importantly, this study did not seek to address whether or not TWA can be used as an
outcome measure to assess the effect of an intervention such as gene therapy in those with atrial
fibrillation. A change in TWA magnitude after an intervention may still reflect an important
change in physiology whether in sinus rhythm or AF.
Chapter 7

SERCA2a Gene Therapy in Patients with Advanced Heart Failure (CUPID-2 Trial Experience in England)

7.1 Introduction

The CUPID-2 trial was a phase 2b multi-national study involving 67 centres in the United States of America, Europe and Israel. This was a double-blind, placebo-controlled study designed to assess the efficacy of SERCA2a gene therapy in patients with advanced heart failure. Full details of the study design can be found in the general methods Chapter 2.2 but in brief, patients with advanced heart failure (symptomatic patients with a LVEF of 35% or less who had either a recent heart failure hospitalisation or a significantly raised NT pro-BNP) were screened for the presence of nAbs to the AAV serotype 1 (AAV1), the viral vector chosen to deliver the SERCA2a gene. Patients in whom nAbs were absent were randomised 1:1 to receive a single ten minute intracoronary infusion of AAV1.SERCA2a or placebo. The primary efficacy endpoint was time-to-recurrent events, namely heart failure hospitalisation or ambulatory treatment for worsening heart failure.
This was the first clinical gene therapy trial for heart failure to recruit patients from the UK and was the largest clinical trial of gene therapy for heart failure to date. Our centre was the only recruitment site in England, while the Golden Jubilee Hospital, Glasgow, recruited in Scotland. These were the only two recruitment sites in the UK. Gene therapy had never before been delivered to heart failure patients in the UK. As a result, there was no streamlined process for gaining approval to conduct such a study or even a previous example to emulate.

Selection of appropriate eligibility criteria for such a trial is critical. The aim is to recruit a population who are likely to benefit from the therapy being investigated and who are likely to experience the trial endpoints. This increases the statistical power of the study so reducing the sample size and/or trial duration required, so minimising costs. This is particularly relevant in an expensive gene therapy study such as CUPID-2. This approach results in eligibility criteria designed to recruit a population with advanced heart failure at high risk of hospitalisations and death. These considerations, however, need to balance against the effect that strict recruitment criteria have on recruitability to the study and the subsequent generalisability of the findings from the study. The eligibility criteria for the CUPID-2 study aimed to achieve the above goals but the effect of such tight criteria on recruitability was unknown.

In addition to the clinical eligibility criteria, patients would be excluded if they had nAbs to the viral vector. Such a criterion is unusual in heart failure trials and it is not well established how restrictive this requirement will be in terms of recruitability to clinical trials or to the delivery of AAV1-mediated gene therapy in clinical practice, should efficacy be proven. Initial data to inform on this in a heart failure population is available from the first CUPID trial in which 509 patients with heart failure were pre-screened for nAbs and approximately 52% of patients would be excluded from receiving this gene therapy product in a clinical trial and clinical practice [189]. During the conduct of the CUPID-2 trial it became apparent that the
prevalence of nAbs observed in the US patients of approximately 50% may not be applicable to non-US countries. This apparent geographic variation in nAb prevalence led me to perform a systematic review of published nAb prevalence as part of this chapter.

The effect of SERCA2a gene therapy on TWA has never been investigated in man. In a guinea pig model of heart failure, treatment with SERCA2a gene therapy reduced the susceptibility of the failing heart to develop cardiac alternans and arrhythmias [159]. The overall hypothesis of this thesis is that SERCA2a gene therapy will reduce ventricular arrhythmias and the magnitude of TWA mediated through normalisation of calcium handling in the sarcoplasmic reticulum (see 1.4). While this effect is not being studied in the main CUPID-2 trial, participants recruited at our centre provide an opportunity to study this in a small population.

### 7.2 Aims

To assess the effect of the clinical eligibility criteria for the CUPID-2 trial on the potential recruitability within a heart failure population.

To estimate the prevalence of nAbs to the AAV1 vector in a UK-based heart failure population.

To describe the baseline and follow-up characteristics of patients recruited from our centre to the CUPID-2 trial and determine if TWA magnitude is reduced by SERCA2a gene therapy in these patients.

To assess the feasibility of carrying out a gene therapy trial in patients with heart failure in the UK.
7.3 Methods

Approval Process for a Gene Therapy Trial in Heart Failure Patients in the UK

Applications for ethical approval for gene therapy studies in the UK are made through the Gene Therapy Advisory Committee (GTAC) which is appointed by the Health Research Authority (for GTAC approval see appendix A) and I assisted in the application to this authority.

Disposal of genetically modified organism (GMO)s, and equipment that has been in contact with GMOs, must adhere to strict legislation. I co-authored the application that gained approval from the Department for Environment Food and Rural Affairs (DEFRA) for deliberate release of GMOs (for DEFRA approval see appendix B). Deliberate release status means there is no requirement to monitor viral shedding. A further requirement for appropriate disposal was to confirm that the incineration process currently employed by the Hospital was adequate. I wrote a number of standard operating procedures (SOP) including one to address the management of spillages in the pharmacy, during transit to the cardiac catheterisation laboratory (cath lab) and while in the cath lab.

Imperial College Gene Therapy Safety Committee require a risk assessment be completed for any activity involving gene therapy and GMOs. All SOPs, safety manuals and other approvals were reviewed before this committee gave a favourable opinion for the trial to commence (for approval see appendix C). Our pharmacy were familiar with preparing, handling and disposing of gene therapy products which was advantageous as a number of protocols and operating procedures were already in place.

Other approvals, more familiar to heart failure studies, such as Medicines and Healthcare Products Regulatory Agency (MHRA) approval and local research and development approval (see appendix D) were also gained.
Patient eligibility determined by clinical enrolment criteria

Patients enrolled in the CUPID-2 trial at our centre were primarily recruited from eight district general hospitals and two tertiary cardiac centres. One of the recruitment criteria for the CUPID-2 trial was to have either been admitted with heart failure within 6 months or to have an NT pro-BNP above a threshold value (see Table 7.1). Natriuretic peptides were not universally available at all recruitment sites so to assess the potential eligibility of patients within a heart failure population for such a gene therapy study I selected patients who had been admitted with a primary diagnosis of heart failure at each recruitment site over a two year period. I reviewed their clinical records and by applying the eligibility criteria established the proportion of patients admitted with heart failure that may be eligible for the CUPID-2 trial. Inclusion criteria for the CUPID-2 trial are displayed in Table 7.1 and exclusion criteria in Table 7.2. Eligibility was assessed based on patient data available at the first outpatient clinic following discharge, providing this was within 6 months of discharge. When screening the databases, if the patient was not reviewed in clinic after the admission then I assumed that the NYHA status, LVEF and optimal medical therapy eligibility criteria were met. This approach would lead to the identification of the maximum number of potentially eligible patients. Some centres did not specify an LVEF, in which case patients were included if reported to have severe LV systolic impairment. Patients were excluded if no assessment of LV function had been performed. Then all patients had review in the research clinic with echocardiography and natriuretic peptide screening if appropriate, to confirm eligibility.

Prevalence of neutralising antibodies to AAV1 in man

In order to estimate the prevalence of nAbs to AAV1 in man I undertook two studies. Firstly, I carried out a systematic review of published data on the reported prevalence of nAbs to AAV1 in human populations. Secondly, I focused on heart failure patients and prospectively analysed
**Inclusion Criteria for the CUPID-2 trial**

18 to 80 years of age  
Chronic systolic heart failure due to ischaemic or non-ischaemic cardiomyopathy  
LVEF less than or equal to 35% during the 60 days before administration of IMP  
NYHA functional class II, III or IV (UK criteria excluded NYHA II)  
Optimal heart failure therapy  
Absence of neutralising antibodies to the AAV1 vector (titre <1:2 or equivocal) within 90 days of screening  
The presence of at least one of the following (added early in the study to increase events rates):  
(i) Hospitalisation for heart failure within 6 months of screening  
(ii) NT proBNP >1200pg/ml (if in AF then NT proBNP >1600pg/ml)

**Table 7.1:** Inclusion criteria for the CUPID-2 trial. AAV1, adenoassociated virus serotype 1; IMP, investigational medicinal product; LVEF, left ventricular ejection fraction; NT proBNP, N terminal pro-B type natriuretic peptide; NYHA, New York Heart Association.

**Exclusion criteria for the CUPID-2 trial**

Intravenous inotropes, vasodilators or diuretics within 30 days before screening  
Restrictive CM, obstructive CM, acute myocarditis, pericardial disease, amyloidosis, infiltrative CM, uncorrected thyroid disease or discrete LV aneurysm  
Cardiac Surgery, PCI or valvuloplasty within 30 days before screening  
MI within 90 days before screening  
Prior heart transplant or implanted LVAD  
Likely need for an immediate heart transplant or LVAD  
Prior CABG not ideal but can be considered case by case  
Liver function tests greater than 3 times upper limit of normal  
Current or imminent need for haemodialysis or GFR less then or equal to 20ml/min/1.73m²  
Bleeding diathesis or platelets less than 50  
Haemoglobin less than 9  
Diagnosis of, or treatment for any cancer other than BCC within the past 5 years

**Table 7.2:** Exclusion criteria for the CUPID-2 trial. BCC, basal cell carcinoma; CABG, coronary artery bypass graft, CM, cardiomyopathy; GFR, glomerular filtration rate; LV, left ventricle; LVAD, left ventricular assist device; MI, myocardial infarction; PCI, percutaneous coronary intervention.
nAb prevalence in a UK heart failure cohort.

Systematic review

To identify published data on the prevalence of nAbs I entered the following search terms in to Pubmed: prevalence, neutralising antibodies, adeno-associated virus, AAV. This identified nineteen studies of which five studies were in man reporting the prevalence of AAV serotype 1 in adults [230–234]. These five studies provided data for fourteen countries and a total of 1984 people.

Prospective analysis of the prevalence of neutralising antibodies to AAV1 in a UK heart failure population

Blood samples from 100 heart failure patients randomly selected from the Biobank at the Royal Brompton Hospital were analysed along with all potential candidates for the CUPID-2 trial (i.e those who met the clinical criteria) at our site. Neutralising antibody titres were determined using an assay developed for the CUPID trial program and a titre >1:2 was considered nAb positive. Demographic data for each subject were collected, scores for social deprivation and population density (to define urban vs rural areas), were based on the patient’s most recent postal address and were derived from the Office for National Statistics. Categorical data were analysed using Fishers exact test and continuous variables using the Mann-Whitney U test. A P-value of < 0.01 was considered statistically significant after a Bonferroni type correction. Ethical approval was provided through the Biobank ethical policy and the CUPID-2 trial ethics.

Characteristics of patients recruited to the CUPID-2 trial at our site and the effect of SERCA2a gene therapy on TWA in these patients

Patients recruited to the CUPID-2 study at our site underwent all investigations detailed in the trial schedule in Table 2.4. In addition, all participants recruited from our site wore an
AECG monitor (GE SEER 12) for 24 hours at baseline (during the screening phase prior to administration of IMP) and this was repeated at 6 and 12 month follow-up visits. TWA was analysed, while blinded to treatment allocation, using the MMA algorithm (GE) as described earlier in section 2.3.1. The TWA value assigned to each recording was the traditional peak TWA during the 24 hour period.

Ethical approval was gained as a separate sub-study of the main CUPID-2 trial.

7.4 Results

Patient eligibility determined by clinical enrolment criteria

Across the ten recruitment sites, over a two year period, 5883 hospitalisations were recorded with heart failure as the primary diagnosis. The effect that applying the recruitment criteria had on the number of eligible patients for the CUPID-2 trial is summarised in Figure 7.1. Thirty seven percent of the patients had an LVEF of 35% or less, six percent had no assessment of LV function performed and fifty seven percent had an LVEF of greater than 35%. Of those who met the LVEF criterion for the trial, 22% were excluded due to the age limit requirement. In the cohort that met the LVEF and age criteria only 19% remained symptomatic to an NYHA 3 or 4 severity when reviewed in clinic. When this group of 317 patients were further refined by application of the exclusion criteria there were 48 patients remaining that fulfilled the clinical criteria for recruitment. This represents 0.8% of the overall population admitted with heart failure. Furthermore, this estimate did not take in to account the exclusion of patients with nAbs to the viral vector.

Prevalence of neutralising antibodies to AAV1 in man

Systematic review

My systematic review included five studies providing data on 1984 people as summarised in
5883 hospitalised with heart failure

2148 with an LVEF ≤35%

1672 aged 18-80

317 NYHA 3/4

After exclusion criteria applied
48 patients met clinical recruitment criteria (excluding screening for nAbs)

**Figure 7.1:** Effect on recruitability of applying CUPID-2 eligibility criteria to a population admitted with heart failure. LVEF, left ventricular ejection fraction; nAbs, neutralising antibodies; NYHA, New York Heart Association.

table 7.3. There is a wide global variation in the prevalence of nAbs to AAV1, ranging from 13% in Italy to 79% in the Netherlands (Figure 7.2). These data are almost exclusively from young healthy volunteers and there is no standard cut-off for what titre constitutes a nAb positive result but a titre of 1:20 was most commonly used (Table 7.3). It is not possible to discern whether any of the apparent global variation in nAb prevalence is confounded by the different titre cut-off levels used in the different countries. A further limitation is that the largest of the five studies, representing 888 patients, was unable to provide any details on the participants from whom the blood samples were taken.

*Prospective analysis of the prevalence of neutralising antibodies to AAV1 in a UK heart failure population*
Table 7.3: Summary of studies describing the prevalence of neutralising antibodies to AAV1 in man, identified by systematic review. IBD, inflammatory bowel disease.

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Subjects</th>
<th>Age (years)</th>
<th>Titre</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcedo et al</td>
<td>888</td>
<td>Unknown</td>
<td>Unknown</td>
<td>1:20</td>
<td>USA (n=100), Italy (n=100), Belgium (n=100), Greece (n=81), Australia (n=100), Rwanda (n=60), Uganda (n=133), Kenya (n=51), Zambia (n=51), South Africa (n=112)</td>
</tr>
<tr>
<td>Liu et al</td>
<td>500</td>
<td>Healthy subjects</td>
<td>≤56</td>
<td>1:10</td>
<td>China</td>
</tr>
<tr>
<td>Van der Marel et al</td>
<td>300</td>
<td>Healthy subjects/subjects with IBD</td>
<td></td>
<td>1:100</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Boutin et al</td>
<td>152</td>
<td>Healthy subjects</td>
<td>25-64</td>
<td>1:20</td>
<td>France</td>
</tr>
<tr>
<td>Mimuro et al</td>
<td>144</td>
<td>Healthy subjects</td>
<td>≥52 (n=37)</td>
<td>1:14</td>
<td>Japan</td>
</tr>
</tbody>
</table>

Percentage of subjects nAb +ve
- 0-20
- 21-40
- 41-60
- 61-80

Figure 7.2: Results of systematic review illustrating the global prevalence of neutralising antibodies (nAb).

Three of the 100 samples sent from the Biobank at the Royal Brompton Hospital could not be analysed leaving 97 valid results. A further 58 samples were collected from patients who were potential candidates for the CUPID-2 trial. Combining these results created a study population of 155 patients with heart failure in whom 60.6% were nAb positive at a titre of 1:2 and hence ineligible for gene therapy. The only characteristic that was associated with being nAb positive was age (Table 7.4.).
<table>
<thead>
<tr>
<th></th>
<th>nAb positive (n=94)</th>
<th>nAb negative (n=61)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66 (56-72)</td>
<td>57 (47-82)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Male</td>
<td>73%</td>
<td>72%</td>
<td>0.8905</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Caucasian</td>
<td>85%</td>
<td>89%</td>
<td>0.6353</td>
</tr>
<tr>
<td>-Non-Caucasian</td>
<td>15%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Aetiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Dilated cardiomyopathy</td>
<td>73%</td>
<td>85%</td>
<td>0.115</td>
</tr>
<tr>
<td>-Ischaemic cardiomyopathy</td>
<td>27%</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>NYHA class</td>
<td>3 (1-3)</td>
<td>2 (1-3)</td>
<td>0.1343</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>29 (21-45)</td>
<td>36 (23-51)</td>
<td>0.0663</td>
</tr>
<tr>
<td>Indices of social deprivation score</td>
<td>11.9 (6.7-62.5)</td>
<td>16.4 (8.3-26.0)</td>
<td>0.2711</td>
</tr>
<tr>
<td>Urban origin</td>
<td>89%</td>
<td>82%</td>
<td>0.2271</td>
</tr>
</tbody>
</table>

Table 7.4: Comparison of neutralising antibody (nAb) positive and negative cohorts within a UK based heart failure population. Statistical comparison made with a Mann-Whitney U test. NYHA, New York Heart Association.

The prevalence of nAbs significantly increases with age [$\chi^2=20.9$, df=5, Fisher-Freeman-Halton exact p=0.0002] (Figure 7.3). Using an age cut-off of 63 years, established through ROC analysis, the prevalence of nAbs in those who are 63 years of age or less is 49% while in those over 63 years of age it is 71%.

![Figure 7.3: Prevalence of neutralising antibodies to AAV1 in a UK population of patients with heart failure, demonstrating the effect of age. [$\chi^2=20.9$, df=5, Fisher-Freeman-Halton exact p=0.0002].](image)
Characteristics of patients recruited to the CUPID-2 trial at our site and the effect of SERCA2a gene therapy on TWA in these patients

Eleven patients were fully enrolled to the CUPID-2 trial at our site. The baseline characteristics of these patients, comparing those who received AAV1.SERCA2a to those in the placebo arm are displayed in Table 7.5. There were no statistically significant differences in baseline characteristics between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>AAV1.SERCA2a (n=6)</th>
<th>Placebo (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>63.3 (58.0-68.8)</td>
<td>40.7 (32.6-58.0)</td>
<td>0.052</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>83</td>
<td>80</td>
<td>0.727</td>
</tr>
<tr>
<td><strong>NYHA 3 (%)</strong></td>
<td>100</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td><strong>Dilated cardiomyopathy (%)</strong></td>
<td>50</td>
<td>80</td>
<td>0.348</td>
</tr>
<tr>
<td><strong>Ischaemic heart disease (%)</strong></td>
<td>50</td>
<td>20</td>
<td>0.348</td>
</tr>
<tr>
<td><strong>Atrial fibrillation (%)</strong></td>
<td>17</td>
<td>0</td>
<td>0.545</td>
</tr>
<tr>
<td><strong>Cardiac resynchronisation therapy (%)</strong></td>
<td>67</td>
<td>20</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Implantable cardioverter defibrillator (%)</strong></td>
<td>67</td>
<td>40</td>
<td>0.392</td>
</tr>
<tr>
<td><strong>6 minute walk distance (m)</strong></td>
<td>343 (195-469)</td>
<td>425 (353)</td>
<td>0.429</td>
</tr>
<tr>
<td><strong>Left ventricular ejection fraction (%)</strong></td>
<td>22 (17-34)</td>
<td>30 (14-33)</td>
<td>1</td>
</tr>
<tr>
<td><strong>B-type natriuretic peptide (ng/L)</strong></td>
<td>972 (325-1767)</td>
<td>705 (267-1741)</td>
<td>0.792</td>
</tr>
<tr>
<td><strong>eGFR (ml/min/1.73m²)</strong></td>
<td>43 (31-58)</td>
<td>73 (60-79)</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>KCCQ score</strong></td>
<td>54.0 (35.0-67.3)</td>
<td>54.2 (31.0-65.4)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Maximum T wave alternans</strong></td>
<td>50 (42-62)</td>
<td>40 (33-56)</td>
<td>0.177</td>
</tr>
</tbody>
</table>

Table 7.5: Baseline characteristics of patients recruited to the CUPID-2 trial from the Royal Brompton Hospital. Continuous variables are displayed as median (interquartile range) and the groups are compared using a Mann-Whitney U test. Categorical data are presented as percentages and compared with chi square. eGFR, estimated glomerular filtration rate; KCCQ, Kansas City cardiomyopathy questionnaire.

At follow-up assessments there remained no significant difference between the AAV1.SERCA2a and placebo groups in all assessments listed in the CUPID-2 trial schedule (see Table 2.4). A representative example of results of three variables collected throughout the trial period are displayed in Figure 7.4. Of note, three patients in each group met the baseline criteria for six minute walk test to include this as a component of their follow-up (see methods section 2.2 for details of the effect of baseline 6 minute walk distance on randomisation and follow-up).
Figure 7.4: Full trial period data of three variables for patients recruited from our site to the CUPID-2 trial. Comparison of treatment group (AAV1.SERCA2a) with control, no significant difference.

There was no significant difference in peak TWA between the AAV1.SERCA2a and placebo groups at any time point (Figure 7.5).
Figure 7.5: TWA measured in participants of the CUPID-2 trial recruited at the Royal Brompton Hospital. Comparison of treatment group (AAV1.SERCA2a) with control, no significant difference.

7.5 Discussion

The main findings from this chapter are:

1. Conducting gene therapy trials in patients with heart failure is challenging,

2. Only 0.8% of a cohort of 5883 patients admitted with a primary diagnosis of heart failure, met the clinical recruitment criteria for the CUPID-2 trial,

3. 60% of heart failure patients in the UK would be excluded from gene therapy trials using AAV1 as a vector due to the presence of nAbs. Furthermore, patients who are older are more likely to have nAbs.

4. No effect of SERCA2a gene therapy on TWA or other parameters of heart failure severity could be detected in the patients recruited at our site.

The findings from this chapter need to be interpreted in light of the main CUPID-2 trial having now published neutral results [235]. The CUPID-2 trial randomised 250 patients with advanced heart failure to receive an intracoronary infusion of either placebo or AAV1.SERCA2a (1 x 10^{13} DRP). There was no significant difference between placebo and AAV1.SERCA2a arms of the trial in the primary outcome, time-to-recurrent events (hazard ratio 0.93, 95% CI 0.53-1.65;
P=0.81) [235]. This was a disappointing outcome and came as a surprise to those who believed that the efficacy of AAV1.SERCA2a had been demonstrated in the CUPID trial. The positive findings of CUPID may, however, have been over interpreted. The CUPID trial recruited 39 patients with advanced heart failure who were randomised to receive an intracoronary infusion of placebo (n=14) or one of three doses of AAV1.SERCA2a: low dose (6 x 10^{11} DRP)(n=8), mid dose (3 x 10^{12} DRP)(n=8) and high dose (1 x 10^{13} DRP) (n=9). The risk of recurrent cardiovascular events (myocardial infarctions, hospitalizations related to heart failure, episodes of worsening heart failure) adjusted for correlated terminal events (cardiovascular death or the requirement for left ventricular assist devices or cardiac transplant) was reduced by 88% in the high dose group compared to placebo (P=0.003) and this effect was maintained for three years (relative risk reduction 82%, P=0.048) [192]. This would suggest that the high dose of AAV1.SERCA2a (the dose also used in the CUPID-2 trial) has a significant effect on outcomes in patients with advanced heart failure. However, there are important limitations in the CUPID trial that could significantly affect the interpretation of this result. Firstly, there was no dose response observed. This is discussed further in the main introduction (section 1.3.8) and can be visualised in figure 1.19 but essentially there was no difference between placebo and either the low or mid dose groups. This means that any positive results of the trial essentially depend upon differences in outcomes observed between placebo (n=14) and high dose (n=9) groups. These are small populations and there were some concerning baseline differences between the two groups. Although these differences did not reach statistical significance, perhaps due to the small sample sizes analysed, the high dose group were numerically younger, had lower creatinine, NT-proBNP and left ventricular end systolic volume and had greater 6MWD, LVEF and VO_{2}max (Table 7.6). These characteristics alone may suggest that the high dose group were less likely to experience cardiovascular events than the placebo group rather than the difference
in event rate being attributable to AAV1.SERCA2a. As a result, it seems that the most likely explanation for the discrepancy between the outcome of CUPID and CUPID-2 is that the results from CUPID were erroneous.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n=14)</th>
<th>AAV1.SERCA2a High dose (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD) yrs</td>
<td>61.0 (11.9)</td>
<td>56.6 (14.0)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>13 (92.9)</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>Heart failure treatment regimen, n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ACEi</td>
<td>8 (57.1)</td>
<td>7 (66.6)</td>
</tr>
<tr>
<td>-ARB</td>
<td>5 (28.6)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>-MRA</td>
<td>8 (57.1)</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>-Beta-blocker</td>
<td>14 (100.0)</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>-Diuretic</td>
<td>12 (85.7)</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>NYHA class III, n (%)</td>
<td>14 (100)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>MLWHFQ total score mean (SD)</td>
<td>48.7 (16.4)</td>
<td>41.4 (26.5)</td>
</tr>
<tr>
<td>Creatinine, mean (SD) mg/dL</td>
<td>1.6 (0.6)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>NT-proBNP, mean (SD), pg/mL</td>
<td>4072 (3906)</td>
<td>2141 (1997)</td>
</tr>
<tr>
<td>Six minute walk distance, mean (SD), m</td>
<td>336 (138)</td>
<td>347 (120)</td>
</tr>
<tr>
<td>VO₂max, mean (SD), mL/kg/min</td>
<td>12.4 (4.2)</td>
<td>15.1 (3.2)</td>
</tr>
<tr>
<td>LVEF, mean (SD), %</td>
<td>22.6 (6.7)</td>
<td>27.9 (5.3)</td>
</tr>
<tr>
<td>LVESV, mean (SD), mL</td>
<td>201 (64)</td>
<td>169 (48)</td>
</tr>
</tbody>
</table>

Table 7.6: Baseline characteristics of patients enrolled in the CUPID trial comparing placebo and high dose groups. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; MLWHFQ, Minnesota living with heart failure questionnaire; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N terminal pro-B type natriuretic peptide; NYHA, New York Heart Association. Data taken from the CUPID trial manuscript [189].

An alternative explanation for the disparity in efficacy of AAV1.SERCA2a between the two trials relates to the potential for less efficient transduction in CUPID-2 compared to CUPID. A review of the manufacturing process, which was different between CUPID and CUPID-2, showed a difference in the proportion of empty viral capsids (containing protein capsid but not single stranded DNA): 85% in CUPID and 25% in CUPID-2. Although it appears counter intuitive, there is data to suggest that a high proportion of empty capsids improves gene transfer (supplementary appendix of the CUPID-2 manuscript [235]), perhaps by acting as a decoy blocking the inhibitory activity of antibodies. It is not possible to accurately assess transduction
efficiency in either of the CUPID trials as cardiac biopsy was not routinely performed in follow-up. Limited data are available from biopsy samples in those who died, received an LVAD or underwent heart transplantation. These results are displayed in Table 7.7 and it is apparent that some biopsy samples from some patients in the CUPID trial contained more AAV1.SERCA2a DNA than samples from some participants in the CUPID-2 trial. It is not, however, consistent that all samples from CUPID participants contained more AAV1.SERCA2a DNA than samples from CUPID-2 participants. Adding further concern regarding the lack of dose response in the CUPID trial, it is noteworthy that the CUPID samples described in Table 7.7 were all from patients in the high dose arm of the trial. All samples from patients in the low and mid dose had undetectable levels of AAV1.SERCA2a DNA (lower limit of detection 20 single-stranded copies of AAV1.SERCA2a DNA per µg total DNA). Differences in transduction between the CUPID and CUPID-2 studies cannot be confirmed from these data but one can be more confident in asserting that the amount of AAV1.SERCA2a DNA found in human samples from the CUPID trial program is significantly less than that found in the animal studies that formed the foundation for CUPID (Table 7.8). Up to 500 copies of the therapeutic gene have been detected in hearts from patients with heart failure treated with the high dose of AAV1.SERCA2a equating to transduction of <1% of cardiomyocytes. In contrast, between 8,000 and 42,000 copies of the therapeutic gene have been identified in animals treated with SERCA2a gene therapy, equating to 30-70% of cardiomyocytes being transduced. This lends plausibility to the concept that the CUPID-2 trial may have been neutral due to inefficient gene delivery transduction of human cardiomyocytes.

Factors that affect transduction efficiency are discussed in the introduction and are summarised schematically in Figure 1.16. Further trials will be required if the question of transduction efficiency is to be addressed but gene therapy trials in this area are expensive, difficult to set
<table>
<thead>
<tr>
<th>Study</th>
<th>Subject</th>
<th>Months post-infusion</th>
<th>Biopsy site</th>
<th>AAV1.SERCA2a (ss copies/microgram DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CUPID</td>
<td>091006</td>
<td>18</td>
<td>Posterolateral wall</td>
<td>&lt;20</td>
</tr>
<tr>
<td>CUPID</td>
<td>091007</td>
<td>11</td>
<td>LVAC</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>Anterior septum</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterior septum</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LVAC</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RVAC</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior wall</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterolateral wall</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td>CUPID</td>
<td>011005</td>
<td>31</td>
<td>LVAC</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td>CUPID</td>
<td>011010</td>
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<td>Posterolateral wall</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior septum</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterior septum</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior wall</td>
<td>&gt;20 to &lt;200</td>
</tr>
<tr>
<td>CUPID-2</td>
<td>021020</td>
<td>12</td>
<td>LVAC</td>
<td>14-62</td>
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<td>CUPID-2</td>
<td>161039</td>
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<td>Anterior septum</td>
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</tr>
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<td></td>
<td></td>
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<td>Posterior septum</td>
<td>71</td>
</tr>
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<td></td>
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<td>Anterior wall</td>
<td>36</td>
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<td></td>
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<td>CUPID-2</td>
<td>501024</td>
<td>1.5</td>
<td>Anterior septum</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Posterior septum</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior wall</td>
<td>40</td>
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<td>Posterolateral wall</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LVAC</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RVAC</td>
<td>&lt;10</td>
</tr>
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<td>CUPID-2</td>
<td>231028</td>
<td>8</td>
<td>Anterior wall</td>
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</tr>
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<td>Heart</td>
<td>72-123</td>
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<td>CUPID-2</td>
<td>251008</td>
<td>14</td>
<td>Anterior septum</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterior septum</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior wall</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterolateral wall</td>
<td>102</td>
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<td>CUPID-2</td>
<td>501002</td>
<td>10</td>
<td>Anterior septum</td>
<td>43</td>
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<td></td>
<td></td>
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<td>Posterior septum</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior wall</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Posterolateral wall</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LVAC</td>
<td>36</td>
</tr>
<tr>
<td>CUPID-2</td>
<td>021009</td>
<td>20</td>
<td>Heart</td>
<td>192</td>
</tr>
<tr>
<td>CUPID-2</td>
<td>111033</td>
<td>16</td>
<td>LVAC</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

**Table 7.7:** Data describing biopsy findings from patients in the CUPID [192] and CUPID-2 (supplementary material [235]) trials in terms of the number of copies of AAV1.SERCA2a DNA identified. LVAC, left ventricular apical core; RVAC, right ventricular apical core; ss, single stranded.
Table 7.8: Comparison of animal and human data illustrating the number of copies of therapeutic DNA and approximate proportion of cardiomyocytes transduced during gene therapy studies. MCARD, molecular cardiac surgery with recirculating delivery; ss, single stranded. Data taken from a presentation by Roger Hajjar at the European Society of Cardiology Congress 2015.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Virus</th>
<th>Delivery</th>
<th>ss copies</th>
<th>Approximate proportion of cardiomyocytes transduced (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>AAV9.SERCA2a</td>
<td>Intravenous</td>
<td>42,000</td>
<td>75</td>
</tr>
<tr>
<td>Rats</td>
<td>AAV9.SERCA2a</td>
<td>Intravenous</td>
<td>30,000</td>
<td>70</td>
</tr>
<tr>
<td>Pigs</td>
<td>AAV1.SERCA2a</td>
<td>Intracoronary</td>
<td>8,000</td>
<td>30</td>
</tr>
<tr>
<td>Sheep</td>
<td>AAV1.SERCA2a</td>
<td>Intracoronary</td>
<td>9,000</td>
<td>33</td>
</tr>
<tr>
<td>Sheep</td>
<td>AAV1.SERCA2a</td>
<td>Surgical MCARD</td>
<td>13,000</td>
<td>42</td>
</tr>
<tr>
<td>Humans</td>
<td>AAV1.SERCA2a</td>
<td>Intracoronary</td>
<td>0-500</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

up and difficult to recruit to. Based on the discussions above about the significant limitations in interpreting the CUPID trial it may have been premature to design CUPID-2 with clinical endpoints using only the high dose from CUPID. Instead, a further dose finding study with higher doses could have been performed. A more suitable endpoint may then have been transduction efficiency. This would not only have been a more scientific approach, it is likely it would have been an overall more cost effective one since it is feasible that a higher dose of gene therapy product may have resulted in positive outcome for CUPID-2.

In order to commence a gene therapy trial in the UK, ethical approval must be given by GTAC. An independent gene therapy safety committee must review all SOPs, approvals and other paperwork. It is helpful if the pharmacy involved is familiar with the preparation, handling and disposal of GMOs. The disposal of GMOs is tightly controlled and permission must be gained from DEFRA. For deliberate release status, this process includes the requirement for an advertisement to be placed in a national newspaper to include details of the GMO, the location of release and relevant dates. This is to allow any public concerns to be raised and addressed. In addition, as with other drug trials in heart failure, approval must be gained from local research and development teams, MHRA and, at our site, the clinical trials oversight committee. In addition to the approval process, education must be delivered to those involved in the trial.
(ward nurses, cath lab nurses, radiographers, physiologists etc). This is to ensure that the trial is carried out in a safe manner and to allow any concerns that staff members may have about this new technology to be aired. This whole process is lengthy and requires significant man power and resilience from any team considering carrying out a gene therapy trial. It is important that centres are willing to persevere with this process as trials like the CUPID-2 trial require multiple centres to be successful, as evidenced by the 67 centres required to recruit 250 patients in a timely fashion.

Another challenging aspect of the CUPID-2 trial was recruitment. This was challenging both in terms of meeting the clinical eligibility criteria and the requirement for patients to be negative for nAbs to the viral vector. The recruitment criteria were designed to identify a high risk population likely to experience the outcomes of the trial. One of the entry criteria was to have either had a heart failure hospitalisation within the previous 6 months or a natriuretic peptide above a threshold value. I examined the records of 5883 patients admitted with a primary diagnosis of heart failure. Only 0.8% of these patients met the clinical eligibility criteria for CUPID-2. There are flaws in these data as coding of hospitalisations is commonly erroneous and the main focus of trials like CUPID-2 is not on all patients admitted with heart failure, rather those with severe left ventricular impairment. That being said, my data demonstrate that in patients admitted with heart failure who have severely impaired left ventricular function (with an LVEF of 35% or less) only 48 out of 2148 (or 2.2%) were eligible for CUPID-2. This attrition of recruitability has an impact on the number of patients that each centre can recruit and means that such trials require a relatively large number of sites to meet their recruitment target which increases the costs associated with the trial. In addition to impacting on the running of a clinical trial, the fact that the vast majority of heart failure patients are not eligible for the CUPID-2 trial does impact on the generalisability of any findings from the trial.
The requirement for participants to be nAb negative further restricts recruitability to this trial and will limit the use of gene therapy in clinical practice should it ever be proven effective. Despite the disappointment of CUPID-2 there is ongoing work to develop AAV based gene therapy. AAVs have become a popular vector as they are non-pathogenic and exhibit tropism for different organs. However, a significant disadvantage of using AAVs is the presence of nAbs. There is evidence in animal studies that nAb titres as low as 1:5 to 1:10 can completely abolish AAV-mediated transduction [236, 237]. An appreciation of the scale of this limitation is important but little data have been published on the prevalence of nAbs in man. The data available are from epidemiological studies which predominantly report on healthy subjects and have used varying titre cut-offs to distinguish between antibody positive and negative: from 1:10 to 1:100. These studies suggest that there may be geographic variability in the prevalence of nAbs; with nAb prevalence ranging from 13% in Italy [234] to 79% in the Netherlands [232], however, minimal demographic data are provided for the subjects in these studies and unmeasured factors such as age may confound the apparent geographic variation. If the geographic variation is borne out by further studies, this could have an impact on which countries are selected to take part in future AAV1-mediated gene therapy trials.

In contrast to the published epidemiological studies, clinical trials of gene therapy in heart failure use a more stringent nAbs titre cut-off of 1:2 to classify an individual as being nAb positive and therefore excluded from trials. I have demonstrated that with this titre cut-off, 60% of UK heart failure patients are nAb positive and so ineligible for clinical trials using an AAV1 vector. The only feature that is associated with nAb status is age with older people being more likely to be nAb positive: the prevalence of nAbs in those who are 63 years of age or less is 49% while in those over 63 years of age it is 71%. These data are important when counselling patients considering enrolment into a clinical trial since the majority of patients will be deemed
ineligible. Another important discussion point when counselling patients who are considering enrolment in a clinical trial of gene therapy is that if they are in the active arm of a trial that uses an AAV vector then they will develop nAbs to the vector administered and therefore will be ineligible for any future gene therapy trials using the same vector and possibly related vectors.

One might consider that a simple solution to nAbs would be to use different AAV serotypes in different people but the prevalence of nAbs to multiple serotypes is high [233], there is cross-reactivity of nAbs across serotypes [234] and not all serotypes exhibit sufficient cardiotropism for use in heart failure [238]. There are strategies in development to overcome the presence of neutralising antibodies. Some of these strategies are in the field of viral engineering to alter known antigenic regions of the AAV [239], or by selecting AAV variants with increased resistance to nAbs [240], or a combination of both strategies [241]. In contrast, another possible strategy to overcome this problem involves removing nAbs from the patient using plasmapheresis [242] prior to delivery of gene therapy. It will, however, take some time for the development of an effective strategy to overcome nAbs and in the meantime this limitation will significantly impeded the expansion of the field of gene therapy for heart failure.

The final finding from this chapter is that there was no effect of AAV1.SERCA2a on TWA in the small number of CUPID-2 participants recruited at our site. Considering that the main CUPID-2 trial was neutral and the possibility that this was due to poor transduction efficiency, one would anticipate that there would be no effect on TWA.

Overall, in this chapter I have demonstrated that gene therapy trials in advanced heart failure are challenging to set up and difficult to recruit to. The findings from the CUPID-2 trial were neutral and this had major financial implications for the company funding it. There are a number of potential explanations for why CUPID-2 was neutral which could be overcome but with the cost, set-up challenges and recruitment challenges it is difficult to know how many companies
will be willing to take a risk on such a strategy in the future.
Chapter 8

Ventricular Arrhythmias in Patients with Heart Failure and the Anti-arrhythmic Effects of SERCA2a Gene Therapy

8.1 Introduction

A range of pathophysiological changes occur in the failing heart that predispose to ventricular arrhythmias (as discussed in section 1.1.9). This predilection to ventricular arrhythmias contributes to SCD being one of the two predominant modes of death in patients with heart failure (See Table 1.5 and Figure 1.5). The pharmacological treatment of ventricular arrhythmias in patients with heart failure is challenging. Beyond the well-established and prognostically proven β-blockade strategy, there are limited other pharmacological options.

Rather than being a direct anti-arrhythmic drug, the beneficial effects of β-blockers are multifactorial in that they reduce the detrimental effects of catecholamine stimulation. These detrimental effects that are diminished by β-blockers include elevated heart rate, increased myocardial energy demands, adverse remodelling due to cardiac myocyte hypertrophy and death, interstitial fibrosis, impaired β-adrenergic signalling, and stimulation of other
detrimental systems such as the renin-angiotensin-aldosterone axis. Several randomised controlled clinical trials have assessed various AADs in patients with heart failure with disappointing results, aside from β-blocker therapy (Table 1.7). Furthermore, some AADs have been associated with increased adverse effects including worse survival [7, 8, 17, 53–61, 243]. Tolerability is an issue with AADs, for example in the OPTIC trial [244], after one year, 23.5% of patients discontinued sotalol and 18.2% stopped amiodarone. The longer term side effects of amiodarone are well established [63]. Medical therapies for heart failure that target the renin-angiotensin-aldosterone system are not traditionally considered to be AADs but have been shown to reduce arrhythmias in heart failure. In the Heart Outcomes Prevention Study, high-risk patients treated with ramipril experienced a significant reduction in sudden cardiac death [245]. In the Randomised Aldactone Evaluation Study (RALES), there was a significant reduction in mortality in those treated with spironolactone and some of this effect was mediated through a reduction in lethal arrhythmias [9]. Eplerenone has also been shown to reduce sudden death in patients with heart failure [246]. The effect of arrhythmia reduction through such agents may be due to reverse remodelling, reduced cardiac hypertrophy and fibrosis.

ICDs have demonstrated the ability to reduce the risk of SCD in patients with heart failure and are indicated for certain groups of patients for primary and secondary prevention of ventricular arrhythmias [12, 16, 17]. ICDs also have limitations which include the short and long term complications of device implantation, the potential for ICD shocks to have a negative psychological effect on patients [65] and there is evidence to suggest that ICD shocks are associated with increased mortality [64].

Given the limited options for managing ventricular arrhythmias in patients with heart failure, a novel therapy that can both improve heart failure outcomes (as had been the hope for AAV1.SERCA2a) and reduce ventricular arrhythmias would be a valuable asset. Experimental
evidence in animal models of heart failure suggests that SERCA2a gene therapy is effective at reducing susceptibility to ventricular arrhythmias by normalising Ca\(^{2+}\) handling, reducing Ca\(^{2+}\) leak from the sarcoplasmic reticulum and preventing pro-arrhythmic after-depolarisations. The CUPID-2 trial was not designed to investigate any anti-arrhythmic properties of SERCA2a gene therapy but it was anticipated that a significant proportion of patients in the trial would already have an ICD implanted at baseline as part of their routine medical care. As such, the CUPID-2 trial provided an opportunity to design the CUPID-2 arrhythmia sub-study to determine if SERCA2a gene therapy is effective at reducing ventricular arrhythmias as assessed by a reduction in appropriate ICD therapy.

8.2 Aims

To determine the proportion of patients that receive appropriate ICD therapy in an observational study of a contemporary heart failure population. These findings were originally intended to guide the design of a prospective study of gene therapy as an antiarrhythmic agent and determine the expected event rate. Instead, these results predominantly provided a context for the findings of the CUPID-2 arrhythmia sub-study.

To determine if AAV1.SERCA2a reduces appropriate ICD therapy in participants of the CUPID-2 arrhythmia sub-study.

8.3 Methods

*Observational study of ICD therapy in a contemporary heart failure population*

I performed an observational, retrospective study of patients under active ICD follow-up at two UK tertiary cardiology centres in 2011 and 2012. Any patient who had an ICD present on the 1st
January 2011 compromised the 2011 cohort. Arrhythmic events experienced by these patients in the next 12 months formed the 2011 data. Some of these patients died or were lost to follow-up but the remaining patients who survived to 1st January 2012 were then included in the 2012 cohort and if they experienced arrhythmic events in 2012 then these were recorded in the 2012 dataset. Other patients were added to the 2012 dataset if they had an ICD implanted between the 1st January 2011 and the 1st January 2012. The focus was on the number of events that could be expected to occur on an annual basis in our population rather than focusing on individual patients as these observational data were partly intended to plan the study design and estimate event rates for a trial to investigate the effect of SERCA2a gene therapy on arrhythmic events. Patients who had heart failure with an LVEF $\leq 35\%$ (or reported as severely impaired) were included. Limited demographic data were collected for each individual. Appropriate ICD therapies were identified either through home monitoring systems or by review of paper records. As this was a retrospective study, all therapies had already been reviewed and recorded as appropriate or inappropriate by a physiologist and/or cardiologist. In borderline cases the original traces were reviewed to determine if the therapy was appropriate. Appropriate therapy was classified as either a shock or anti-tachycardia pacing (ATP). If a shock was immediately preceded by ATP that failed to cardiovert the ventricular arrhythmia, then this was classified as a shock. If ATP was successful at cardioverting the arrhythmia then this was classified as ATP. Patients who had separate episodes that each met one of the above criteria i.e. an episode of successful ATP and a separate episode of shock were classified as having had ATP and shock in that year.

**CUPID-2 arrhythmia sub-study**

The methods for the main CUPID-2 study are described in detail elsewhere (see section 2.2). Data from ICD interrogation in the main CUPID-2 trial was collected for the purpose of a safety
outcome. The sponsors had not considered using these data for an antiarrhythmic substudy. I wrote a proposal to the steering committee detailing a pre-specified statistical plan and it was agreed that the raw data would be provided to me for analysis. Patients recruited to the main CUPID-2 study who already had an ICD in situ at baseline formed the study population for this sub-study. As this was a post hoc analysis, the selection of data fields collected was already established in the CUPID-2 trial protocol (Figure 8.1). ICD interrogation took place at baseline (to include the previous 90 days), and during follow-up months 1, 3, 6, 9 and 12. As such, the data fields displayed in Figure 8.1 were completed on six occasions with each occasion describing varying time frames: 90 days, 1 month, 2 months, 3 months, 3 months and 3 months. The primary outcome event was appropriate ICD shock with a secondary outcome event being any appropriate therapy (shock or ATP). Data were not collected to record dates of ICD therapy, rather the total number of events that occurred in the relevant time period was recorded. This prevented the data being analysed as time-to-first ICD therapy. Instead crude event rates, for AAV1.SERCA2a and placebo groups, were calculated as the total number of events in a time period divided by the sum of the patient days of follow-up. Patients with heart failure can experience VT storm in which one individual may experience multiple shocks in a 24 hour period. Such an individual could significantly affect the crude event rate for their group. Rather than exclude these patients I performed a secondary analysis of the data categorising individuals as either having had a therapy or not in the relevant time period rather than an absolute number of therapies. Statistical comparisons were made using chi square.

8.4 Results

*Observational study of ICD therapy in a contemporary heart failure population*

Characteristics of the patients included in the retrospective observational study are displayed in
### Table 8.1

<table>
<thead>
<tr>
<th>Non-sustained Ventricular Tachycardia (NSVT)</th>
<th>4</th>
<th>Number of non-sustained VT episodes since last interrogation</th>
<th>Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>Maximum ventricular rate during VT episodes</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Number of beats</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Duration</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**Sustained Ventricular Tachycardia (VT)**

|                                             | 8 | Number of sustained VT episodes requiring intervention since last interrogation | Not applicable |
|                                             | 9 | Maximum ventricular rate during VT episode                     | Not applicable |
|                                             | 10| Number of beats                                                | Not applicable |
|                                             | 11| Duration                                                       | Not applicable |

**VT/Ventricular Fibrillation (VT/VF) Therapy Summary**

|                                             | 12| Pace terminated episodes                                       | Not applicable |
|                                             | 13| Shock-terminated episodes                                     | Not applicable |
|                                             | 14| Total shocks                                                  | Not applicable |
|                                             | 15| Aborted charges                                                | Not applicable |

**Atrial Tachycardia/Atrial Fibrillation (AT/AF) Summary**

|                                             | 16| Number of mode switches (SR to AF)                            | Not applicable |
|                                             | 17| Maximum duration of the mode switches (maximum duration of AF) | Not applicable |

**Atrial Tachycardia/Atrial Fibrillation (AT/AF) Therapy Summary**

|                                             | 18| Pace terminated episodes                                       | Not applicable |
|                                             | 19| Shock-terminated episodes                                     | Not applicable |
|                                             | 20| Total shocks                                                  | Not applicable |
|                                             | 21| Aborted charges                                                | Not applicable |

**Bradycardia Information**

|                                             | 22| % Atrial Paced                                               | < |
|                                             | 23| % Ventricular Paced                                          | < |

**Figure 8.1:** Data fields collected from ICD interrogation during the CUPID-2 trial.

Table 8.1. 479 patients were identified in 2011 and 537 in 2012. The proportions of patients receiving at least one appropriate shock, ATP or both in each year are displayed in Figure 8.2. There was a trend for a greater burden of ICD therapy in patients with a secondary prevention indication but this did not reach statistical significance (Table 8.2). On average 9% of patients received an appropriate shock each year.

*CUPID-2 arrhythmia sub-study*

Patients enrolled in to the CUPID-2 study who had an ICD in situ at the time of enrolment.
Male 84%
Age at implant (years) 67 (58-73)
Aetiology
- Ischaemic 74%
- DCM 15%
- Unknown/not recorded 11%
Biventricular device 66%
Ejection Fraction (%) 25 (20-30)
Indication
- Primary prevention 55%
- Secondary prevention 43%
- Unknown/not recorded 2%

Table 8.1: Characteristics of heart failure patients with an ICD identified for observational data analysis. DCM, dilated cardiomyopathy.

<table>
<thead>
<tr>
<th></th>
<th>Primary Prevention</th>
<th>Secondary Prevention</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indication</td>
<td>Indication</td>
<td></td>
</tr>
<tr>
<td>2011 Appropriate Shock</td>
<td>8.20%</td>
<td>14.10%</td>
<td>0.052</td>
</tr>
<tr>
<td>2011 Appropriate ATP</td>
<td>14.30%</td>
<td>15.70%</td>
<td>0.743</td>
</tr>
<tr>
<td>2012 Appropriate Shock</td>
<td>7.10%</td>
<td>8.90%</td>
<td>0.413</td>
</tr>
<tr>
<td>2012 Appropriate ATP</td>
<td>12%</td>
<td>14.40%</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Table 8.2: The Proportion of patients who received appropriate implantable cardioverter defibrillator (ICD) therapy in 2011 and 2012 stratified by primary or secondary prevention indication for ICD implantation. ATP, anti-tachycardia pacing.
Figure 8.2: The proportion of heart failure patients receiving appropriate ICD therapy in 2011 and 2012 (observational study). Shock only: patients who received at least one appropriate shock in the year (this may or may not have been preceded by unsuccessful ATP), shock & ATP: received at least one shock and at least one successful ATP therapy, ATP only: received at least one successful ATP therapy in the year but no shocks.

comprise the study population for the CUPID-2 arrhythmia sub-study. This population is described in Table 8.3. There were no statistical differences in baseline characteristics between the AAV1.SERCA2a and placebo groups. ICD interrogation was not performed for every patient on every visit. For example, if a patient was too unwell to attend the appointment or had been admitted to hospital, ICD data may not have been available.

It was apparent that several of the data fields displayed in Figure 8.1 were interpreted differently by different centres resulting unreliable data. The two most important data fields for ventricular arrhythmias appeared to be reliably completed: the number of pace terminated episodes (i.e. ATP) and the number of shock terminated episodes of VT or VF.

Crude event rates, described as the number of appropriate ICD therapies per patient days, are displayed in Figure 8.3. These results suggest that at baseline the incidence of ICD therapies is significantly greater in the placebo than AAV1.SERCA2a group there is then no difference between the two groups until the 9 and 12 month checks when placebo again have a higher rate than AAV1.SERCA2a. The highest incidence of ICD shocks occurred in the three
Table 8.3: Baseline characteristics of patients in the main CUPID-2 who already had an ICD at baseline (the CUPID-2 arrhythmia sub-study population). Continuous variables are described as mean (standard deviation) and compared using Student’s independent t-test while categorical variables are described as percentages and compared using chi square. ICD, implantable cardioverter defibrillator; NT pro-BNP, N terminal pro-B type natriuretic peptide; NYHA, New York Heart Association.

<table>
<thead>
<tr>
<th></th>
<th>AAV1.SERCA2a n=102</th>
<th>Placebo n=95</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>77</td>
<td>84</td>
<td>0.184</td>
</tr>
<tr>
<td>Ischaemic aetiology (%)</td>
<td>51</td>
<td>54</td>
<td>0.406</td>
</tr>
<tr>
<td>Prescribed amiodarone (%)</td>
<td>18</td>
<td>17</td>
<td>0.428</td>
</tr>
<tr>
<td>Prescribed β-blockers (%)</td>
<td>100</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Six minute walk distance (m)</td>
<td>354 (110)</td>
<td>354 (111)</td>
<td>0.979</td>
</tr>
<tr>
<td>NYHA Classification (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-NYHA 2</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>-NYHA 3</td>
<td>81</td>
<td>82</td>
<td>0.984</td>
</tr>
<tr>
<td>-NYHA 4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>NT proBNP (pg/ml)</td>
<td>365 (522)</td>
<td>281 (312)</td>
<td>0.177</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>22 (6)</td>
<td>23 (6)</td>
<td>0.430</td>
</tr>
<tr>
<td>Cardiac resynchronisation therapy (%)</td>
<td>49</td>
<td>39</td>
<td>0.155</td>
</tr>
<tr>
<td>Primary prevention indication for ICD (%)</td>
<td>82</td>
<td>87</td>
<td>0.328</td>
</tr>
</tbody>
</table>

month interval recorded at the 12 month check. A histogram of these data demonstrates that the vast majority of participants received no shocks, 6 participants received one shock, one participant had two shocks and one participant had 25 shocks (Figure 8.4). This individual with 25 shocks was in the placebo group and skewed the results to increase the incidence of shocks in the placebo group to the extent that it became significantly greater than the AAV1.SERCA2a group. The individual with 25 shocks may have had one arrhythmic event with all shocks in a short period of time (VT storm) or they may have had 25 separate events in the three months but this difference cannot be discerned without dates for therapies being available. While it is numerically true that the incidence of appropriate therapies in the placebo group was greater than for the AAV1.SERCA2a group, this is not a clinically useful way of describing a treatment effect since one individual with a VT storm can inappropriately weight the result heavily. Exclusion of this patient nullified any significant difference in incidence
Figure 8.3: Crude event rates for ICD therapy in patients in the CUPID-2 arrhythmia sub-study. The total number of events and the number of patients with an ICD check in each time period are presented. The number of events may be greater than the number of patients experiencing an event if a patient has more than one event and the number of patients with ICD data may increase from one time period to the next if any patients missed ICD interrogation at a follow-up time but survived to have a check at the next scheduled follow-up. Comparison of treatment (AAV1.SERCA2a) and placebo groups. * indicates significant to P<0.05.

between the two groups. Analysis as categorical data (i.e. therapy or no therapy during the monitoring period) demonstrates that there is no significant difference between placebo and
Figure 8.4: Histogram of number of appropriate shocks at the 12 month follow-up in the CUPID-2 arrhythmia sub-study.

AAV1.SERCA2a groups (Table 8.4).

When all ICD shocks over the full 12 month trial period are combined then 12.7% of the patients in the AAV1.SERCA2a group and 13% of the patients in the placebo group received at least one appropriate shock during the 12 month follow-up of the trial and this was not statistically different (P=0.95). When assessing any appropriate therapy (ATP or shock) over the 12 month follow-up, 20.6% of patients in the AAV1.SERCA2a group and 28.3% of patients in the placebo group experienced this outcome and there was no statistical difference between the groups (P=0.21).

8.5 Discussion

The main finding from this chapter is that AAV1.SERCA2a had no effect on the proportion of patients receiving ICD therapy during 12 months follow-up in the CUPID-2 arrhythmia sub-study. This is not unexpected in light of the neutral results published for the primary
<table>
<thead>
<tr>
<th></th>
<th>Proportion of patients with an appropriate ICD shock</th>
<th>Proportion of patients with an appropriate ICD shock OR ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAV1.SERCA2a n=102</td>
<td>Placebo n=92</td>
</tr>
<tr>
<td>Baseline</td>
<td>3/100 (3.0%)</td>
<td>5/90 (5.6%)</td>
</tr>
<tr>
<td>Month 1</td>
<td>3/93 (3.2%)</td>
<td>2/85 (2.4%)</td>
</tr>
<tr>
<td>Month 3</td>
<td>1/92 (1.1%)</td>
<td>3/86 (3.5%)</td>
</tr>
<tr>
<td>Month 6</td>
<td>3/88 (3.4%)</td>
<td>2/79 (2.5%)</td>
</tr>
<tr>
<td>Month 9</td>
<td>3/84 (3.6%)</td>
<td>3/74 (4.1%)</td>
</tr>
<tr>
<td>Month 12</td>
<td>4/77 (5.2%)</td>
<td>4/73 (5.5%)</td>
</tr>
<tr>
<td>Summed FU</td>
<td>13/102 (12.7%)</td>
<td>12/92 (13.0%)</td>
</tr>
</tbody>
</table>

Table 8.4: ICD therapy presented as categorical data comparing treatment (AAV1.SERCA2a) to placebo at each follow-up time point and then all events over the 12 month follow-up combined in Summed FU for patients in the CUPID-2 arrhythmia sub-study. Categorical data statistically compared using chi square test.

outcomes of the main CUPID-2 trial [235], particularly considering the hypothesised explanations for why CUPID-2 may have been neutral described in the discussion of Chapter 7.

Even if the main CUPID-2 trial had been positive, it remains doubtful that the event rate for the CUPID-2 arrhythmia sub-study (particularly with appropriate shock as the primary outcome) would have been sufficient to test the hypothesis of whether AAV1.SERCA2a has an anti-arrhythmic effect. This study was underpowered due to an insufficient event rate in the primary outcome. While this is a moot point in the current study it may be relevant for planning future studies when ICD therapy is used as an outcome measure. In the CUPID-2 arrhythmia sub-study, during the 12 months follow-up 13 patients in the AAV1.SERCA2a group and 12 in the placebo group received at least one appropriate ICD shock. Combining the two groups this equates to just under 13% of the participants receiving a shock per year. The equivalent figure in my observational study was 9%. Published data can also inform on this. The Evaluation Médico-Economique du Défibrillateur Automatique Implantable (EVADEF) study was a prospective, multi-centre cohort study [247] investigating 2,296 patients who received an ICD in the years 2001-2003. Secondary prevention implants comprised 82% of participants and 18% of implants were for primary prevention, participants were
followed up for two years. Within this population 2009 patients had a cardiomyopathy defined as either ischaemic cardiomyopathy (n=1313), dilated cardiomyopathy (n=416) or hypertrophic cardiomyopathy/arrhythmogenic right ventricular cardiomyopathy (n=280). In this sub-population with cardiomyopathy 14% of patients received at least one appropriate shock annually and 22% received at least one appropriate therapy (i.e. ATP or shock) annually. Patients with a secondary prevention indication were significantly more likely to receive at least one appropriate shock annually than those with a primary prevention indication: 15% vs 4% respectively. The OPTIC study demonstrated that the pharmacological therapy patients receive can affect the burden of ICD therapy. Patients receiving a secondary prevention ICD were randomised to $\beta$-blocker therapy (n=138), sotalol (n=134) or $\beta$-blocker and amiodarone (n=140) and in one year follow-up the proportions of patients receiving an appropriate shock in each group were respectively; 22.0%, 15.1% and 6.7% [244]. This variation in event rates for ICD therapy represents a challenge if one is to plan a trial using ICD therapy as an outcome measure. Recruitment of patients with a secondary prevention indication for their ICD may be an appropriate approach to increase event rates but it is otherwise very challenging to anticipate who will receive an ICD shock, indeed the fact that the majority of patients who undergo ICD implantation do not receive therapy reflects our inability to predict who is at risk of ventricular arrhythmias.

If one were to change the primary outcome from appropriate shock to any appropriate therapy (i.e. shock or ATP) then this would increase the event rate. This approach approximately doubled event rates in the CUPID-2 arrhythmia sub-study and in my observational study. The clinical significance of receiving ATP is less robust than a shock. This is in part because ICD programming significantly influences whether an individual receives ATP. There is evidence that ATP is able to terminate fast VT (which is likely to be a clinically significant arrhythmia)
and avoid a shock [248, 249]. Furthermore, evidence suggests that individuals who receive ATP only have a similar survival to those who receive no therapy which contrasts with the worse survival of those who receive shocks [250]. Since the publication of the Multicenter Automatic Defibrillator Implantation Trial-Reduce Inappropriate Therapy (MADIT-RIT) trial many centres changed the way they programmed their ICDs in an effort to minimise therapy. MADIT-RIT demonstrated that mortality could be reduced if ICDs were programmed to only deliver therapy if a fast ventricular arrhythmia is detected (over 200 bpm) or if therapy is delayed when the arrhythmia is slower (170 bpm) [251]. These differences in programming strategies are likely to affect the incidence of ICD therapy in a clinical trial. While it may be considered reasonable to combine ICD shock and ATP as an outcome measure in a drug trial it is important that ICD programming is consistent throughout the trial. This was not feasible in the CUPID-2 arrhythmia sub-study.

The studies described above predominantly focus on the first ICD shock during follow-up. Using this event, the proportion of patients during follow-up who receive a shock can be determined and a Kaplan-Meier curve can be plotted. This allows populations to be compared either categorically as proportion of patients receiving a shock or using survival statistics as event free survival. Neither method takes in to account that patients may receive multiple shocks during follow-up which may have a significant effect on the individual. Recurrent events are more challenging to analyse. A simple approach used in the OPTIC trial, as a secondary analysis, was to categorise patients in to groups depending on how many shocks they received in the follow-up period: 0 shock, 1 shock, 2-5 shocks, 6-10 shocks, >10 shocks. An alternative approach could be to adopt a similar approach to the CUPID-2 trial analysing recurrent events using a joint frailty model (as discussed in section 2.2).

If one were designing a study to test the anti-arrhythmic properties of AAV1.SERCA2a
prospectively then it could be argued that the use of ICD therapy as an end point is simply a surrogate measure of a more important endpoint, SCD. The use of SCD as an end point would also expand the recruitable patient population beyond those with an ICD in situ. The use of SCD as an end point, however, also has drawbacks as illustrated in the recently published Danish Study to Assess the Efficacy of ICDs in Patients with Non-ischemic Systolic Heart Failure on Mortality (DANISH) study [19]. In this study a population of 1116 patients with non-ischaemic cardiomyopathy were randomised to receive conventional therapy or conventional therapy and an ICD. Over a median follow-up of 5.6 years there was no difference in the primary outcome of all-cause mortality. There was also no difference in cardiovascular death. There was a significant reduction in SCD, a secondary end point, with 8.2% of patients in the conventional therapy arm experiencing SCD and 4.3% in the ICD arm (HR 0.5 (95% CI, 0.31-0.82), P=0.005). The risk of SCD was low in this population in part because the non-ischaemic population is recognised to be at lower risk of SCD than those with ischaemic cardiomyopathy but also because participants were receiving excellent conventional heart failure therapy. An intervention like an ICD, which is only intended to prevent one mode of death, had no effect on all-cause mortality because so few patients died from SCD. The hope had been that AAV1.SERCA2a would have a positive impact on pump failure in patients with heart failure and if it also had an anti-arrhythmic mode of action then the effect on survival could have been additive. In the current climate of patients receiving excellent conventional heart failure treatment, designing trials with SCD or ICD therapies as end points is challenging.
Chapter 9

Conclusions

The main findings from this thesis are:

1. Analysis of more than one ECG lead can significantly affect the interpretation of TWA as determined using the MMA method on AECG.

2. The current iteration of AAV1 mediated SERCA2a gene therapy was ineffective at reducing appropriate ICD therapy in a population with advanced heart failure.

3. The proportion of patients receiving an appropriate ICD shock in unselected observational data and in an advanced heart failure population from the CUPID-2 trial was approximately 9-13% annually. This event rate has implications on sample size for any trials that rely on this as an end point.

In my efforts to refine the MMA method of TWA analysis I demonstrated in Chapter 3 that low level, non-peak, TWA values appeared to measure physiological perturbations by demonstrating a significant increase in TWA during a tilt table test. Values other than the peak TWA over 24 hours could be measured with sufficient reproducibility but these variables were unable to distinguish between groups of heart failure patients any better than the traditional peak TWA
The main refinement to the MMA method of TWA analysis that I found may impact significantly on the interpretation of this result in clinical studies was the interrogation of more than one ECG lead to find the peak TWA value on AECG as described in Chapter 4. The sensitivity to detect high levels of TWA increased with more leads being used. This could have the effect of reclassifying a TWA negative patient as positive. The challenge of using this approach currently is that there are no set values for what is considered a significantly elevated TWA in each ECG lead. 12-lead AECGs are more commonly available now and the prognostic cut-points for each lead could be tested. The ongoing Risk Estimation Following Infarction Noninvasive Evaluation - ICD Efficacy (REFINE-ICD) study presents a possible opportunity to do this. The REFINE-ICD trial will assess whether prophylactic ICD implantation, guided by non-invasive risk assessment tools, reduces mortality in post-MI patients with an LVEF 36%-50%. Patients will be randomised to ICD or control if they have an abnormal heart rate turbulence and TWA on AECG. The study is expected to enrol 1000 patients and the expected completion date is December 2021. A sub-study could be conducted to assess the effect of using more than one ECG lead in predicting outcomes in each treatment group.

Concerns have been raised that the MMA method of TWA analysis does not take into consideration the heart rate at which TWA is recorded or the magnitude of the T waves. If either of these parameters are important in the interpretation of TWA then an indexed value of TWA would need to be considered and this could affect all subsequent and previous TWA studies. The data I presented in Chapter 5 suggest that during ambulatory monitoring there is no significant correlation between TWA and either heart rate or T wave magnitude. It is generally accepted that heart rate is important in the development of TWA but I would hypothesise that the heart rates achieved during a 24 hour AECG in a heart failure patient are not sufficient for
this relationship to be important.

Chapter 6 was unfortunately underpowered. The aim of this chapter was to assess if the results of TWA analysis while in normal sinus rhythm are comparable to those while in AF or while receiving biventricular pacing. The presence of AF and paced rhythms are both exclusion criteria for TWA analysis. The utility of TWA is significantly limited in heart failure patients when these two exclusion criteria are applied. The trend from my data would suggest that TWA results in sinus rhythm are not comparable to those in AF or while receiving biventricular pacing. Due to problems with recruitment, this study was underpowered and should only be used for hypothesis generating. A larger study is required to reliably address the question.

During my PhD the main CUPID-2 trial published neutral results. There is some evidence that there was inadequate transduction of cardiomyocytes as limited amounts of the therapeutic DNA could be identified on the biopsy samples available. With neutral results from the main trial, the expectation was that the CUPID-2 arrhythmia sub-study would also be neutral as was the case. No safety concerns were raised which is particularly relevant in a gene therapy study. The financial impact on the company funding the CUPID-2 trial was profound. They ultimately required a merger with another biopharmaceutical company and it is unknown whether this iteration of AAV1.SERCA2a will ever be developed further. It would be unfortunate if this product is abandoned as it may simply be a case of needing to use a higher dose or different delivery method. Perhaps a better approach after the CUPID study would have been to perform a further dose finding study and demonstrate adequate transduction with biopsy samples before launching into a larger clinical trial. In addition, one might consider the most appropriate target population for such a therapy might be those with non-ischaemic cardiomyopathy since these hearts should have more surviving cardiomyocytes to transduce than an individual who has had a large MI. Further studies will be needed if such therapies are going to progress and
this means overcoming the problem of nAbs. I have demonstrated that more than half of heart failure patients in the UK are ineligible for gene therapy using AAV1 as a vector. This makes recruitment challenging and will limit the number of patients who can be treated should the therapy be proven efficacious. Further challenges surrounding the conduct of gene therapy trials includes the legislation and approvals that are required, which may be a deterrent for many centres agreeing to take part. Even if there is no future for this iteration of AAV1.SERCA2a gene therapy, there are other promising approaches to gene therapy in heart failure. SDF-1 is a promising target and the method of delivery for this therapy has the advantage that it can be directly injected in the areas of myocardium required or given as a retrograde infusion through the coronary sinus. AC6 is another target for gene therapy in heart failure and a phase I/II study of safety and efficacy in patients with a LVEF of $\leq 40\%$ is due to complete in October 2017. The gene therapy product being tested is Ad5.hAC6 which is an adenoviral vector with the human AC6 gene. This will delivered by an intracoronary route with dose escalation. The primary outcome is exercise treadmill time and LV function on echocardiography before and after dobutamine infusion. Although this is the same delivery route as the CUPID-2 trial it is a different vector and different molecular target, but this is still in the early stages of clinical development.

With the main CUPID-2 trial being neutral, it was expected that the CUPID-2 arrhythmia sub-study would also be neutral. However, even had the main study demonstrated a reduction in hospitalisations, it is unlikely that the event rate of appropriate ICD shocks would have been sufficient to reliably test the hypothesis that AAV1.SERCA2a is an anti-arrhythmic. Even if the therapy had been highly effective and reduced the annual event rate from 13\% of participants receiving an appropriate shock to 5\% then a total sample size of 394 would be needed (197 in each group) to provide a power of 80\% and confidence interval of 95\%. This is the sample size...
size that would be required to detect a difference at 12 months and an alternative to increasing
the sample size could be to extend the follow-up time. In smaller studies like the CUPID-2
study, less clinically relevant outcomes such as appropriate ATP or changes in the magnitude
of TWA could be used as surrogate measures as a plausibility approach before designing a
larger study. The approach of using arrhythmic end points in heart failure studies may not
be of much clinical relevance in certain populations. The recently published DANISH study
investigated the effect of ICD implantation versus standard care in a population of patients
with non-ischaemic cardiomyopathy. The trial was neutral for its primary outcome of all-cause
mortality. Despite more than five years follow-up only only 8.2% of patients in the control group
experienced SCD. This illustrates the low risk nature of some populations with heart failure. If
one did want to assess the anti-arrhythmic properties of a new therapy then the most appropriate
population, from the perspective of event rates, may be those with ischaemic cardiomyopathy
who have a secondary prevention ICD. For smaller studies, surrogate markers such as TWA
may be appropriate for testing plausibility.

The neutral outcome of the CUPID-2 trial was disappointing. Gene therapy studies are
expensive to run, difficult to setup and high risk for those financing them. Hopefully, progress
will continue to made in efforts to create a new treatment for patients with heart failure and gene
therapy may ultimately prove to be an effective approach.

Small sample size has been mentioned in individual chapters but it is a critical and important
weakness of some of my studies that weakens the robustness of these studies. As such, a final
mention of this was appropriate in this main conclusions chapter. In particular, Chapter 6 is
underpowered and the results of this chapter can only be used as preliminary data for hypothesis
generation and planning future studies. Another study that was underpowered was the effect of
SERCA2a gene therapy on TWA. While this investigation would be critical for addressing the
title of this thesis, the direction of the thesis changed, as described in the introduction, making this study less relevant. Furthermore, with the neutral results of the CUPID-2 trial it is clear that the current iteration of SERCA2a gene therapy is ineffective so one might not expect there to be an effect on TWA which further lessens the importance of this study.
Bibliography


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Gunjan Chaudhry, G Muqtada Orlov, Michael Hoffmeister, Peter Haffajee, Charles Case


[89] Murphy CF, Lab MJ, Horner SM, Dick DJ, Harrison FG. Regional electromechanical


[179] Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, et al. Early and


[250] Sweeney MO, Sherfesee L, DeGroot PJ, Wathen MS, Wilkoff BL. Differences in

Appendices
Appendix A

GTAC Approval
21 November 2012

Alexander Lyon
Royal Brompton Hospital
Sydney Street
London
SW36NP

Dear Alexander Lyon

Study title: A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomised Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure

REC reference: GTAC195
Protocol number: CELL-004
EudraCT number: 2012-001700-37

Thank you for your letter of 29 October 2012, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the interim Chair

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion
does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

*Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.*

*Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.*

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

*Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (“participant identification centre”), guidance should be sought from the R&D office on the information it requires to give permission for this activity.*

*For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.*

*Sponsors are not required to notify the Committee of approvals from host organisations.*

*Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).*

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

*It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).*

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

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**Statement of compliance**

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**After ethical review**

**Reporting requirements**

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

**Feedback**
You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

GTAC195 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Prof Andrew George
Chair

Email: NRESCommittee.GTAC@nhs.net

Enclosures: “After ethical review – guidance for researchers”

Copy to: Mr Jamie Pearson

Dr. Ginette Hoare
The Royal Brompton & Harefield NHS Foundation Trust
Appendix B

DEFRA Approval
Applicant: Celladon Corporation (with separate trial sponsorship from Imperial College London and the British Heart Foundation)

Application: Application for Part B consent to carry out clinical gene therapy trials using a GM adeno-associated viral vector (AAV1/SERCA2A) expressing a human heart calcium transporter gene.

Ref: 13/R46/01 and 13/R46/01/S

Date: 25th March 2013

Advice of the Advisory Committee on Releases to the Environment under section 124 of the Environmental Protection Act 1990 to the Secretary of State for Environment, Food and Rural Affairs and Ministers of the Scottish Government.

ACRE is satisfied that the information provided by the applicant in accordance with the current regulations on the Deliberate Release of GMOs, demonstrates that the ‘release’ of this GMO under the conditions of the trial will not have an adverse effect on human health or the environment. ACRE therefore sees no reason for the release not to proceed.

The GMO

1. ACRE recently considered an application from the Celladon Corporation for clinical trials of a GM gene therapy agent based on a replication defective, highly depleted adeno-associated virus (AAV1/SERCA2A) designed to improve Calcium ion transport across the cell membranes of cardiac tissue in heart failure patients. Members assessed the environmental risks associated with the release of this GMO under the conditions of the trial set out in the application, including risks to humans who have not been administered the vector.

2. Two studies are planned for English sites and one for Scotland. In England, one study is to be undertaken at the Royal Brompton Hospital in London where approximately 8 to 10 patients with systolic heart failure will receive the MYDICAR® product that is based on the AAV1/SER2A viral gene therapy agent. A second study will take place at two sites, the Harefield (Middlesex) and Papworth (Cambridge) heart transplant centres resulting in 16 patients who have been fitted with left ventricular assist device (LVAD) receiving the same dose of AAV1/SERCA2a as in the Brompton study. The Scottish study will take place at the Golden Jubilee National Hospital, Glasgow in 8 to 10 patients.
3. The agent administered in the gene therapy trials is a protein-encased viral capsid containing DNA for the human SERC2A gene driven by the human Cytomegalovirus immediate early enhancer/promoter (CMVie) (609 bases) and hybrid intron (319 bases). This cassette is flanked at either end by the AAV1 terminal repeat sequences (145 bp per end).

4. The wild type AAV1 virus is a small non-enveloped single stranded DNA virus that is non-pathogenic and not known to be associated with any human diseases (although over 90% of people are seropositive to AAV before entering adulthood indicating that they have been exposed).

5. The UK ACDP has not categorised wild type AAV, consequently the UK Scientific Advisory Committee on Genetic Modification states that Containment Level 1 is sufficient except in cases where a potentially harmful transgene may require higher containment. (see Section 2.6.5 of the SACGM Compendium of Guidance). The SERC2A gene is not considered harmful and thus no increase in the basic level of containment is required.

The clinical trial
6. The principal aim of the clinical trials is to evaluate and confirm the clinical safety and efficacy of the GMO gene therapy agent versus placebo added to an optimal Heart Failure (HF) regimen. Celladon believes that targeted SERCA2a transporter protein replacement in advanced HF patients will correct imbalances in Ca2+ cardiac metabolism, resulting in enhanced cardiac function and energetics, which will in turn translate to improved clinical outcomes.

7. The principal aim of the study on patients fitted with a left ventricular assist device (LVAD) is to determine 1) the safety and efficacy of SERCA2a gene transfer in patients with advanced chronic heart failure and LVAD support, 2) the magnitude of viral gene transfer to the human failing myocardium and 3) the influence of circulating neutralising antibodies to AAV1 upon myocardial gene transfer.

8. Study sites will be evaluated and personnel trained on drug assignment, receipt, dispensing, storage and accountability procedures. In addition to receiving a site initiation visit by the sponsor that reviews investigational product storage, handling, dilution and administration according to the Study Pharmacy and Interventionalist manuals, the sites will complete in-service training on use of the administration syringe pump and complete an administration ‘dry run’. Thus personnel involved in delivery of the drug will only be those familiar with procedures that minimize undue exposure to themselves and to the environment.

Administration and fate of the GMO
9. The release will be performed at the investigator’s centres, in a hospital catheterization laboratory. Subjects will be observed for a recovery period either in a room near the catheterization lab or in a normal hospital room. Release will be a single intracoronary infusion of 50ml containing $1 \times 10^{13}$ capsid particles for each study subject.

10. After administration the puncture wound created for arterial access for the administration of investigational product will be monitored in the cardiac catheterization laboratory, during the overnight hospitalization, and then just
before discharge from the hospital. Use of an Angio-Seal vascular closure or similar medical device may be used to aid in rapid closure and sealing of the puncture site; the protocol allows radial, brachial or femoral arterial access as determined by the treating interventionist. After closure and sealing of the puncture site it will be bandaged accordingly.

11. Preclinical data indicate that the biodistribution and persistence of AAV1/SERCA2a is similar to other AAV1- and AAV2-based vectors. The persistence of vector DNA is limited to the injection/infusion site (the heart) and highly perfused tissues and decreases with dose administered and time. AAV1/SERCA2a is expected to spread to other parts of the body before it is cleared. After intra-coronary (IC) delivery of AAV1/SERCA2a, particles which are not taken up in the heart are first passed through the lung via the coronary sinus, where they are thought to be cleared by the reticuloendothelial system (RES). Based on animal studies and clinical studies of other AAV gene therapy agents, it is expected that concentrations will decrease quickly over time. In studies of AAV2 vectors in cystic fibrosis and HIV vaccines administered via aerosol or intramuscularly, respectively, at doses as high as $1 \times 10^{13}$ DRP, most samples were negative and those that were positive were at less than 1/1,000,000 of the dose administered even at 2 hours after dosing. Stool and urine samples were negative for all samples. In summary shedding is to be expected to varying degrees from some patients and continuing for as long as 150 days post administration.

**Waste material and excess inoculant**

12. All disposable materials (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the investigational product will be disposed of as biohazardous materials according to individual institutional practices and policies. In general the materials will be disposed of in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

13. The unused investigational product and vial, stopper and crimp seal can be decontaminated with a 10% aqueous solution of household bleach (5000 ppm sodium hypochlorite), autoclaved or incinerated according to institutional practice. Following decontamination, materials will be disposed of as biohazardous waste. If excess investigational product is destroyed by bleach it can be poured down a sink with running water or otherwise in compliance with local and institutional disposal and cleaning procedures. Non-disposable materials, equipment and surfaces will be decontaminated with a 10% solution of household bleach. Some non-disposables may be autoclaved.

**Detection methods and monitoring**

14. The GMO can be identified by qPCR. The PCR primers only detect the transgene sequence within the GMO. The primers are designed to detect a 106 bp fragment in the SERCA2a transgene in the GMO. The donor (human) DNA in humans is not detected because there is an intervening 1.1 kb intron. The primers do not hybridise to the recipient DNA. The assay has been shown to detect less than 30 DNA copies in blood and tissue. The sensitivity and reliability of the assay in other matrices should be similar.

15. The health of patients enrolled in the study will be monitored for two years, or longer, over the course of the study. On Day 0, subjects will undergo cardiac catheterization and angiography, followed by infusion of investigational product. In the Phase 2b study at Months 1, 3, 6, 9 and 12 (12-Month Active
Observation Period), subjects will undergo a battery of safety, efficacy and economic assessments, followed by quarterly visits (Months 15, 18, 21, 24, etc.) in the Long-Term Follow-Up for collection of information on clinical events and resource utilization until the last enrolled subject completes 12 months of observation and at least 180 adjudicated HF-related hospitalizations have occurred, whichever comes later. All subjects will be observed and followed for a minimum of 24 cumulative months. The 24 cumulative months includes the amount of time in the 12-Month Active Observation Period plus the amount of time in Long-Term Follow-Up.

16. In the LVAD study subjects will be monitored weekly including a clinical evaluation, record of all medications and blood tests. Then subjects will be monitored monthly to month 6 including a clinical evaluation, record of all medications and blood tests followed by an annual follow up including a clinical evaluation and record of all medications for 10 years. While some viral shedding by subjects following administration is expected, there is negligible risk from shedding and exposure of family members or other casual contacts from infectious AAV1/SERCA2a so shedding and effects will not be monitored in the present study.

17. However, prior to submission of the Marketing Approval Application for MYDICAR Celladon will conduct a vector shedding study to monitor vector shedding in an open label study. qPCR assay for vector DNA in saliva, buccal swab, urine, and faeces Day 1, Day 3, Day 7; followed by weekly for 1 month, and then monthly for 3 months until there are two consecutive negative results.

Comment

18. ACRE discussed the application for Deliberate Release of the AAV/SERC2A GMO gene therapy agent at its committee meeting on the 7th February 2013.

19. ACRE agree that the genetic composition of the viral product is well characterised and the methods used to produce the inoculant are such that the actual GMO delivered to the patients would be as described in the application. ACRE note that, although low levels of the antibiotic resistance genes used in the intermediates of production of the gene therapy agent are detected by PCR, this does not present any significant risk.

20. The risk of recombination and replication of the AAV/SERC2A virus is negligible since the viral genome has had virulence genes and other sequences removed (only 6% of the genome was present) and the virus can only be propagated in the presence of a ‘helper’ virus.

21. Data on the likely amounts and timing of shedding are limited to studies in which the same or a very similar vector was used to carry different human genes. The lack of robust data on shedding would normally be a significant issue (and ACRE would certainly want to see more information if the application reached the Market Authorisation Application phase). However, taking into account the likely amounts of shed vector and that AAV1/SERC2a is a non-pathogenic, non-replicating entity, encoding a human gene, the risk from shedding is considered negligible for the purposes of the current trials. This is because even if small amounts of vector were shed as expected, the ‘biological containment’ inherent to it would make this essentially an environmental ‘dead-end’, ie incapable of replication in the environment.
22. Overall ACRE is satisfied that the application should be allowed to proceed as is it content that potential risks to the environment and non-patient humans have been sufficiently considered and that the appropriate risk management procedures have been described and will be applied.
Appendix C

Imperial College GM/GT Approval
Dear Dr Lyon,

I am pleased to confirm that you now have consent to proceed with the project referred to below. The project registration, licenses or letters related to this consent are appended so as to comprise a single locked pdf record of the approved work. A summary of the individual consents and signatures collected can be found to the rear of this consent certificate. Also attached, as a separate file, is the original Word document that can be used when changes need to be made.

As principal investigator, please take note of the following requirements;

- This project registration, consent certificate and associated documentation must be provided to all those at risk of exposure or harm.
- All those involved in the work must be adequately trained and their competency assessed in accordance with the risk assessment and the associated operating procedures. Those registered to work on this project must be recorded on the personnel registration form available from the Safety Department website.
- Wherever this following box □ is hatched, you will ensure that all those registered to work on this project have obtained health clearance before their work on this activity begins.
- This risk assessment must remain accurate and valid at all times and it must be reviewed regularly.
- Where changes to the assessment are necessary then these must be notified to the Biosafety Administrator (biosafety@imperial.ac.uk) by completing a Form C and by making changes to the original project risk assessment and risk assessment covered by this current consent.
- Note that this consent certificate is invalidated as soon as any of the details provided change through alterations in the experimental procedures, control measures available, location, or people involved.
- This consent applies to you as named PI. This cannot be transferred to any other person without formal consent being provided by the Safety Department. Should you wish to end this project, or transfer it to another PI then you must do so by completing a Form E before submitting this to the Biosafety Administrator (biosafety@imperial.ac.uk).

If you have any questions with regard to this consent or as to your work in general then please do not hesitate to contact either your DSO/CSM/FSM or a member of the Safety Department Biosafety Team.

Yours sincerely

Ian Hackford

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**Principal Investigator**

Name: Alexander Lyon
CID: 424703

Position: Imperial College Senior Lecturer/ RB&HFT Honorary Consultant

Department / Section: Myocardial Function

Division: NHLI
Faculty: Medicine

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**Project Registration & risk assessment submitted by**

Name:
CID:

Position:

Department / Section:

Division:
Faculty:

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**Project summary**

Title: A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure (The CUPID2 Trial)

Class: 1
N/R □
Containment Level: 1
N/R □
GM Centre number: GM309 N/R □
# Record of Necessary Consent

This section to be completed by Safety Department only

The following table allows identification of all those parties that must provide consent, license or approval prior to a project being given final consent to begin.

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<td>25/03/2013</td>
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1. **GOALS OF THE TRIAL** *(maximum 300 words)*

The goals of the trial should be explained and justified. This information provides a useful background and puts the work in context. Details of the patient pathway should be provided.

The principal aim of the study is to evaluate and confirm the clinical safety and efficacy of a single intracoronary infusion of $1 \times 10^{13}$ DNase Resistant Particles (DRP) MYDICAR® (AAV1/SERCA2a) versus placebo added to an optimal heart failure (HF) regime in the treatment of subjects with New York Heart Association (NYHA) class III/IV symptoms of systolic heart failure.

2. **AN OVERVIEW OF THE DIFFERENT GENETICALLY MODIFIED MICRO-ORGANISMS (GMMs) THAT WILL BE CONSTRUCTED**

2.1.1 An adequate overview of the different types of GMM that will be constructed must be provided. Outline the scope of the project and define the boundaries of the work to be carried out. Diagrammatic representation can be used and a plasmid map, if relevant, attached to this form. Where a project includes multiple hosts for genetic material, e.g. cloning of genes into bacteria and then into mammalian cells for further studies, all individual GMMs to be created must be listed.

Recombinant adeno-associated virus serotype 1, with rep and cap genes replaced by those for the cytomegalovirus promoter (CMV) and sarcoplasmic reticulum calcium ATPase 2a (SERCA2a).

MYDICAR® is a recombinant adeno-associated viral vector (AAV), which consists of an AAV serotype...
1 capsid and the human SERCA2a cDNA flanked by Inverted Terminal Repeats (ITRs) derived from AAV serotype 2. The SERCA2a protein is the only protein expressed after MYDICAR® treatment, and is a fully human, intracellular, endoplasmic protein that is naturally expressed in cardiomyocytes. MYDICAR® refers to AAV1/SERCA2a drug product intended for intracoronary administration.

MYDICAR® is formulated at a concentration of $2 \times 10^{13}$ DRP/mL in a buffer containing sodium chloride, L-histidine, magnesium chloride, polysorbate 20 and Water for Injection. The buffer without the active ingredient AAV1/SERCA2a serves as the matching placebo. Both MYDICAR® and matching placebo are clear, colourless solutions provided in clear glass vials and sealed with butyl rubber stoppers and crimp seals and labelled with the sponsor’s identification, protocol information, appropriate precautionary statements and other information, as required. MYDICAR® and placebo are visually indistinguishable from each other.

2.1.2 List of all recipient strain(s) to be used.
List all species and strains that will be recipients for any genetic material. For each species, list the name of any strains and the name of the wild-type organism(s) from which it is derived and the extent to which it is disabled.

Human

2.1.3 List of vector(s) to be used
List the names and any associated disabling mutations.

| rAAV1 | Replication deficient |

2.1.4 Names and functional properties of all inserted gene(s)
Describe the listed genes in such a way that an outside reviewer will have a general idea of their function i.e. providing an abbreviation may not be sufficient. Provide details of any known homologues if the function of a gene is unknown.

The CMV promoter, a strong but non-tissue specific promoter.
SERCA2a, the calcium pump protein of the internal calcium stores in the cardiac myocyte

2.2 An indication of the most hazardous GMM
Identify the most hazardous GMM to be constructed in this work giving consideration to both human health and the environment. This will be the most hazardous combination of recipient strain, vector or virus and inserted material from the lists made above. With some projects it will not be clear that one GMM will be more hazardous than any of the others (e.g. if all the work is Class 1). If this is the case, this should be stated.

AAV1-CMV-SERCA2a

3. Identification of the hazard to human health

3.1.1 In which hazard group is each host organism placed by the Advisory Committee on Dangerous Pathogens? Class 1

AAV does not produce any apparent human disease, although the existence of neutralising antibodies demonstrates that around 40-50% of the population have been infected with it. It lies dormant in the cell as an episomal circularised concatamer, and the recombinant form has been designated as non-integrating by the EMEA. It does not therefore insert into the host DNA, eliminating the possibility of insertional mutagenesis. The wild-type is re-expressed only when the cell is infected with the helper virus, adenovirus. However, the recombinant form used in CUPID2 has had the viral proteins rep and cap removed, so there is no possibility of production of new viral particles after the first infection. Approximately 300 patients have been treated with AAV in clinical trials without reported problem. However, in one study, two patients (receiving a higher titre of the virus than in the present study) showed release of liver enzymes. The evidence in one patient was that cytotoxic T-cells against the viral capsid had produced release of enzymes from hepatocytes transiently presenting the capsid on their surface after infection. The previous trial in the US using the same AAV1.SERCA2a vector as CUPID2 injected 51 patients percutaneously into the coronary vessels using the same technique (14 placebo, 37 active in 3 different vector doses). Safety was demonstrated in this phase 2 trial, including in the 12 patients receiving the ‘high dose’ which will be used in this CUPID2 trial.

3.1.3 What are the hazards associated directly from the inserted gene product?

SERCA2a gene expression produces SERCA2a protein only in cells with functional sarcoplasmic reticulum (SR), i.e. smooth, skeletal and cardiac muscle. The effect in cardiac muscle is limited in normal subjects by the amount which can be inserted into the SR, so increases are less in normal than failing heart. The functional effect is to increase contractile force and speed relaxation, which is generally beneficial. Animals experiments show that
arrhythmias are reduced in failing heart, but may under some circumstances be increased in non-failing heart. Smooth muscle proliferation is reduced by SERCA2a overexpression, which may prevent atherosclerosis. Rescue of skeletal muscle function in muscular dystrophy models has been demonstrated with direct intramuscle injection, but there is no evidence of direct skeletal muscle transfection following intracoronary infusion.

3.1.4 If the function of the inserted gene is unknown, describe the function of any known homologues.
N/A

3.1.5 Hazards arising from the alteration of existing traits of the host
N/A

3.1.6 Hazards arising from the sequences within the GMM being transferred to related microorganisms
N/A

3.2 Provisional Assignment of a Containment Level According to the Contained Use Regulations
Consider the containment level necessary to control the risk of the host to human health, making a judgment about whether the modification will result in a GMM which presents hazardous, less hazardous or about the same.

<table>
<thead>
<tr>
<th>Containment Level</th>
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4. Identification of the Hazard to the Environment

4.1.1 Hazards associated with the recipient microorganism (e.g. bacterial host or viral vector)
The virus is replication defective. Animals are not natural hosts of AAV, and would have to be exposed to both AAV and a helper virus for infection to occur. Hazards to the animal would be as described for human subjects, above.

4.1.2 Hazards arising directly from the inserted gene product
Hazards to the animal would be as described for human subjects, above.

4.1.3 Hazards arising directly from the alteration of existing traits (e.g. alteration of pathogenicity, host range or tissue tropism)
N/A

4.1.4 The potential hazards of sequences within the GMM being transferred to related microorganisms
N/A

5. Risks & Control Measures

5.1.1 Most hazardous procedure
Identify and describe the most hazardous procedure(s) involving the handling of the gene therapy product...

Removal of the full dose of vector from the vial into the syringe (Royal Marsden Clinical Trials Pharmacy).
Attachment of the syringe to the syringe pump connected to the patient's catheter (Cardiac Catheter laboratory).

5.2 Preventing Exposure

5.2.1 Substitution with a safer alternative
Is substitution with a safer gene therapy product practical? Provide reasons for your answer.

Cardiac myocytes are difficult to transfect using non-viral vectors

5.2.2 Isolation/ segregation (Administration of gene therapy product)
a) Where will the gene therapy product be administered to the patient?
In the cardiac catheter laboratory in the Cardiovascular Biomedical Research Unit (BRU), Royal Brompton Hospital (RBH).
b) Is this room adequately separated from other areas? Provide details.
Yes - this catheter laboratory is adequately separated from the waiting area, anaesthetic and recovery rooms by an intervening corridor. It is within a swipe card-restricted access section of the BRU not accessible to the public.
c) Is/are the room(s) to be used for this trial to be shared with other patients not involved directly in this trial? If yes, provide details and explain why separate room(s) are not being used.

Due to the high volume of cardiac procedures performed in the BRU catheter laboratory at RBH, it is not possible to dedicate one cardiac catheter laboratory specifically for the Gene Therapy (GT) trial. To prepare the cardiac catheter laboratory for subsequent patients the GMO will be inactivated (Procedure 2 of CUPID 2 Study Manual) and disposed of in line with Sponsor’s validated procedure for inactivation of the GMO (see Procedure 12 of CUPID 2 Study Manual).

At the end of each trial procedure, the catheter laboratory will be cleaned in line with RBH procedures (see Procedure 9 of CUPID 2 Study Manual).

d) Is access to this room restricted? Provide details.

Access is limited to clinical staff involved in the catheterisation procedure, the trial PI, study coordinator and sponsor representative (e.g. CRO).

e) How long after the administration of the gene therapy product will the patient have to remain in hospital for? Where will they be transferred to?

From the Cardiac Catheter laboratory patients will be transferred to one of the cardiology wards at the RBH (Level 5 - York ward or Paul Wood ward). The likely duration of hospital stay is 1 night after the procedure.

f) Is this room adequately separated from other areas? Provide details.

The hospital ward is accessed by swipe card, and trial patients will be placed in either open bays or side-rooms depending upon clinical need.

g) Is/are the room(s) to be used for this trial to be shared with other patients not involved directly in this trial? If yes, provide details and explain why separate room(s) are not being used.

This aspect of the trial will be conducted under EU Directive for Deliberate Release of GMOs. Monitoring of shedding is not a study requirement and will not be performed. Trial patients will be placed in single sex open bays shared with non-trial patients. Patients will only be placed in a side-room if clinically indicated. Precautions beyond the hospital’s procedures for infection control are not required (Procedure 6 of CUPID 2 Study Manual).

h) Is access to this room restricted? Provide details.

Access to the ward is restricted with swipe card access. Access to the bay or side room is available from the ward corridor.

5.3 CONTROLLING EXPOSURE

5.3.1 Storage

a) Who will receive the gene therapy product? Provide contact details.

Royal Brompton Hospital Pharmacy Staff

The name of the person receiving IMP:
Nancy Jones, Jermaine Wright and/or Vibha Teli
Telephone: 0207 351 8121 Ext. 8737 or 8905 or 0207 351 8368
Fax: 0207 351 8985
Email: v.teli@rbht.nhs.uk; J.Wright2@rbht.nhs.uk; N.Jones@rbht.nhs.uk

b) How will the product be transported to the storage location?

The GT product will arrive by courier to the RBH Pharmacy in a vial on dry ice. It will be transported in sealed plastic bags in a designated sealed container to the storage location (2-8°C refrigerator) in the RBH Pharmacy Clinical Trials room.

c) Where will the gene therapy products be stored? Are there any particular requirements for the room ventilation e.g. negative pressure, temperature control?

GT product will be held in a dedicated 2-8°C Clinical Trial fridge in the Clinical Trials room, RBH Pharmacy Department.

d) If to be stored in Liquid Nitrogen, describe the precautions to be taken to prevent a release of infectious material whilst either loading or removing a sample from storage.

N/A
e) In the event of breakdown of the storage equipment is backup storage available? If yes, where is this located?

There is a locked Pharmacy Cold Store available for use in the event of a fridge breakdown.

f) In the event of breakdown, is this equipment alarmed? Who will be alerted by this alarm?

All fridges are monitored continuously and set to alarm if the temperature deviates outside of the acceptable 2 - 8°C range for >1 minute. During working hours, the alarm will trigger hospital switchboard who will notify a senior member of Pharmacy to investigate the cause. Outside of normal working hours, an alarm will trigger switchboard to notify the on-call Pharmacist to investigate the cause.

g) What security measures are in place? Would you be able to easily and rapidly identify that a sample was missing?

The GM product will be held in the RBH Pharmacy, Clinical Trial room which is alarmed and locked by key code. The Clinical Trial room is kept locked at all times and only accessible to authorised members of pharmacy staff. Each vial is supplied by the study Sponsor randomised and numbered and storage will be logged in the accountability documents (filed in the site Pharmacy File) and recorded on the fridge. When required, each vial will be logged out and the number recorded on the fridge door (and in the Pharmacy File). Each vial is delivered in single units from the RBH Pharmacy to the Royal Marsden Hospital (RMH) Clinical Trials Pharmacy for assembly into a single syringe.

The remaining contents of each vial will be inactivated and destroyed in line with Sponsor’s procedure for inactivation of the GMO (see Procedure 11 of CUPID 2 Study Manual).

The syringe is then transferred back to the RBH Pharmacy fridge where it is stored and logged. Once confirmed that the trial patient is ready for GM product infusion, the syringe stored in the RBH Pharmacy fridge will be logged out and transferred to the RBH BRU cardiac catheter laboratory.

Following infusion and on completion of the procedure, each completed infusion and the destruction of any remaining GM product will be documented in the site Pharmacy File.

5.3.2 Preparation of gene therapy product

a. Containment and Ventilation

i. Is the use of a microbiological safety cabinet required for the preparation of the gene therapy product material? Will aerosols or splashes be generated during any stage of the activity and do these aerosols pose a risk of infection to personnel? If yes, specify the type(s) and when it/they will be used.

The GM product will be delivered in a sealed vial, and will be reconstituted and withdrawn into the administering syringe in the GT room inside the licensed GM safety cabinet at RMH. The trained pharmacists conducting the assembly of the GM product will wear specialised personal protective equipment (outlined in section 4.2.1 of the Study Manual).

The GT room in the RMH Pharmacy is specifically designed for GT manipulation and handling. It provides a sterile environment with adequate ventilation and in the event of any spillages the pharmacists are trained to follow the specific procedures outlined in the Clinical Trials Information Sheet (Sponsor Pharmacy File).

The syringe containing the GT product will then be transferred to the RBH BRU cardiac catheterisation laboratory in sealed plastic bags within the designated sealed plastic container. The syringe will be capped and no needles attached to the syringe during transfer. There should be little to no possibility of aerosols or splashes.

The designated member of the clinical trials team transporting the syringe will be trained to minimise and treat spillages in line with the Clinical Trials Information Sheet (Sponsor Pharmacy File) and will carry a GM spill kit as outlined in section 4.1.3 of the CUPID 2 Study Manual.

ii. Is any other form of Local Exhaust Ventilation required?

No

iii. Are there any particular requirements for the room ventilation e.g. negative pressure, temperature control?

No

iv. With reference to the room where the gene therapy product will be administered to the patient. Are there any particular requirements for the room ventilation e.g. negative pressure, temperature control?

No

5.3.3 Centrifugation

a) Will the gene therapy product need to be centrifuged? If yes, describe amounts (conc. & volume) to be centrifuged?

No

b) Will sealed rotors and buckets be used for this? If yes, where will these rotors/buckets be opened?

© Imperial College Safety Department  Page 5 of 14  18/10/2012
5.3.4 Use of incubators
Will the gene therapy product be cultured in an incubator? If yes, what type of incubator (e.g., shaking or static shelf) is this and describe the measures to be used to prevent and contain any spillages therein.

No

5.3.5 Administration of GMMs to patients
Will the GM material be administered to humans? If yes, how long will the GM organism persist following administration?

AAV transfection has been shown to persist in primates after gene therapy for 8 years. Persistence in solid organs is likely to be in the months to years timescale. Persistence of circulating AAV2 has been seen for up to 14 weeks in serum at the top dose studied (~10^{15} DRP), with shedding in urine up to 4 weeks. In the patients receiving a similar viral dose to trial patients in CUPID2 (~10^{15} DRP), circulating viral DNA in the serum was detectable up to 4 weeks post infusion (Manno, Nature 2006). However, this is based on PCR analysis for vector genome, and it is not known if viable viral particles persist. This trial will be governed as a 'deliberate release' trial, and therefore no monitoring of shedding will be performed. Patients will have an overnight stay on the Cardiology ward and if medically stable will be discharged within 24 hours of GT delivery.

a) What is the route of shedding?
AAV is likely to be in faeces or urine. Virus may also be in blood samples taken for diagnostic purposes within 14 days of administration.

b) What level of shedding could occur?
The 2007 joint EMEA and ICH workshop on viral/vector shedding determined that while rAAV is extensively bio-distributed and shedding is known, the virus is non-pathogenic and risks are estimated to be very low. In studies of AAV2 vectors in cystic fibrosis and HIV vaccines, administered via aerosol or intramuscularly respectively, at doses as high as 1 x 10^{13} DRP, most samples were negative and those that were positive were at less than 1/1,000,000 of the dose administered even at 2 hours after dosing. Stool and urine samples were negative for all samples. In studies of AAV vectors for haemophilia B patients that were administered doses as high as 1 x 10^{14} DRP, persistence in saliva, urine and semen was 1 week or less. Since rAAV is non-replicating, even in the presence of helper virus, there is no reason to believe, or any current evidence, to indicate that the added DNA will spread from the human subject to other persons or to the environment.

c) How will shedding be monitored?
Shedding will not be monitored in this trial.

d) How will the shedding of the gene therapy product be contained?
This trial is conducted in compliance with the Contained Use of GMOs 2000 Regulation and EU Directive 2001/18/EC for Deliberate Release of GMOs. No procedures will be performed to actively contain release after vector infusion into the trial subject. Normal clinical care pathways will be followed for a patient undergoing an invasive cardiac procedure such as coronary angiography.

e) What are the consequences for other body systems (i.e. non-target tissues) from the systemic administration of the gene therapy product?
Potential expression of viral capsid and consequent T cell-mediated cytotoxicity from AAV. Increased calcium cycling into sarcoplasmic reticulum in muscle tissue, but no evidence of non-cardiac transfection has been demonstrated in preclinical models as the coronary endothelium is exposed to the high viral dose before dilution in the systemic vasculature. Cells types which lack a sarcoplasmic reticulum are unable to express the SERCA2a protein.

f) What is the normal mode of transmission of the GMM?
Respiratory, gastrointestinal and possibly sexual transmission

g) What other routes of transmission are possible? E.g. needlestick injuries, how will these be minimized?
Potential Needle stick Injury: Blunt needles will be used to withdraw the undiluted GM product from the vial. No other needles will be used during administration of the GM product. For blood sampling, usual clinical precautions will be taken e.g. no re-sheathing of needles, gloves and aprons to be worn. Used needles will be disposed of in sharps bins waste. Needle stick injuries should be encouraged to bleed and then washed with soap and water. In the case of a needle stick or ingestion both the trial team and occupational health doctor must
CONFIDENTIAL INFORMATION

be informed (see section 10.3 of the CUPID 2 Study Manual).

Personal protective equipment including safety glasses, gloves, and gown or lab coat will be worn when working with the GMO. If skin or eyes are exposed they should be rinsed with copious amounts of water.

h) What are the possible consequences of an accidental exposure? (to the person administering the gene therapy product)

Introduction of virus at a significantly lower titre than that in the patient is not predicted to cause gene transfer to the recipient, given the high titres required for direct intracoronary infusion to achieve efficient target organ transfection. The risk of causing accidental gene transfer via a needle stick is extremely low given the doses required for effective target tissue transduction.

i) What samples are required to be taken from the patients following administration of the gene therapy product?

Blood samples will be taken from the patients, both trial specific samples (see Procedure 1 of CUPID2 Study Manual) and as part of standard patient care (see Section 9.2 of the study manual). In the future myocardial samples may be taken from samples at transplant, left ventricular assist device implantation or post-mortem.

j) How will these samples be removed from the patient? Will sharps be used? How and where will these sharps be disposed of?

Normal syringes and needles will be used. Syringes will be disposed of in clinical waste. Used needles will be disposed of in sharp bins waste.

k) Who will take these samples?

These samples will be taken by members of the clinical team including doctors, nurses and phlebotomists and also by members of the research team.

l) What Personal Protective Equipment will they be required to use?

Disposable gloves will be worn according to standard clinical practice handling clinical samples such as blood samples and tissue specimens.

m) Where will these samples be taken to for analysis? How will the samples be transported?

Trial specific blood samples will be taken in the BRU then prepared and stored in a designated freezer or fridge in the BRU. These samples will then be transferred to the core laboratory by courier according to the LabCorp Manual.

Routine blood samples taken as part of standard patient care will be transported in sealed bags in the metal container to the Biochemistry and Haematology Laboratories, by trained phlebotomy porters and in line with Trust policy for transport of blood samples. These samples will be processed and stored in the Royal Brompton Hospital (RBH) Laboratories.

n) Who will analyse these samples? How and where will this waste be disposed of?

These samples will be sent to the core laboratory LabCorp Mechelen in Belgium for analysis as outlined in the study protocol.

5.3.6 Sharps

Are sharps to be used at any stage during this activity? If yes, what sharps, justify their use and describe their use and disposal. Also describe any additional precautions required to ensure their safe use

Needle sharps generated in the cardiac catheterisation laboratory before vector delivery (e.g. when obtaining intra-arterial and intravenous access) will be placed in a designated sharps bin and sent for incineration (see Procedure 2 of Study Manual).

Blood samples will be taken from patients for both the clinical trial and usual clinical care. Disposable gloves will be worn by the staff member undertaking phlebotomy. Some laboratory procedures may require sharps e.g. slicing of myocardial samples from patients after transplantation, LVAD implantation or at post-mortem. All sharps from phlebotomy and pathology studies will be placed in a separate sharps bin for incineration with clinical waste.

5.3.7 Other hazardous procedures

Describe any other hazardous procedures to be undertaken and the control measures to be implemented

None

5.3.8 Security

Is this work notifiable under the Anti Terrorism Crime and Security Act?

No

5.3.9 Personal Protective Equipment (used for handling the gene therapy product)
a) Describe protective clothing to be worn, where will they be stored and the procedures for decontamination and laundering.

RBH Pharmacy staff will prepare the vector solution in the gene therapy suite of the RMH Pharmacy. Pharmacy staff will wear disposable sterile all-in-one coveralls with hoods and boots, mask, eye protection and double gloved in the clean room to prepare the product. At the end of the session all items are to be placed into an incinerator bin and taken with the Gene Therapy (GT) waste stream at the RMH for incineration.

b) Will gloves be worn? If yes, what type are these and where will they be stored?

All operators will be double gloved with sterile nitrile and sterile latex gloves.

c) Is any other type of personal protective equipment to be used?

Plastic sealed goggles

5.3.10 Personal Protective Equipment (used for administration of the GT/GM material to patients)

a) Describe protective clothing to be worn, where will they be stored and the procedures for decontamination and laundering.

Disposable gowns, gloves and surgical hat will be worn by the interventionalist and scrub nurse during the catheterisation procedure. All disposable PPE will be placed in the incineration waste bin for disposal via high temperature incineration in accordance with Trust’s waste disposal policy (see Procedure 10 of Study Manual).

b) Will gloves be worn? If yes, what type are these and where will they be stored?

Yes. Surgical grade sterile gloves as worn by all operators for cardiac catheterisation procedures.

c) Is any other type of personal protective equipment to be used?

Surgical masks with protective visors will be worn by the clinical operators during the procedure to administer the GT product. This is best clinical practice to protect against exposure to arterial blood loss during coronary angiography.

5.3.11 Risks to personnel other than patients

a) Consider personnel potentially exposed to the gene therapy product at all stages of the trial i.e. preparation, transport, administration, follow-up care, removal of samples, through to inactivation.

The risks to others involved in the trial are extremely small. In the event of direct inoculation, via a spill, needle stick, inhalation or other route, the total number of infective viral particles will be low, many log scales lower the dose directly infused into the trial participant’s coronary artery. Effective SERCA2a protein expression is restricted to striated and smooth muscle, and this requires direct delivery with high viral vector titres to ensure efficient tissue transfection e.g. \( >10^{11} \). The virus is non-replicating and cannot therefore amplify infective particle numbers. The risk of inadvertent gene expression in another person is therefore very low (see Appendix 2 for risk score estimate).

b) Will visitors to the patients be allowed? How will their risk of exposure to the gene therapy product be minimised? What information will they be provided with?

Visitors will be allowed to visit the patient after they have returned to the cardiology ward. No specific protective measures will be required, and therefore the visitors will not be provided with additional information beyond that for visiting patients on the cardiology ward.

5.4 Administration Controls

5.4.1 Maintenance

Describe the maintenance and testing procedures for:

Microbiological Safety Cabinets: Tested and serviced every 6 months by ELS under contract to the Royal Marsden Hospital

Autoclaves: will not be used

5.4.2 Information, Instruction and Training

a) Describe the training of all staff identified as being at risk of exposure. Include details on record keeping.

All staff involved in the clinical trial and those specifically involved in the handling of the GT product will receive training from the Study Sponsor on the handling of GM materials as part of the Site Initiation Visit. This will include trial pharmacy staff (both Royal Brompton Hospital (RBH) and Royal Marsden Hospital (RMH) teams), designated staff transporting the GT material, those staff involved in the cardiac catheterisation procedure in the Royal Brompton Hospital (RBH) BRU cardiac catheter laboratory.

All records of staff training will be filed in the Investigator Site File (ISF).

b) Has a Local Code of Practice been prepared? If yes, is this available to all those at risk of exposure?
5.5 **MEDICAL ISSUES** (for staff involved in this GT/GM Clinical Trial)  
*This section must be completed by the Occupational Physician for work at Containment Level 2, or higher. (NOT/APPLICABLE)*  
*This section is only completed once approval for the project is given from the GMSC.*

5.5.1 **Health effect**

5.5.2 **Medical risk assessment**

5.5.3 **Pre-exposure**

5.5.4 **Post-exposure action**

5.5.5 **Antibiotic treatment or chemoprophylaxis**

5.5.6 **What health surveillance is required?**

5.5.7 **Additional notes/ comments**

### 5.6 ASSIGNMENT OF FINAL CONTAINMENT LEVEL AND CLASSIFICATION

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<thead>
<tr>
<th>Containment Level</th>
<th>Classification</th>
<th>With derogation from certain controls (list these, if relevant. If required seek advice from your DSO or the College GMSO)</th>
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<tbody>
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<td>3</td>
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<td>☐ HSE approval is required prior to commencement of work</td>
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### 6. **ROUTINE DECONTAMINATION**

6.1 **Waste handling**

All waste potentially contaminated with GM culture material must be rendered non-viable prior to leaving the site for final disposal. This includes GM material disposed of after the preparation of the gene therapy product stage, administration to patient stage, waste generated when removing and analysing samples.

a) If chemical disinfection is used to treat GM waste

<table>
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<th>Containment Level</th>
<th>Classification</th>
<th>With derogation from certain controls (list these, if relevant. If required seek advice from your DSO or the College GMSO)</th>
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## CONFIDENTIAL INFORMATION

<table>
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<tr>
<th>Liquid waste</th>
<th>Residual GT solution in syringe</th>
<th>Royal Marsden Hospital Pharmacy</th>
<th>BRU Cardiac Catheterisation Laboratory</th>
<th>Any remaining investigational product in the vial will be diluted and mixed with fresh 0.5% hypochlorite solution allowing a minimum contact time of 20 minutes. This will then be disposed of in an incineration waste bin.</th>
<th>SRCL (Pollution Prevention and Control-[England and Wales] Regulations 2000).</th>
<th>Incineration bin. Collected and incinerated according to Waste Management Policy, RMH, 2012.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid waste</td>
<td>N/A</td>
<td>Royal Marsden Hospital Pharmacy</td>
<td>BRU Cardiac Catheter Laboratory</td>
<td>Solid waste from the Royal Marsden gene therapy suite, including the investigational product vial, will be placed into an incinerator bin and taken with the gene therapy waste stream at the Royal Marsden Hospital.</td>
<td>RMH NHS Trust policy – Waste Management Policy, Version 7, dated October 2012</td>
<td>Incineration bin. Collected and incinerated according to the Disposal of Clinical Waste by GM Pharmacetical waste, RBHT, 2013.</td>
</tr>
<tr>
<td>(Sharps should be included)</td>
<td></td>
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</table>

b) If autoclaving is used to inactivate the GM waste, please provide the following details:

<table>
<thead>
<tr>
<th>Liquid waste</th>
<th>Storage location of waste prior to inactivation</th>
<th>Autoclave Cycle</th>
<th>Monitoring of treatment e.g. Chart recorder attached to autoclave</th>
<th>Validation of treatment e.g. Annual 12 point thermocouple testing of autoclave</th>
<th>Route of Disposal</th>
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<tr>
<td>Solid waste</td>
<td>No autoclaving will be used</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>(Sharps should be included)</td>
<td></td>
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</table>

© Imperial College Safety Department  Page 10 of 14  18/10/2012
### 7. TRANSPORT

**a)** How will the gene therapy product be transported within the laboratory e.g. between incubator and safety cabinet? Detail the containment measures which will be used to prevent or contain accidental splashes or spillages.

In sealed plastic bags within a designated plastic specimen container between fridge and clean room in the RMH Clinical Trials Pharmacy. The sealed plastic bags are only opened when ready to be disinfected into the safety cabinet for manipulation.

**b)** Will viable GT/GM material be transported to or from these labs? If yes, describe the route of transportation and describe in detail the containers to be used. Note that this includes the movement of waste containing viable GT/GM material e.g. to an autoclave elsewhere in the building.

The GT/GM product will be delivered by courier to the RBH Pharmacy. It will be transported to the RMH Pharmacy for assembly and manufacturing. Following assembly into a syringe the GT/GM product will be returned for storage in the RBH Pharmacy prior to transport to catheterisation laboratory for administration. The GT/GM product will be placed into sealed plastic bags and then put into a designated plastic container for transport. GT/GM waste generated in the RMH Pharmacy will be disposed of in line with RMH waste procedure (see Procedure 11 of Study Manual). Any GT/GM waste generated in the RBH Pharmacy and cardiac catheterisation laboratory will be sealed in incinerator bins labelled biohazard and destroyed in the relevant waste stream at RBH immediately after each procedure in line with RBH waste disposal procedures (see Procedures 2 and 10 of Study Manual respectively).

**c)** How will the gene therapy product be transported to the administration location? Detail who will transport the material and the containment measures to be used.

The GT/GM product will be transported from the RBH Pharmacy to the BRU cardiac catheterisation laboratory by a member of the research team, in the plastic container as in b above. The designated member of staff will carry a GT/GM spill kit as outlined in the Study Manual Section 4.1.3.

**d)** How will specimens be transported around the hospital? Describe the containment measures to be used.

As in c. above

**e)** Will any GT/GM material or specimens be transported to other College campuses or external locations? If yes, please describe the transportation and containment measures to be used.

GT/GM material will not be transported to other Imperial College sites. Any tissue samples taken at cardiac transplant/post mortem will be transferred to the US for PCR testing. Biopsy specimens to be transported will follow procedures outlined in the LabCorp Manual provided by the study Sponsor.

GM waste materials will be inactive and disposed (see Procedure 2, 10, 11 of CUPID 2 Study Manual).

### 8. EMERGENCY PROCEDURES

**a)** Describe the procedures in place for dealing with spillages of GT/GM material;

**Within the microbiological safety cabinet (if relevant)**

- Notify others and isolate the area.
- If not already wearing, put on appropriate personal protective equipment: gown or lab coat, gloves, surgical or procedure mask and safety glasses, shield or goggles.
- Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
- Decontaminate the area of the spill.
- Place absorbent material over the spill.
- Working from the outside to the centre, saturate the absorbent material with fresh 0.5% sodium hypochlorite solution.
- Allow to stand for at least 20 minutes.
- Place the absorbent material in an appropriate biohazard waste container and dispose as a biohazard material.

Within the laboratory but outside of any primary control measure (e.g. safety cabinet, spill tray, etc)

- Notify others and isolate the area.
- If not already wearing, put on appropriate personal protective equipment: gown or lab coat, gloves, surgical or procedure mask and safety glasses, shield or goggles.
- Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
- Decontaminate the area of the spill.
- Place absorbent material over the spill.
- Working from the outside to the centre, saturate the absorbent material with fresh 0.5% sodium hypochlorite solution.
- Allow to stand for at least 20 minutes.
- Place the absorbent material in an appropriate biohazard waste container and dispose as a biohazard material.

Outside of the laboratory (e.g. during transport to a centrifuge facility)

1. Notify others and isolate the area.
2. If not already wearing put on appropriate PPE: gown or lab coat, gloves, and surgical or procedure mask and safety glasses, shield or goggles.
3. Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
4. Decontaminate the area of the spill:
   a. place an absorbent material (e.g., paper towel) over the spill
   b. saturate the absorbent material with fresh 0.5% sodium hypochlorite solution starting at the outside and working toward the center
   c. allow to stand for at least 20 minutes
5. Dispose of the absorbent material in an appropriate biohazard waste container and dispose as a biohazard material.

Contact:
Study Coordinators: Dr Carl Hayward and Miss Sophie Welch or
Study PI: Dr. Lyon (PI)
The study team will also inform the BSO.

Within the centrifuges (if relevant)
N/A

b) **Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)**

**Immediate action:**
Accidental exposure to blood by whatever route should be treated as for any exposure to human blood.
Thorough irrigation of the affected body part with fast flowing running cold water.

**When and to whom to report the incident:**
Lilian Zoubiri (Occupational Health Nurse) Harefield Hospital ext 5721
To the Quality and Safety Department via Datix as defined in the Trust’s Adverse Incidents Management and Reporting Policy (see Procedure 12 of Study Manual).
To the Study Coordinators – Dr Carl Hayward and/or Miss Sophie Welch

**Medical intervention/ Prophylaxis:**
Standard for blood exposure.

c) **Describe the specific arrangements required to evacuate a patient in the event of a fire.**

**Immediate action:**
The patient is removed to a safe area within hospital and member of staff stays with him/her during emergency.

d) **Describe the actions to be taken in the event of death of the patient before the end of the treatment period.**

**Action:**
The patient will wear a wristband tag to indicate participation in the trial, with the contact details of the Principal Investigator (PI). A postmortem will be performed with tissue biopsies of the heart taken if prior consent obtained.

e) **Describe whether any specific procedures are required to be followed in the event of the patient requiring resuscitation following cardiac arrest or other acute medical emergency**

**Action required:**
The patient will wear a wristband tag to indicate participation in the trial, with the contact details of the Principal Investigator (PI). Normal clinical procedures for cardiac resuscitation should be followed.

f) **Describe the procedures to be followed if the patient suffers from post-operative infection. Would the patient require transfer to another location? Detail the potential for exposure to other personnel and the control measures in place to minimize this.**

**Action required:**
Aseptic procedures on the wards, and in clinical areas with higher levels of care e.g. AICU, should be sufficient to prevent exposure of personnel to blood or bodily fluids. The patient will wear a wristband tag to indicate participation in the trial, with the contact details of the PI.

9. **ACCOMMODATION**

9.1 **WHERE WILL THIS WORK TAKE PLACE?**

<table>
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<tr>
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<th>Building</th>
<th>Campus</th>
<th>Person in control of area</th>
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<td>Royal Brompton Hospital</td>
<td></td>
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10. **PERSONNEL**

10.1 **NAMES OF ALL OTHER PERSONNEL INVOLVED IN THE PROJECT**

<table>
<thead>
<tr>
<th>Surname</th>
<th>Initials</th>
<th>CID</th>
<th>Position (e.g. PG student, research associate etc)</th>
<th>Employer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyon</td>
<td>AR</td>
<td>424703</td>
<td>PI</td>
<td>Imperial College</td>
</tr>
<tr>
<td>Hayward</td>
<td>C</td>
<td>792150</td>
<td>Study coordinator</td>
<td>Imperial College</td>
</tr>
<tr>
<td>Sloane</td>
<td>G</td>
<td></td>
<td>BRU manager</td>
<td>RBHT</td>
</tr>
<tr>
<td>Di Mario</td>
<td>C</td>
<td>535261</td>
<td>Interventional Cardiologist</td>
<td>RBHT</td>
</tr>
<tr>
<td>De Silva</td>
<td>R</td>
<td></td>
<td>Interventional Cardiologist</td>
<td>Imperial College</td>
</tr>
<tr>
<td>Welch</td>
<td>S</td>
<td></td>
<td>Research Nurse</td>
<td>RBHT</td>
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10.2 **OTHER PEOPLE WHO MAY BE AT RISK FROM THE ACTIVITY**

*For example, other researchers, cleaners or maintenance workers*

<table>
<thead>
<tr>
<th>Details (including their names if known)</th>
<th>Employer</th>
<th>Involvement with this trial and exposure opportunity</th>
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<tr>
<td>Carol Rayne, Safety Manager, Q&amp;S Lead</td>
<td></td>
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10.3 **Who will be responsible for managing Health and Safety risks for non-College personnel involved in this clinical trial?**

Lead Occupational Health Nurse, Lilian Zoubiri

11. **DECLARATIONS**

☒ To be completed by the PI responsible for this project. By ticking this box I confirm that all information contained in this assessment meets the requirements of the College GM policy and is correct and up to date. Any changes to the project that alters the information supplied on the assessment will invalidate this assessment and approval granted by it. All such work must cease and changes notified to the Local GM/QT Safety Committee using GM Form C.

I also undertake to ensure that no work will be carried out until this assessment has been completed and approved and that all necessary control measures are in place. Also, I accept that a statutory notification period may be required before work can commence.

☒ I confirm that this information has been discussed with the Departmental/Divisional Safety Officer.
I confirm that the information detailed on this risk assessment form has been provided to the relevant person with responsibility for the clinical care of patients and also to the person with managerial responsibility for Trust staff involved in this clinical trial.

12. ELECTRONIC APPROVALS

Relevant approvals (as detailed below) MUST be in place BEFORE work commences

### Divisional/Departmental Safety Officer (as per local arrangements)

<table>
<thead>
<tr>
<th>Name:</th>
<th>Position:</th>
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<tr>
<td>Signature:</td>
<td>Date:</td>
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</tbody>
</table>

The signatures above must be obtained PRIOR to approval by the GT/GM Chair.

### GT/GM Chair approval

<table>
<thead>
<tr>
<th>Name:</th>
<th>GM Centre no:</th>
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<tbody>
<tr>
<td>Signature:</td>
<td>Date:</td>
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</tbody>
</table>

HSE notification of this work

The College GMSO will inform the PI if the project must be notified to the HSE. All notifications MUST be made by the College GMSO and MUST NOT be made by the individual Depts/Divs or Principal Investigators. If notification is required then form CU2 (available at [https://www.hse.gov.uk/forms/genetic/cu2.pdf](https://www.hse.gov.uk/forms/genetic/cu2.pdf)) must be completed and submitted to the College GMSO once all other approvals have been obtained.

To be completed by the College GMSO

<table>
<thead>
<tr>
<th>Is HSE notification required for any aspect of this project?</th>
<th>Yes ☐ No ☐ (Such notification must only be carried out by the Safety Dept)</th>
</tr>
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<tbody>
<tr>
<td>Date HSE consent was given:</td>
<td>HSE reference:</td>
</tr>
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CU2 Title (if different to the project title in Part 1)

**DATA PROTECTION**

The information provided on this form will be processed by the Safety Department in accordance with the College’s Data Protection Policy.

<table>
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<th>Review history</th>
<th>The PI responsible for this project must ensure that this risk assessment remains valid</th>
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<td>Review 2</td>
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<td>Due date</td>
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<td>Date conducted</td>
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<tr>
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Appendix D

Research and Development Approval for CUPID-2
Dear Dr Lyon,

Project Title: CUPID II - A Phase 2b, DoubleBlind, PlaceboControlled, Multinational, Multicenter, Randomised Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® AAV1/SERCA2a) in Subjects with Heart Failure
Project Reference: 2012CS006B

Thank you for registering your Research Project with the R&D office. The project details have been entered on our Research Management Database. Please ensure you keep the R&D office informed of the following:

- changes to the status of the project e.g. abandoned, completed etc
- changes to the funding arrangements
- changes to the original application e.g. change in personnel or amendments requiring ethical review

RESEARCH GOVERNANCE
Royal Brompton & Harefield NHS Foundation Trust manages all research in accordance with the requirements of the research governance framework. Whilst working as an employee of the Royal Brompton and Harefield NHS Foundation Trust, or holding an Honorary Contract to do research which involves NHS staff or patients, their organs tissue or data, you must comply with all reporting requirements, systems, and duties of action put in place by the Trust to deliver research governance. As such if you are acting as either Chief/Principal Investigator your responsibilities under this framework include:

- ensuring compliance with protocol and advising of any changes to the protocol
- reporting any adverse events whether related to research or not to clinical governance/ethics/R&D
- taking appropriate urgent safety measures
- ensuring adherence to the principles of ICH GCP
- ensuring researchers have necessary expertise
- ensuring compliance with the Data Protection Act
- ensure adequate monitoring arrangements are in place
- ensure compliance with the Human Tissue Act

The Trust routinely audits a minimum of 10% of its research activity. This is to ensure that research is progressing satisfactorily and to guard against research fraud. You are requested to maintain and retain appropriate records of your research, and assist the Trust as and when required should any such audit take place in your area.

CLINICAL TRIALS REGISTRATION
The majority of research journals will now only publish research that has been registered at a publicly accessible database before the enrolment of the first patient. Where research projects are sponsored by either the Trust or Imperial College it is recommended that the project is registered at www.ClinicalTrials.gov which is free of charge. For Trust sponsored projects please contact 020 7351 8575 and an account will be set-up for you to register your project. Similarly, please contact clinical.researchoffice@imperial.ac.uk for an account to register Imperial College sponsored projects.

In receiving this letter you are agreeing to abide by the terms as outlined above. Please accept this letter as the Trust’s authorisation to commence your research.

Yours sincerely,

[Signature]
Dr Angela Cooper
Associate Director of Research
Appendix E

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Expected completion date Oct 2016
Estimated size (number of pages) 300
Elsevier VAT number GB 494 6272 12
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