Protocol

Written to comply with all aspects of ICH Chapter 6 – Guideline for Good Clinical Practice (CPMP/ICH/135/95)

Version : 4

Date : Tuesday, 09 September 2014

Study Title: **Isoprenaline Infusion as a method of Induction of Atrial Fibrillation; A randomised controlled trial investigating the use of Isoprenaline to induce an episode of atrial fibrillation**

Short Title: Isoprenaline Infusion as a method of Induction of Atrial Fibrillation (Iso AF) Study

Trial Registration Numbers

- Local R&D Reference : ISO1
- EU Clinical Trials Register (EudraCT) : 2014-002290-11
- ClinicalTrails.gov Reference
- Local NRES REC Reference : 14/SC/1171

Sponsor

The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust will act as the Sponsor for this study

Sponsor's Authorised Representative

Caroline Jamieson-Leadbitter

Acting Head of Research for Royal Bournemouth Hospital
RM&G Office
5th Floor,
Royal London House
Christchurch Road
Bournemouth
BH1 3LT
Tel: 01202 962376

Investigatory Team

Chief Investigator

Dr John R Paisey DM BM FRCP

Consultant Cardiologist and Electrophysiologist
Dorset Heart Centre
The Royal Bournemouth Hospital
Castle Lane East
Bournemouth
BH7 7DW

Honorary Senior Lecturer
University of Southampton

Coordinating Investigator

Dr Robert W Bowers MBBS BSc MRCP

Research Fellow in Cardiac Electrophysiology
Dorset Heart Centre
The Royal Bournemouth Hospital
Castle Lane East
Bournemouth
BH7 7DW

Co-Investigators

Dr S Mark Sopher MD MBBS MRCP

Consultant Cardiologist and Electrophysiologist
Dorset Heart Centre
The Royal Bournemouth Hospital
Castle Lane East
Bournemouth
BH7 7DW

Dr Richard N Balasubramaniam PhD MBChB MRCP

Consultant Cardiologist and Electrophysiologist
Dorset Heart Centre
The Royal Bournemouth Hospital
Castle Lane East
Bournemouth
BH7 7DW

Dr Tim R Betts MD MBChB FRCP

Consultant Cardiologist and Electrophysiologist
John Radcliffe Hospital
Oxford University Hospitals NHS Trust
Headley Way
Oxford
OX3 9 DU

Study Sites

The Royal Bournemouth Hospital
Castle Lane East
Bournemouth
BH7 7DW
01202 303 626

John Radcliffe Hospital
Oxford University Hospitals NHS Trust
Headley Way
Oxford
OX3 9 DU
01865 741166

Investigational Medicinal Product (IMP) Supply

IMP Manufacturer

South Devon Healthcare NHS Foundation Trust
Licence Holder MIA(IMP) 13079

Head Office:

Hengrave House
Torbay Hospital
Torquay
United Kingdom
TQ2 7AA

Pharmaceutical Manufacturing Contact Details:

Torbay Pharmaceuticals
South Devon Healthcare NHS Foundation Trust
Unit 7 Torbay Business Park
Long Road
Paignton
Devon
TQ4 7HP

Email	torbaypharmaceuticals@nhs.net
Telephone	01803 664707
Fax	01803 664354

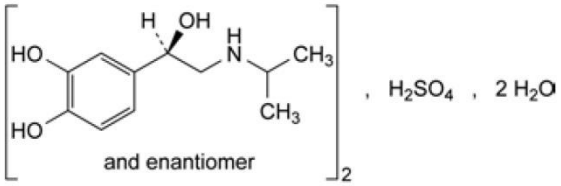
Manufacturing Site:

Pharmaceutical Manufacturing Unit
Kemmings Close
Paignton
Devon
United Kingdom
TQ4 7TW

Investigational Medicinal Product (IMP)

Isoprenaline Sulphate

Isoprenaline is a synthetic, sympathomimetic amine that is structurally related to epinephrine but acts almost exclusively on beta-adrenergic receptors. It is a short acting, non-selective beta agonist with very low affinity for alpha adrenoceptors. The drug is produced in two forms, isoprenaline sulphate and isoprenaline hydrochloride. The hydrochloride form is more potent with 1mg of the hydrochloride equivalent to 1.125mg of the sulphate(1). The chemical formula for Isoprenaline Sulphate is $(C_{11}H_{17}NO_3)_2,H_2SO_4$. It has a relative molecular mass of 556.6. Synonyms include Isoproterenol and Bis[(1RS)·1·(3,4·dihydroxyphenyl)·2·[(1·methylethyl)·amino]ethanol] sulphate dehydrate. Its structure, in common with other catecholamines, is that of a benzene ring with hydroxyl group substituted at positions 3 and 4, and an ethalyamine side chain.



It is believed that β-adrenergic effects result from stimulation of the production of cyclic adenosine-3′,5′-monophosphate (AMP) by activation of the enzyme adenyl cyclase. The main effects of therapeutic doses of isoproterenol are relaxation of smooth muscle of the bronchial tree, cardiac stimulation, and peripheral vasodilation.

Isoprenaline induced peripheral vasodilation leads to a fall in systemic vascular resistance and a drop in diastolic blood pressure. Mean arterial pressure typically drops although systolic pressure is supported by myocardial inotropic and chronotropic responses facilitating an increased cardiac output. Myocardial Oxygen demand rises which may lead to ischaemia if in conjunction with obstructive coronary disease.

Cardiac effects can include sinus tachycardia, palpitations and arrhythmia. Flushing sensations are common, side effects can also include headaches. Isoprenaline is not known to cause QT prolongation.

The pharmacologic actions of isoprenaline appear to be terminated principally by tissue uptake. The drug is metabolized by conjugation in the GI tract and by the enzyme catechol-*O*-methyltransferase in the liver, lungs, and other tissue. The major metabolite after IV administration is 3-*O*-methylisoproterenol (which has been reported to have weak β-adrenergic blocking activity) and its conjugates. The duration of action is brief(2).

In America, Isoprenaline (in the hydrochloride form) has been licenced under the trade name Isuprel™ by the Food and Drug Administration (the FDA) for use since 1956. Licensed routes of administration of Isuprel™ include central or peripheral intravenous administration as an infusion or bolus injection, intramuscular, subcutaneous or intracardiac routes. As an infusion, recommended

dosing ranges are between 0.5mcg/min to 30mcg/min. The drug should be stored at between 20 and 25°C(3).

Licensed indications include;

- For mild or transient episodes of heart block that do not require electric shock or pacemaker therapy.
- For serious episodes of heart block and Adams-Stokes attacks (except when caused by ventricular tachycardia or fibrillation)
- For use in cardiac arrest until electric shock or pacemaker therapy, the treatments of choice, are available.
- For bronchospasm occurring during anaesthesia.
- As an adjunct to fluid and electrolyte replacement therapy and the use of other drugs and procedures in the treatment of hypovolemic and septic shock, low cardiac output (hypoperfusion) states, congestive heart failure and cardiogenic shock.

Although unlicensed in the UK, Isoprenaline is extensively used. Its indications in modern practice include its use for patients presenting to hospital with abnormal, slow heart rhythms and as an infusion to provide physiological stress to the heart for diagnostic scans. Most commonly, Isoprenaline is used in the cardiac electrophysiology laboratory as part of routine clinical electrophysiology studies to diagnose abnormal heart rhythms with a view to abolishing them using an ablation technique. It is used in this setting numerous times every day in every centre in the UK with specialist electrophysiology services. It is considered a standard part of the procedure and is used in combination with programmed electronic stimulation to induce arrhythmias that they might be categorised and treated(4).

Background

Atrial Fibrillation

Atrial fibrillation is an abnormal heart rhythm where electrical activation of top chambers of the heart (the atria) is chaotic and occurring essentially constantly(5). This leads to deterioration of atrial mechanical function. Atrial fibrillation is very different to normal, sinus rhythm where electrical excitation happens in a specific and highly ordered pattern and results in effective contraction of the chambers. Episodes of atrial fibrillation can come and go (paroxysmal atrial fibrillation) or the abnormal rhythm can persist (persistent atrial fibrillation).

Atrial fibrillation is the most common cardiac arrhythmia in humans affecting almost 1.5% of people living in the UK(6) and over 33 million people worldwide(7). It is a significant cause of cardiovascular morbidity and mortality and is estimated to have cost the UK health economy £1,873 million in 2008 alone(6). In particular, the presence of atrial fibrillation confers a 5 fold increase in the risk of stroke for the sufferer, with strokes in patients in whom atrial fibrillation has been previously diagnosed

shown to be more debilitating than in those without that co-morbidity. The NHS stroke improvement programme estimates that up to 4,500 strokes and 3,000 deaths could be prevented per year through improvement in the risk assessment of patients with atrial fibrillation.

Treatment of atrial fibrillation for symptomatic individuals centres around restoration and maintenance of sinus rhythm(5). This is achieved using antiarrhythmic drug therapy or by modifying the electrical properties of the heart with a catheter ablation procedure. There remains a significant group of patients refractory to all currently available therapies.

Given the difficulty of finding effective therapy for this condition, attention has naturally fallen on the pathophysiological mechanisms involved in the initiation and propagation of atrial fibrillation and on the substrate in which it occurs. One avenue for this investigation has studied the effect of pharmacological manipulation of the heart using the drug isoprenaline.

Inducing Atrial Fibrillation and Atrial Fibrillation Ablation

Electrical isolation of the pulmonary veins has become the cornerstone of current ablation strategy for atrial fibrillation(8). Despite this consensus, evidence demonstrates that as a single procedure therapy, the technique is not sufficiently effective(9). In groups where ablation is used as treatment for persistent atrial fibrillation, the results are particularly disappointing.

This need for improvement in procedural techniques has led to a number of different approaches to guide additional ablation lesion sets. One such approach has developed from the observation that the ability to induce an iatrogenic episode of atrial fibrillation following ablation predicts the outcome of that procedure(10)(11)(12). Thus developed a technique of continuing to perform ablation until the patient is rendered sufficiently resistant to atrial fibrillation that the operator can no longer induce the abnormal rhythm(13). Results reported after using this technique have varied with some demonstrating benefit and others not(14)(15).

One explanation for the disparity in results from different investigators is the wide heterogeneity in the stimulation protocols delivered, the sites of stimulation, the use of concurrent pharmacological stimulation and indeed the definition of induced atrial fibrillation dependent on its duration. Perhaps more importantly though, it seems the variation in outcomes reported by those utilising this approach may be derived from the lack of understanding of the pathophysiological meaning of being able to induce atrial fibrillation. One study has attempted to characterise myocardial substrate associated with inducibility and has demonstrated correlation between higher bi-atrial voltage and negative inducibility in those who have undergone pulmonary vein isolation(16). The evidence however, clearly shows that atrial fibrillation can be induced in those where it does not occur spontaneously(17). Using repeated, aggressive stimulation protocols, investigating normal heart subjects with no prior history of atrial fibrillation Kumar and colleagues demonstrated an ability to induce an iatrogenic episode of atrial fibrillation in almost 50%(18). This bore no relevance to the occurrence of clinical atrial fibrillation during follow-up. A firm understanding of what interpretation can be made from atrial fibrillation inducibility is yet to be elucidated.

The seminal work in the pathogenesis and ablation of atrial fibrillation demonstrated that focal triggers from the pulmonary veins could initiate an episode of the rhythm disturbance(19). Further

work demonstrated that ectopic foci elsewhere in the atria could also initiate atrial fibrillation(20). Convincingly ablation of these ectopic foci appeared to effectively treat atrial fibrillation(21)(22)(23).

The electrophysiological effects of beta adrenoceptor agonists such as isoprenaline include increased automaticity and an increase in both early and delayed after-depolarisations(24). These properties have meant that clinicians have used an infusion isoprenaline to increase the likelihood of focal ectopic activity during ablation procedures for organised atrial tachycardia(25) as well as atrial fibrillation(23)(26).

Wide area circumferential pulmonary vein isolation has largely superseded targeting of pulmonary vein triggers(27)(28). There is still interest in ablation of non-pulmonary vein triggers to retain some direct electrophysiological basis for atrial fibrillation ablation. Non-pulmonary vein triggers are identified, mapped and then ablated, facilitated by using an isoprenaline infusion to increase the frequency of ectopy.

The potential to stimulate increased atrial ectopic activity, coupled with its ability to reduce action potential duration, shorten the atrial effective refractory period and increase conduction velocity(29) has widened the utility of isoprenaline still further. These characteristics have prompted investigators to use the drug as a method of iatrogenic induction of an episode of atrial fibrillation(30).

Groups have subsequently experimented with adding non-inducibility of atrial fibrillation by isoprenaline infusion to their ablation technique. This has been used in addition to non inducibility by atrial pacing(31), as a separate endpoint focussing on non-pulmonary vein triggers prior to pacing inducibility testing(15) or in place of programmed stimulation(32) with results that, in general, show modest benefit from the technique.

Isoprenaline Infusion as a Method of Inducing Atrial Fibrillation

From this and other published literature, the use of an isoprenaline infusion to induce an episode of atrial fibrillation has become part of clinical practice. However, the question of whether an isoprenaline infusion can induce an episode of atrial fibrillation has not yet been answered by rigorous scientific evaluation. This is especially important when it is considered that the technique is being used to guide additional radiofrequency ablation which may result in life threatening complications.

The pathophysiological mechanism by which isoprenaline can induce atrial fibrillation in humans is not yet elucidated. It is known that some arrhythmogenic foci are derived from remnant embryonic sinus venosus tissue and might be directly catecholamine sensitive in the same way as sinoatrial nodal tissue(32). Specifically within the pulmonary veins, the increase in heart rate and shortening of action potential duration caused by isoprenaline may combine to cause an increase in intracellular calcium and so phase 3 early after-depolarisations(24) promoting additional ectopy. Accumulation of intracellular calcium can also lead to spontaneous release from the sarcoplasmic reticulum, activating calcium sensitive membrane ionic currents and causing delayed after depolarisations and triggered activity(33). The direct relevance of this to atrial rather than ventricular myocytes is less clear however.

All of these potential mechanisms demonstrated in animal models promote firing from ectopic foci as a method of atrial fibrillation induction. However, there is clear evidence in humans that, although linked to long term risk(34), ectopy alone is insufficient to induce atrial fibrillation(35) and removal of ectopy is insufficient to abolish atrial fibrillation(36). Further, in the study by Elayi et al, the group in whom atrial ectopy but not atrial fibrillation occurred during isoprenaline infusion demonstrated a higher rate of recurrence during follow-up(32). Potentially suggesting the ability to induce atrial fibrillation with isoprenaline might not have direct relevance to clinical susceptibility to the arrhythmia.

Animal studies directly investigating isoprenaline as a method of induction of atrial fibrillation also suggest that it may not be as efficacious as its widespread use might suggest. There is strong evidence that in the animal model at least, an interaction between the two arms of the autonomic nervous system precedes atrial fibrillation occurrence in spontaneous episodes of the arrhythmia(37), that triggered firing within canine pulmonary veins requires mixed autonomic stimulation(38) and that the ability of an infusion of isoprenaline to induce atrial fibrillation is abolished with abolition of parasympathetic nervous system influence but not vice versa(39). The interplay between the opposing actions of the autonomic innervation to the myocardium seems to have a far stronger association with atrial fibrillation than sympathetic stimulation alone.

The idea that a mixed autonomic response rather than pure sympathetic stimulation might be the pathophysiological basis for spontaneous atrial fibrillation in the human heart is supported by analysis of heart rate in the time preceding paroxysms(40). Further, in a population where paroxysmal atrial fibrillation has been demonstrated to be dependent on pulmonary vein ectopy, holter electrocardiographic analysis suggests a mixed autonomic state and shift to vagal predominance precedes episodes of sustained arrhythmia(41). In addition, stimulation of human cardiac ganglionic plexus results in a vagal response and can initiate atrial fibrillation(42). Cardiac ganglionic plexi have also been demonstrated to be a therapeutic target for atrial fibrillation ablation with successful results on subsequent arrhythmia burden(43).

Even proponents of the use of isoprenaline as a method of induction of atrial fibrillation recognise that this interplay might be important. A potential hypothesis for the ability of a pure sympathomimetic to induce atrial fibrillation is that the neurological and peripheral vascular effects of the drug lead to a vagal response which can trigger atrial fibrillation. This may be an explanation for the low rates of atrial fibrillation induction seen without protracted infusions of high dose isoprenaline(32).

Studies looking specifically at the ability of isoprenaline to induce atrial fibrillation in humans are few in number. The most robust is a study by the group from the University of Michigan published in 2008(30). They administered an isoprenaline infusion prior to ablation in 80 patients with a past history of paroxysmal atrial fibrillation. The infusion protocol lasted for 18 minutes including a washout phase and was delivered with the patients under conscious sedation. The authors reported that atrial fibrillation was induced in 84% of participants. The authors also reported on outcomes in a control group who had undergone an ablation for a regular supraventricular tachycardia (SVT). These participants were used as normal heart controls despite evidence which has previously

demonstrated that following an ablation for SVT, they may be particularly resistant to atrial fibrillation(44). From 20 participants, 1 experienced an episode of atrial fibrillation during the infusion protocol. The main limitation of this study is the fact that no control investigation was used, allowing a major confounder come into play; what would have happened if the participants had simply been observed for 18 minutes prior to their ablation? There is no information given on the frequency of clinical episodes of atrial fibrillation in the patients studied. We know that they had a long history of paroxysmal atrial fibrillation (7±6 years) without progression to persistent arrhythmia, but the frequency of paroxysms is not reported. We do not know what proportion of episodes of atrial fibrillation documented in the study as precipitated by isoprenaline would have occurred spontaneously. This concern is supported by results from a group who were deliberately looking for spontaneous episodes of atrial fibrillation. In their study of patients undergoing ablation under general anaesthesia, 27% (23 of 85 participants) had a spontaneous episode of atrial fibrillation(45). Interestingly, in only 7 of those 23 could atrial fibrillation be induced using iatrogenic means. The mechanism for an excess of episodes of spontaneous atrial fibrillation around the time of an ablation is easily hypothesised. Participants in the study were sedated but conscious and about to undergo a procedure that for many would have held significant trepidation. The autonomic interplay occurring at such times is exactly the type that has been demonstrated to be associated with initiation of paroxysms of fibrillation.

The same cofounding influence of the patient's own autonomic nervous system and the random occurrence of an episode of atrial fibrillation can be applied to many of the studies using isoprenaline induction. Early studies on atrial fibrillation initiation that used isoprenaline within their protocols investigated patient pools which were deliberately enriched with participants suffering from frequent episodes of arrhythmia (6±4 episodes per day(23)) often despite medication (had to have failed more than 2 drugs and still have an episode at least every 48 hours(19)).

Justification for Research

The use of an isoprenaline infusion to induce an episode of atrial fibrillation has become part of clinical practice without the rigorous scientific evaluation usually considered necessary for adoption.

Animal studies have demonstrated a number of mechanisms by which this drug might precipitate atrial fibrillation if the substrate for that arrhythmia is present. However, there is good evidence to suggest that a sympathomimetic alone might not be sufficient to induce atrial fibrillation. Proposed instead is that chance fluctuation in vagal tone during administration of the drug creates the autonomic milieu required.

Although numerous studies have utilised an isoprenaline infusion in the context of ablation of atrial fibrillation seemingly to good effect, without a placebo control it is difficult to interpret the relative effect in a setting where the patient's own autonomic nervous system is likely to be stimulated. It is also impossible to build an understanding of the meaning of inducibility by isoprenaline without first definitively establishing the effect.

Administration of an isoprenaline infusion is safe, however the additional ablation that it is being used to guide carries risk including of life threatening perforation. It is imperative that there is a clear understanding of the magnitude of effect of an isoprenaline infusion in this context so that clinicians can use this potentially beneficial ablation protocol with confidence whilst keeping patient safety paramount.

Our research proposal will provide an answer to the question of “Can an infusion of isoprenaline be used to induce an episode of atrial fibrillation?” We will answer this question using a double blind, placebo controlled trial and will answer it for subjects at very high risk for an episode of atrial fibrillation, those at intermediate risk and in those with a low risk separately.

The second important question this research will answer; “Is the response to an isoprenaline infusion consistent in the same person on different occasions?” This has huge importance if isoprenaline inducibility is to be used to guide therapy. To answer this question our protocol uses open label administration of an isoprenaline infusion to subjects on two separate occasions and compares the results. This will be performed for a group with a history of previous paroxysmal atrial fibrillation.

The protocol for this study will answer a number of subsidiary questions to further improve the scientific quality of the evidence regarding this use for Isoprenaline. We will collect observational data as part of this protocol which will directly inform the design of planned future studies in this area.

Future Application for Findings and Further Investigation

This project represents a foundation to future studies investigating the clinical utility of an isoprenaline infusion. If, backed by the sound, scientific evidence that our project will deliver, isoprenaline infusion is validated as able to induce atrial fibrillation, we intend to investigate its potential ability to identify patients with substrate for atrial fibrillation, especially in subjects where that arrhythmia has not before been seen clinically.

The group where this use is potentially most interesting is in assessment of those attending for common type atrial flutter ablation in whom atrial fibrillation had not before been documented. Meta analysis[3] suggests as many as 25% of these patients will go on to get atrial fibrillation in the 12 months after their ablation. We propose that non-inducibility of atrial fibrillation by high dose isoprenaline infusion following a cavotricuspid isthmus ablation (as treatment for atrial flutter) might predict a low risk group for clinical atrial fibrillation. This might then be able to be used to inform anticoagulation or further treatment.

We plan to secure national level funding for a 350 patient, cohort study with the aim of commencing recruitment in 2015/6. If this technique allows us to characterise a very high or very low risk population, it could quickly be applied to clinical decision making on issues such as preventative medication, anticoagulation and treatment timing for atrial fibrillation, potentially reducing stroke risk in a substantial cohort of patients. We feel this offers a clear route to future patient benefit.

In addition to this direct clinical application of the results of this investigation, a more mechanistic line of future study is also apparent. Once the ability of this sympathomimetic to induce atrial fibrillation is accurately and robustly characterised, its effects in conjunction with other pharmacological agents can also be characterised. One example of such an experiment would be to characterise the ability of isoprenaline infusion to induce atrial fibrillation in patients who had been pre-treated with atropine to abolish any involvement from the parasympathetic nervous system. Such data would improve our understanding of the exact role of the branches of the autonomic nervous system in initiation of atrial fibrillation.

Potential Risks and Benefits for Study Subjects

There are a number of specific potential risks to study subjects which have been considered. They relate to the administration of isoprenaline in specific populations, to the consequences of inducing an episode of atrial fibrillation and to the methods of restoration of sinus rhythm if required.

Administration of Isoprenaline

The effects of isoprenaline in participants will include palpitations and in some cases feelings of light-headedness, anxiety or headaches. These are detailed as part of the consent process in the participant information sheet along with some accounts of patient experiences of being given isoprenaline. Isoprenaline administration at the dose given in this study protocol is safe and well tolerated(46). Expected potential side effects of the drug include nausea, non-specific discomfort and chest discomfort. These are uncommon and will cease within moments of the drug infusion being stopped.

The licensed product literature for Isuprel™ (Isoprenaline) lists administration in patients with tachyarrhythmias as a contraindication to use. This advice is applicable within the remit of the licensed uses for the drug and the expected environment of administration for those uses but is not directly relevant to our protocol. Our project administers isoprenaline to participants, some known to have a history of tachyarrhythmia, with the desired aim of induction of tachyarrhythmia in an environment where it is safe to do so. As described already, this is a common clinical use for the drug, albeit outside the licensed indications. There is an excellent safety profile demonstrated for use in this way and a large body of clinical experience which justifies the application in this study. Most importantly, if sustained tachyarrhythmia occurs during administration of isoprenaline, this protocol dictates the drug infusion be immediately discontinued. Coupled with the short duration of action of the drug, this means that isoprenaline will not be delivered during tachyarrhythmia and so adheres to the advice of the published product literature.

As discussed previously, one of the actions of isoprenaline is to increase heart rate and improve myocardial contractility with consequent increase in myocardial oxygen demand. Where there is coexistent obstructive coronary artery disease, this increase might lead to myocardial ischaemia with consequent angina pain for the subject and possible increase in the risk of myocardial infarction. There is published literature demonstrating the safety of using isoprenaline in the setting of obstructive coronary disease from its use in functional myocardial imaging(47)(48) and further evidence from a similar catecholamine; dobutamine with aggressive dosing regimens in the same setting(49). To safeguard participant safety, subjects with known obstructive coronary disease or active symptoms suggestive of angina will be excluded from enrolment. Given the available safety data, there is no requirement to rule out any other, undiagnosed obstructive coronary disease nor is there need to exclude subjects with non-obstructive disease.

Although the actions of isoprenaline mean that its administration increases cardiac output, there is concern regarding increased myocardial strain in subjects with severe impairment of left ventricular function, active heart failure syndrome or severe valvulopathy. In addition there is concern regarding the potential to induce ventricular arrhythmias in high risk groups such as those know to have hypertrophic cardiomyopathy. All of these groups of participants have been excluded from enrolment with this study.

Induced episodes of atrial fibrillation

The goal of this study is to use a drug to precipitate an episode of the abnormal heart rhythm atrial fibrillation. In some cases the subjects have a known diagnosis of the arrhythmia, in some they will have never experienced the rhythm disturbance. Specific risks to be addressed from inducing an episode of atrial fibrillation include the potential to render that person more susceptible to atrial fibrillation in the future, the risks posed by being in the arrhythmia and any risk from iatrogenic restoration of sinus rhythm, most dramatically being the need for direct current cardioversion. In these participant groups, given the setting and duration of the episodes of atrial fibrillation being induced, we believe that we will be able to terminate atrial fibrillation and restore sinus rhythm if required in all subjects. We do not believe there is a risk of inducing a state of permanent atrial fibrillation.

The study by Kumar et al offers excellent evidence to dispel the concern that an episode of induced atrial fibrillation can increase the future susceptibility to clinical atrial fibrillation. Using aggressive pacing protocols this group were able to induce atrial fibrillation lasting greater than 1 minute in 49.5% of subjects none of whom had a past history of atrial fibrillation or were considered to have underlying structural heart abnormality. There was no demonstrated tendency to clinical atrial fibrillation in the study subjects during a mean follow up period of 28±22 months (median 23 months)(18). Despite this direct evidence, the concept of atrial fibrillation begets atrial fibrillation is well known in the setting of the clinical disease. Reassuringly, a pervious study by Daoud and colleagues demonstrated that the changes in atrial refractoriness that are thought to be the electrophysiological substrate for this concept are transient and are dependent on the length of the episode of atrial fibrillation(50). They demonstrated that for short episodes of atrial fibrillation, atrial refractoriness returns to normal within a few minutes. These studies allay concern regarding those considered without substrate for atrial fibrillation and can be extrapolated to include those who have been previously susceptible to the arrhythmia. The short duration of action of isoprenaline is further reassurance that an episode of atrial fibrillation induced as part of this study will not affect the future clinical course for that subject.

For participants where atrial fibrillation is managed using antiarrhythmic therapy, cessation of these medications as part of the study may cause a deterioration in clinical and symptomatic status of the participant, however this is clearly signposted on the patient information sheet and considered part of the informed consent process. There is no reason to believe that reinstatement of the medication will not return the participant to their previous level of symptom control after involvement in the study.

It is well documented that atrial fibrillation confers an increase in an individual's risk of stroke. This risk is based on a number of variables and importantly is measured over a number of years of suffering with the diagnosis. Short duration atrial fibrillation does not confer this risk. International guidance regarding management of clinical atrial fibrillation suggests that it takes more than 24 hours for the formation of intra-cardiac thrombus which is the mechanism for stoke risk(5). In individuals where atrial fibrillation is induced and present for only a short duration (more than 15 minutes but less than 20 minutes), left atrial mechanical dysfunction resolves immediately after return to sinus rhythm even in a very high risk population(51). This removes the concern that there may be ongoing increase risk even after restoration of sinus rhythm.

The study by Sparks et al also addresses the concern that the use of direct current cardioversion may induce atrial “stunning” and so a risk of stroke in the time directly after. This is not the case with short duration atrial fibrillation as mechanical function immediately returns to baseline in both those returning to sinus rhythm spontaneously and those returned by DC cardioversion(51). Immediate DC Cardioversion of arrhythmia within the context of the cardiac electrophysiology laboratory is known to be safe and effective(52).

Where a participant is not already on anticoagulant therapy and has had short duration atrial fibrillation induced, there is no need for anticoagulation regardless of the mechanism of their return to sinus rhythm or background stroke risk.

Cessation of antiarrhythmic medications

We will be asking participants to stop any medications that control the rhythm of the heart for 6 half lives before each study infusion administered in Part A of the protocol. During these few days participants may experience a worsening of any palpitation symptoms. We do not believe this short cessation of medication confers any long term threat to the participants. Recruits are told of the risk and are advised that they “should consider this point carefully before agreeing to take part”. Although symptoms may worsen for those few days, we would expect things to return to normal once the tablets are restarted after the study visit.

Benefits of Involvement in Study

It is not considered that there will be any direct benefit to the study participants from the administration of Isoprenaline in this context. Benefit from involvement in the study includes increased access to healthcare professionals for participants and additional ambulatory electrocardiographic monitoring available to a subject’s usual physicians which may contribute to decision making regarding further treatment and the need for ongoing anticoagulation.

Study Objectives

This study protocol has two parts. Both parts consist of a series of experiments studying the effects of an infusion of the drug isoprenaline with particular reference to its ability to induce the abnormal heart rhythm atrial fibrillation. Part A investigates the consistency of effect of an isoprenaline infusion, Part B is a placebo controlled investigation of the ability of isoprenaline to induce atrial fibrillation.

Included in this section is a sample size calculation relating to each individual experiment.

Protocol Part A

Experiment 1

- Does an infusion of the drug Isoprenaline consistently induce or fail to induce atrial fibrillation in a patient with a diagnosis of paroxysmal atrial fibrillation?
    - Null hypothesis under investigation is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is not less than 20%.
    - Alternative hypothesis is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is less than 20%.
    - Open label. Paired Statistics. Event rate >50%. Clinically significant difference in repeatability of test decided to be >20%
    - A 95%CI of +/- 10% around an expected % disagreement of 10% would require 35 participants

Protocol Part B

Experiment 2

- Does an infusion of the drug Isoprenaline induce atrial fibrillation in a patient known to have had previous episodes spontaneous atrial fibrillation?
    - Null hypothesis under investigation is that there is no significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.
    - Alternative hypothesis is that there is significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.

- Double blinded. Event rate in Isoprenaline group likely >30%. Event rate in placebo group likely <5%. For 90% power and 95% significance projected to need 31 participants per group.
- For higher event rates, fewer participants required. 15 participants per group for event rate >50%, 6 participants per group for event rate >80%.
- Aim to recruit 30 participants per group.

Experiment 3

- Does an infusion of the drug Isoprenaline induce Atrial Fibrillation in subjects following an ablation procedure for common type atrial flutter?
  - Null hypothesis under investigation is that there is no significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.
  - Alternative hypothesis is that there is significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.
  - Double blinded. Event rate in Isoprenaline group likely >30%. Event rate in placebo group likely <5%. For 90% power and 95% significance projected to need 31 participants per group.
  - Aim to recruit 35 participants per group.

Experiment 4

- Does an infusion of the drug Isoprenaline induce Atrial Fibrillation in subjects following an ablation procedure for either Atrio-Ventricular Re-entrant Tachycardia (AVRT) or Atrio-Ventricular Nodal Re-entrant Tachycardia (AVNRT)?
  - Null hypothesis under investigation is that there is no significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.
  - Alternative hypothesis is that there is significant difference in the proportion of subjects who experience the occurrence of an episode of atrial fibrillation during the infusion protocol when comparing those who receive the isoprenaline infusion with those who receive the placebo infusion.
  - Double blinded. Event rate in Isoprenaline group likely <10%. Event rate in placebo group likely <5%. For 90% power and 95% significance projected to need 133 participants per group.
  - Descriptive data only, not feasible to power properly for this endpoint but may be reasonable to use a 1 sided test. Aim to recruit 35 participants per group.

Experiment 5

- Does a Cavo-tricuspid isthmus ablation for atrial flutter alter inducibility of atrial fibrillation using an isoprenaline infusion?
    - Null hypothesis under investigation is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is not less than 20%. Where one administration is before and one is after an atrial flutter ablation procedure.
    - Alternative hypothesis is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is less than 20%. Where one administration is before and one is after an atrial flutter ablation procedure.
    - Double blinded, placebo controlled. Event rate >30%
    - A 95%CI of +/- 10% around an expected % disagreement of 10% would require 35 participants

Experiment 6

- Does an ablation procedure for a AVRT or AVNRT alter inducibility of atrial fibrillation using an isoprenaline infusion?
    - Null hypothesis under investigation is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is not less than 20%. Where one administration is before and one is after an SVT ablation procedure.
    - Alternative hypothesis is that the proportion of subjects in whom there is a change in the occurrence of atrial fibrillation between two administrations of an infusion of isoprenaline is less than 20%. Where one administration is before and one is after an SVT ablation procedure.
    - Double blinded, placebo controlled. Event rate <10%
    - A 95%CI of +/- 10% around an expected % disagreement of 10% would require 35 participants

This protocol will also collect data regarding the inducibility of atrial fibrillation by an isoprenaline infusion in participants prior to atrial flutter and SVT ablation and after a pulmonary vein isolation procedure. In the pulmonary vein isolation group this will allow analysis of the change in inducibility associated with that procedure. These data will be reported as secondary, hypothesis generating experimental analyses and hence are not associated with detailed power calculations as in the defined analyses above.

Study Design

This project investigates different aspects of the use of isoprenaline to induce an occurrence of atrial fibrillation through a number of different experiments. Data from any one participant may be used in a number of the experimental analyses, however participation in the study will follow one of 2 schedules for any given subject. As this is the case the project has been presented in two parts, with participants in Part A sharing a similar experience which will be different to those participating in Part B and vice versa.

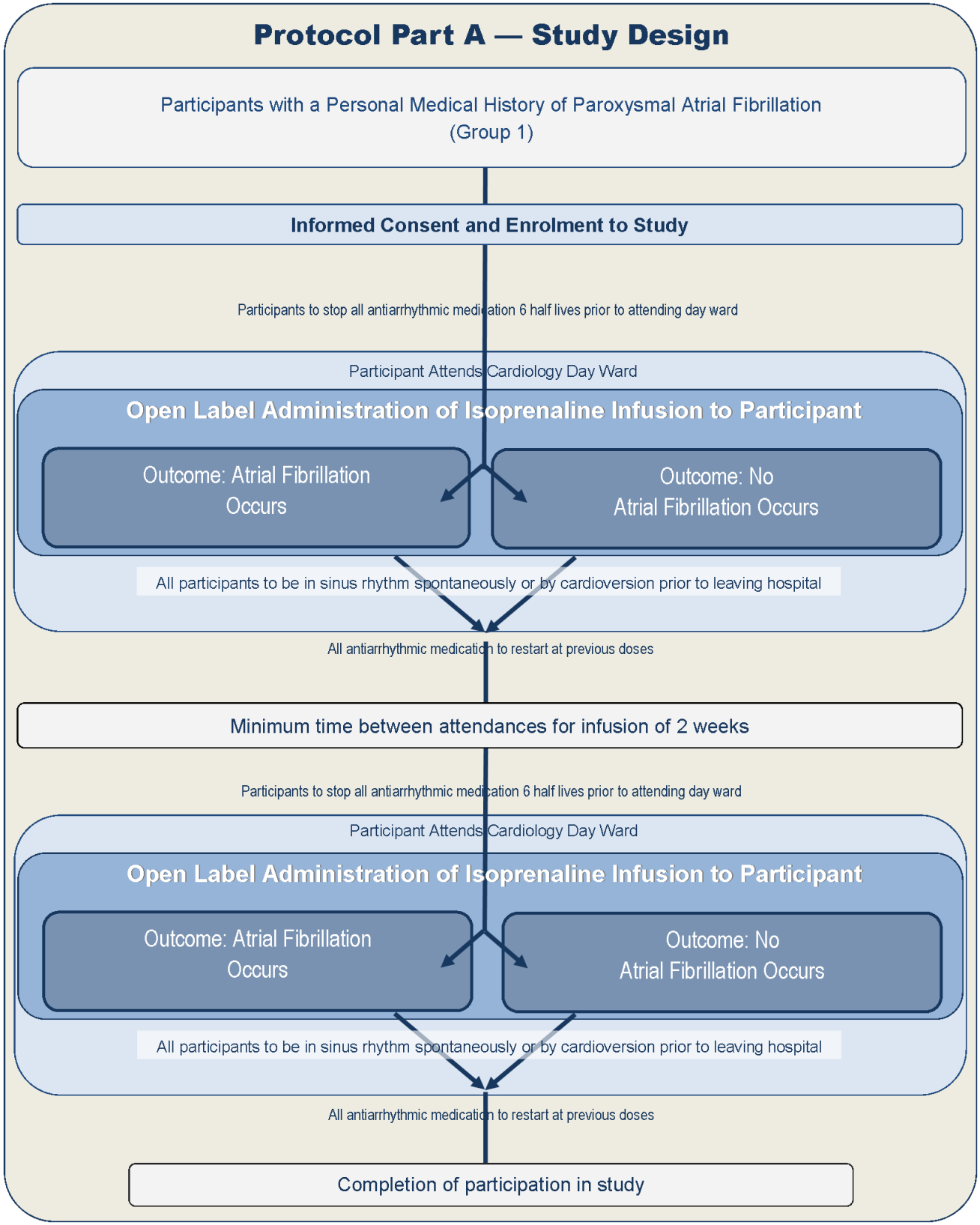
Of note, where eligible, subjects will be offered the opportunity to participate in more than one part of the study. For example, a participant with a diagnosis of paroxysmal atrial fibrillation could take part in Protocol Part A and then go forward for an ablation procedure, associated with which they could take part in Protocol Part B. To avoid possible contamination of results, this will not be allowed where it will mean that study drug infusions for different parts of the study will occur closer than 2 weeks apart.

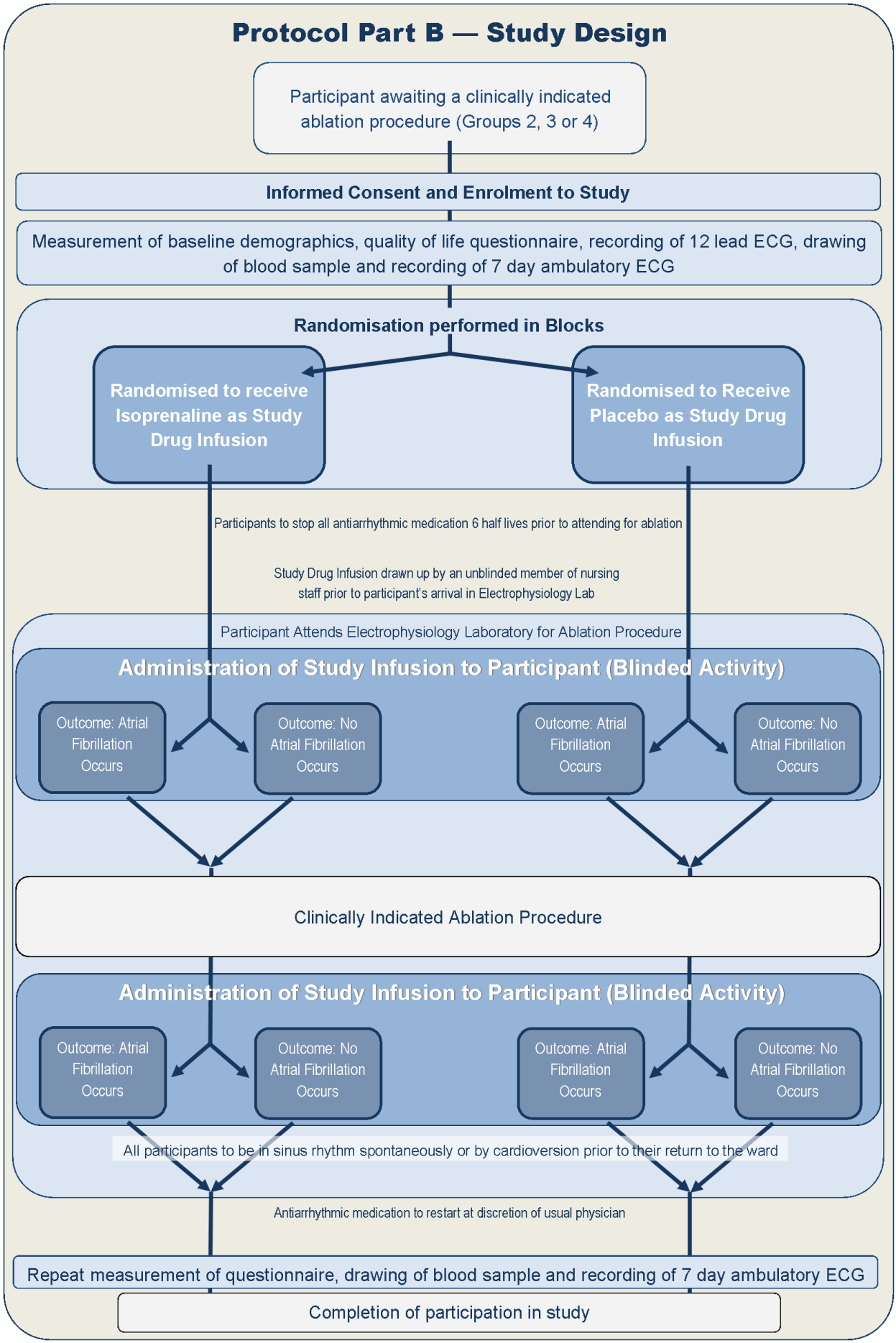
Protocol Part A

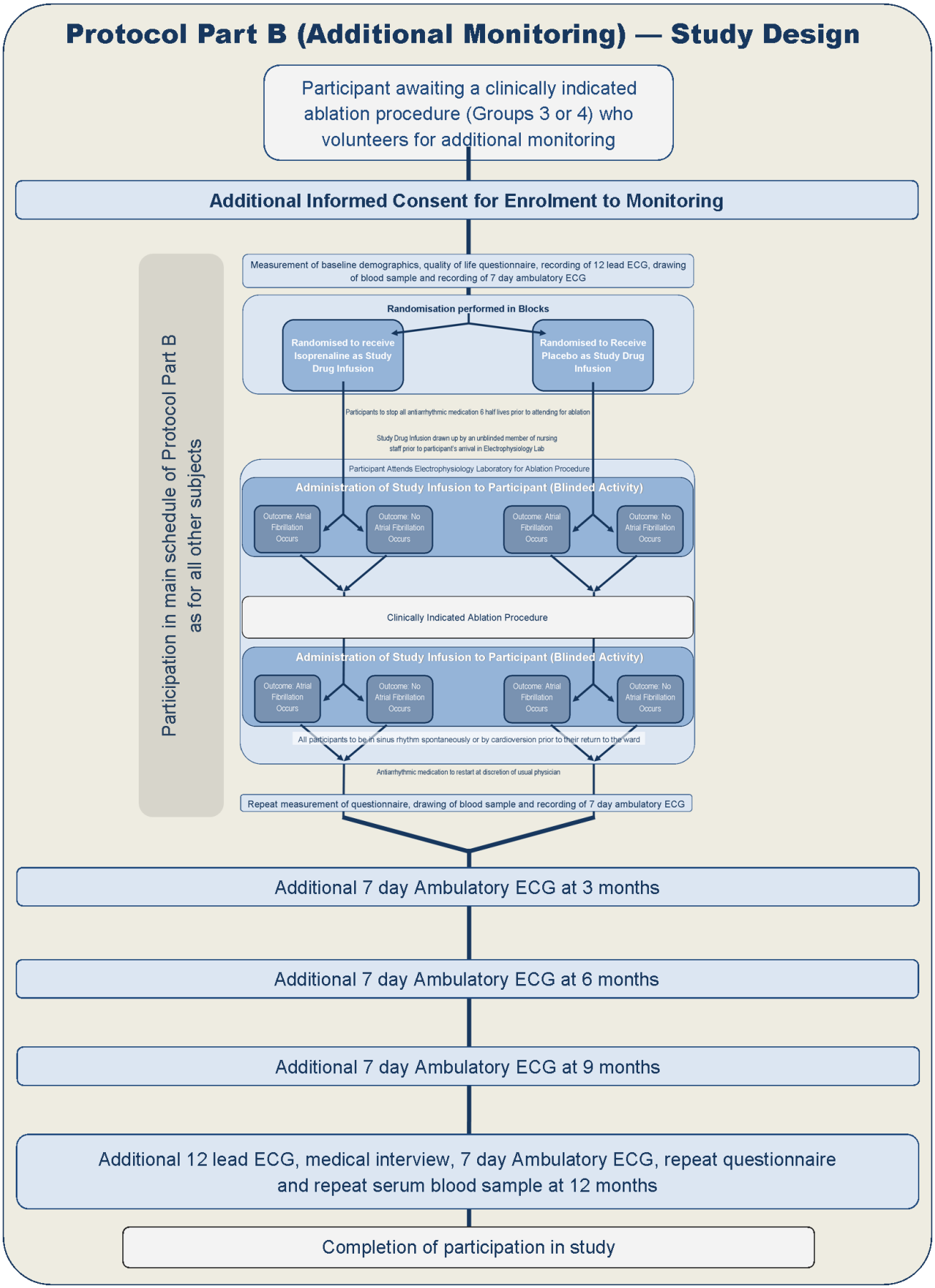
This part of the project investigates the consistency of the outcome of repeated administrations of an isoprenaline infusion. Subjects for this part will be recruited independent of their need for an ablation procedure. The isoprenaline, study drug infusion is administered on an open label basis on two separate occasions at least 2 weeks apart. Administration will be in a ward based environment. No follow up visits will be required.

Protocol Part B

Part B of the project investigates the ability versus placebo of an isoprenaline infusion to induce atrial fibrillation and also any change in that inducibility associated with different ablation procedures. Subjects will be recruited based on their medical history and need for an ablation procedure. Subjects will be randomised in a double blinded fashion to receive isoprenaline or placebo infusions. The study drug infusion that they are randomised to will be delivered before and after their clinically indicated ablation procedure in the cardiac catheter laboratory environment whilst the subject is under sedation for that procedure. There will be follow up requirements for study subjects over and above the standard clinical follow up procedure.


Protocol Part A — Study Design
Participants with a Personal Medical History of Paroxysmal Atrial Fibrillation (Group 1)
Informed Consent and Enrolment to Study
Participants to stop all antiarrhythmic medication 6 half lives prior to attending day ward
Participant Attends Cardiology Day Ward
Open Label Administration of Isoprenaline Infusion to Participant
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
All participants to be in sinus rhythm spontaneously or by cardioversion prior to leaving hospital
All antiarrhythmic medication to restart at previous doses
Minimum time between attendances for infusion of 2 weeks
Participants to stop all antiarrhythmic medication 6 half lives prior to attending day ward
Participant Attends Cardiology Day Ward
Open Label Administration of Isoprenaline Infusion to Participant
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
All participants to be in sinus rhythm spontaneously or by cardioversion prior to leaving hospital
All antiarrhythmic medication to restart at previous doses
Completion of participation in study


Protocol Part B — Study Design
Participant awaiting a clinically indicated ablation procedure (Groups 2, 3 or 4)
Informed Consent and Enrolment to Study
Measurement of baseline demographics, quality of life questionnaire, recording of 12 lead ECG, drawing of blood sample and recording of 7 day ambulatory ECG
Randomisation performed in Blocks
Randomised to receive Isoprenaline as Study Drug Infusion
Randomised to Receive Placebo as Study Drug Infusion
Participants to stop all antiarrhythmic medication 6 half lives prior to attending for ablation
Study Drug Infusion drawn up by an unblinded member of nursing staff prior to participant's arrival in Electrophysiology Lab
Participant Attends Electrophysiology Laboratory for Ablation Procedure
Administration of Study Infusion to Participant (Blinded Activity)
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Clinically Indicated Ablation Procedure
Administration of Study Infusion to Participant (Blinded Activity)
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
All participants to be in sinus rhythm spontaneously or by cardioversion prior to their return to the ward
Antiarrhythmic medication to restart at discretion of usual physician
Repeat measurement of questionnaire, drawing of blood sample and recording of 7 day ambulatory ECG
Completion of participation in study


Protocol Part B (Additional Monitoring) — Study Design
Participant awaiting a clinically indicated ablation procedure (Groups 3 or 4) who volunteers for additional monitoring
Additional Informed Consent for Enrolment to Monitoring
Participation in main schedule of Protocol Part B as for all other subjects
Measurement of baseline demographics, quality of life questionnaire, recording of 12 lead ECG, drawing of blood sample and recording of 7 day ambulatory ECG
Randomisation performed in Blocks
Randomised to receive Isoprenaline as Study Drug Infusion
Randomised to Receive Placebo as Study Drug Infusion
Participants to stop all antiarrhythmic medication 6 half lives prior to attending for ablation
Study Drug Infusion drawn up by an unblinded member of nursing staff prior to participant's arrival in Electrophysiology Lab
Participant Attends Electrophysiology Laboratory for Ablation Procedure
Administration of Study Infusion to Participant (Blinded Activity)
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Clinically Indicated Ablation Procedure
Administration of Study Infusion to Participant (Blinded Activity)
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
Outcome: Atrial Fibrillation Occurs
Outcome: No Atrial Fibrillation Occurs
All participants to be in sinus rhythm spontaneously or by cardioversion prior to their return to the ward
Antiarrhythmic medication to restart at discretion of usual physician
Repeat measurement of questionnaire, drawing of blood sample and recording of 7 day ambulatory ECG
Additional 7 day Ambulatory ECG at 3 months
Additional 7 day Ambulatory ECG at 6 months
Additional 7 day Ambulatory ECG at 9 months
Additional 12 lead ECG, medical interview, 7 day Ambulatory ECG, repeat questionnaire and repeat serum blood sample at 12 months
Completion of participation in study

Endpoints

Study Endpoints

Experiments 1, 5 and 6

Primary Endpoint

- Change in outcome between two administrations of the study drug infusion protocol

Secondary Endpoints

- Change in time to outcome between two administrations of the study drug infusion protocol
- Change in maximum rate of infusion between two administrations of the study drug infusion protocol

Experiments 2, 3 and 4

Primary Endpoints

- Frequency of the occurrence of atrial fibrillation lasting greater than 30 seconds during study drug infusion protocol
- Frequency of the occurrence of an arrhythmia other than atrial fibrillation, sinus tachycardia or junctional rhythm lasting greater than 30 seconds during study drug infusion protocol

Secondary Endpoints

- Frequency of the occurrence of atrial fibrillation lasting less than 30 seconds during study drug infusion protocol
- Frequency of the occurrence of an arrhythmia other than atrial fibrillation, sinus tachycardia or junctional rhythm lasting less than 30 seconds during study drug infusion protocol
- Time from start of infusion protocol to occurrence of atrial fibrillation
- Rate of infusion at time of occurrence of atrial fibrillation
- Maximum tolerated rate of study drug infusion prior to its cessation for any reason

Infusion Endpoints

All parts of this investigation utilise a standard infusion protocol of a study drug. This study drug will be isoprenaline or placebo delivered in a double blinded fashion or will be open label isoprenaline dependent on which experiment it is being administered for. In all cases the infusion protocol will be identical as will the associated outcomes recorded. Although different experiments within the protocol call for different, statistically driven endpoints, the endpoints for every administration of a study drug infusion protocol are the same.

Outcome after which infusion would be stopped

- Occurrence of atrial fibrillation lasting greater than 30 seconds during study drug infusion protocol
- Occurrence of an arrhythmia other than atrial fibrillation, sinus tachycardia or junctional rhythm lasting greater than 30 seconds during study drug infusion protocol
- Discontinuation of the study drug infusion
    - Severe tremulousness or anxiety
    - Complaints of severe chest tightness
    - Decrease in systolic blood pressure to consistently < 70mmHg
    - Electrocardiographic changes suggestive of myocardial ischaemia

Pre-specified outcomes to be recorded but which would not trigger cessation of infusion

- Occurrence of atrial fibrillation lasting less than 30 seconds during study drug infusion protocol
- Occurrence of an arrhythmia other than atrial fibrillation, sinus tachycardia or junctional rhythm lasting less than 30 seconds during study drug infusion protocol
- Time from start of infusion protocol to occurrence of atrial fibrillation
- Rate of infusion at time of occurrence of atrial fibrillation
- Maximum tolerated rate of study drug infusion prior to its cessation for any reason

Safety Endpoints associated with each infusion administration

- Death within 24 hours of start of study drug infusion protocol
- Stroke within 24 hours of start of study drug infusion protocol
- Myocardial Infarction within 24 hours of start of study drug infusion protocol

Observational Data

Collected from Additional Monitoring Group

Participants from some groups recruited will be offered the chance to have some additional follow up monitoring after their ablation procedure. This data will be purely descriptive and unpowered. It will be analysed looking at the following endpoints.

Observational Endpoints

- An occurrence of an episode of atrial fibrillation lasting greater than 30 seconds and documented on either 12 lead ECG or on ambulatory ECG monitoring in the 12 months following an ablation procedure
- Time in days from ablation procedure to the occurrence of an episode of atrial fibrillation lasting greater than 30 seconds and documented on either 12 lead ECG or on ambulatory ECG monitoring
- An occurrence of an episode of atrial fibrillation lasting less than 30 seconds and documented on either 12 lead ECG or on ambulatory ECG monitoring in the 12 months following an ablation procedure
- Recurrence of the ablated arrhythmia
- Occurrence of a novel arrhythmia other than atrial fibrillation
- Discontinuation of Anticoagulation
- Stroke
- Myocardial Infarction
- Hospitalisation for heart failure
- Hospitalisation for bleeding episode
- Hospitalisation for any cause
- CV Death
- Death from any cause
- Change in quality of life questionnaire

Subject Identification and Recruitment

Subject Groups
The study is organised with subjects divided into specific groups which will be analysed separately. These groups include;

1. Paroxysmal Atrial Fibrillation Group
2. Supraventricular Tachycardia (SVT) Ablation Group
3. Atrial Flutter Ablation Group
4. Pulmonary Vein Isolation Group

The participant's past history of abnormal heart rhythms will be used to indicate their risk for developing atrial fibrillation based on previous epidemiological studies. Those with known clinical paroxysmal atrial fibrillation will represent the highest risk group, those undergoing ablation for an SVT will be considered low risk and those undergoing ablation for atrial flutter will form an intermediate risk group.

Potential participants will be identified as being possible study candidates at the time of clinical review as part of their usual care. Clinicians or other healthcare professionals performing such a clinical review will identify the subject as a potential candidate for recruitment, will mention the study to the potential participant and highlight their case to the study team for so that they may approach that person to recruit them. For participants in Part B, it is anticipated that this will be the review from which they are listed for an ablation procedure. The clinical team will also review the case notes for patients already listed for an ablation procedure in their centre and potential candidates identified in this manner will be approached.

If an individual is identified as a potential study candidate, the attending clinician will ask a member of the research team to meet the patient, determine if they meet the inclusion criteria and discover if they are interested in participating in the study. This meeting with research staff may be directly after the NHS clinical review, or be remote to it. If the meeting is remote to the NHS review, the clinician may give written information to the patient about the study and the research team may then contact them by phone to establish if the patient is eligible and willing to participate.

Occasionally, the clinician performing the review may also be directly involved with implementation of the trial. If this is the case, they will not be the member of the research team to discuss recruitment to the study. This will keep clear the distinction between NHS care and participation in the study.

Potential participants who meet the eligibility criteria and indicate that they are interested in taking part will be invited to enrol with the study. They will be given an information leaflet to keep and advised that they may ask any questions of the research team and that they should discuss their participation in the study with others. There will be a minimum of 24 hours between a person being invited to enrol as a subject in the study and their signing the consent form. Where potential participants meet the eligibility criteria but do not wish to take part in the study, anonymous details of the patient will be recorded and the patient will not be enrolled into the study.

All authorised persons taking consent will be medical professionals with working knowledge of the Mental Capacity Act and have completed GCP training.

Consent will only be sought from those potential participants who demonstrate a clear understanding of the study and the purpose. Individuals will be advised that a decision not to take part will not affect their care in any way.

Inclusion Criteria

All Participants in All Groups

- The participant must be willing to comply with the protocol requirements including travelling to the investigating hospital for the attendances required for the study.
- Provision of informed consent
- Participants must be over 18 years of age

Group 1 – Paroxysmal Atrial Fibrillation Group

- Participants must have a history of arrhythmia with 12 lead ECG or ambulatory ECG monitoring recordings documenting a diagnosis of atrial fibrillation
- Participants must have a pattern of symptoms and investigation results consistent with a diagnosis of paroxysmal atrial fibrillation

Group 2 – SVT Ablation Group

- Participants must have a history of arrhythmia with documented regular, narrow complex tachycardia available on either 12 lead ECG or ambulatory ECG monitor recording
- Participants must be listed to undergo an Electrophysiology study with a view to performing an ablation procedure
- Participants must go on to have an ablation procedure for either Atrio-Ventricular Re-entrant Tachycardia (AVRT) or Atrio-Ventricular Nodal Re-entrant Tachycardia (AVNRT) with a defined procedural endpoint

Group 3 – Atrial Flutter Ablation Group

- Participants must have a history of arrhythmia with 12 lead ECG documentation fulfilling pre-specified criteria for diagnosis of common type (cavotricuspid isthmus dependent) atrial flutter
- Participants must be listed to undergo a cavotricuspid isthmus ablation for common type atrial flutter
- Participants must go on to have only a cavotricuspid isthmus ablation for common type atrial flutter
- Sustained bi-directional cavotricuspid isthmus block must have been demonstrated as the endpoint for the ablation procedure

Group 4 – Pulmonary Vein Isolation Group

- Participants must have a history of arrhythmia with 12 lead ECG or ambulatory ECG monitoring recordings documenting a diagnosis of atrial fibrillation
- Participants must be listed to undergo an ablation procedure for atrial fibrillation with the intent of the attending physician to perform pulmonary vein isolation alone as an ablation strategy
- Participants must be in sinus rhythm at the time that they enter the Cardiac Electrophysiology Laboratory for their ablation procedure.
- Participants must have an ablation procedure for atrial fibrillation and this must have involved only ablation to achieve pulmonary vein isolation
- Pulmonary vein isolation must have been demonstrated as the endpoint for the ablation procedure

Exclusion Criteria

All Participants in All Groups

- Allergy to Isoprenaline
- Any treatment with Amiodarone in the 3 months prior to ablation procedure
- Hypertrophic cardiomyopathy
- Suspected acute myocarditis
- Uncorrected, severe valvulopathy graded by transthoracic echocardiographic parameters
- An Acute Coronary Syndrome within the last 6 months
- Recent (within the last 6 months) or scheduled coronary revascularisation
- Ongoing angina symptoms without investigations demonstrating the absence of myocardial ischaemia
- Left ventricular ejection fraction measured at <30%
- Symptoms of decompensated heart failure syndrome in the last 3 months
- Severe obstructive lung disease
- Pregnancy at the time of enrolment or a desire to become pregnant during the study period
- Women who are breastfeeding
- Reduced life expectancy not associated with cardiovascular disease (less than 1 year)
- Unable to provide informed consent

Group 1 – Paroxysmal Atrial Fibrillation Group

- Any past history of episode of persistent atrial fibrillation at the time of enrolment to the study
- Treatment with any antiarrhythmic agent within six half-lives of that agent from before an administration of the study drug infusion

Group 2 – SVT Ablation Group

- Any past history of atrial fibrillation documented on 12 lead ECG or ambulatory ECG monitor
- Any past history of atrial flutter documented on 12 lead ECG or ambulatory ECG monitor
- Characterisation of SVT as any arrhythmia other than ANRT or AVNRT
- Treatment with any antiarrhythmic agent within six half-lives of that agent from the ablation procedure (including intra-procedural use) with the exception of isoprenaline and adenosine

Group 3 – Atrial Flutter Ablation Group

- Characterisation of arrhythmia as any arrhythmia other than cavo-tricuspid isthmus dependent atrial flutter at the time of ablation
- Treatment with any antiarrhythmic agent within six half-lives of that agent from the ablation procedure (including intra-procedural use) with the exception of isoprenaline and adenosine

Group 4 – Pulmonary Vein Isolation Group

- Requirement for a more extensive ablation strategy than pulmonary vein isolation alone
- Intra-procedural treatment with any antiarrhythmic agent with the exception of isoprenaline and adenosine
- Treatment with any antiarrhythmic agent in the days before the ablation within six half-lives of that agent from the ablation procedure

Participant Information

The Participant Information Sheet (PiS) will be specific to the part of the study a subject is being recruited to. The "Participant Information Sheet for those being recruited to the Cardiac Ward based Infusion Protocol" will be for those recruited to protocol Part A and includes subjects in group 1. The "Participant Information Sheet for those being recruited to the Cardiac Electrophysiology Laboratory Infusion Protocol" will be for those recruited to protocol Part B and includes those from groups 2, 3 and 4. The "Additional Information Sheet for those undergoing an ablation for Atrial Flutter or Atrial Fibrillation" will be given to participants recruited to groups 3 and 4.

Informed Consent

Informed consent to enter the study must be sought from each participant. This will be taken only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected.

Sample Size Calculation

The sample size calculation for estimation of the proportion of disagreement between repeated isoprenaline infusions in terms of ability to induce atrial fibrillation is based on the width of a 1-sided 95% confidence interval (CI). Assuming that the proportion of disagreement will be $\leq$ 10%, a sample size of 35 patients would enable the proportion to be estimated with an upper limit of the 1-sided 95%CI for the proportion of $\leq$ 20%.
A 1-sided CI is used as we only wish to ensure that the upper limit of the estimated proportion of disagreement is less than a given cut-off (20% has been chosen to indicate clinically significant disagreement); the lower limit of the CI is immaterial as the lower the proportion of disagreement the better, so we do not need to restrict this.

The sample size calculation for the randomised comparison of proportion of participants inducible in the placebo and isoprenaline arms is based on the chi-squared test. In the Pulmonary Vein Isolation Group, assuming that almost no participants in the placebo arm will be inducible (1%), and that the proportion of participants in the active drug (isoprenaline) arm who are inducible will be 30%, then 31 patients in each arm will be required for 90% power (assuming 2-sided significance of 5%).

It is noted that previous literature suggests that the proportion of patients with a past history of atrial fibrillation who are inducible with isoprenaline is greater than 80%. This does not fit with our interpretation of day to day clinical practice and hence the study is powered to detect a far lower arte of inducibility. We will however perform a pre-specified interim analysis of the Pulmonary Vein Isolation Group once 10 participants with a past history of atrial fibrillation have been randomised to each arm of the study (Experiment 2). If a significant result is detected, enrolment to that block will be discontinued.

Statistical analysis

The analysis of the disagreement between repeated isoprenaline infusions in terms of ability to induce atrial fibrillation will estimate the proportion of disagreement with a 1-sided 95%CI for the proportion. A 2-sided 90%CI is first calculated and the upper limit of this interval gives the required upper limit of a 1-sided 95%CI. If the estimated upper limit is < 20% (the chosen cut-off for clinically significant disagreement) then agreement between repeated infusions will be considered acceptable.

The randomised comparison of proportion of patients inducible between placebo and isoprenaline will use the chi-squared test or the Fisher's exact test if numbers of patients in some categories are small.

It is anticipated that the rate of induced episodes of arrhythmia in a number of the groups where they are being given placebo infusion will approach zero. If the results of the study demonstrate that the rate of inducibility in the SVT group or atrial flutter group being given placebo infusion is zero, we would consider it acceptable to use one sided tests of significance.

Study Duration

We aim to recruit 235 participants over a 24 month recruitment period. It is anticipated that the majority of subjects will be recruited from the Bournemouth site.

- Start Date: 01/09/2014
- Estimated Date of End of Trial: 01/09/2017
- Maximum Duration of Participation: 12 months

Study Procedure

The study will require a minimum of 2 encounters with the study team. For subjects in the group of participants requiring the most visits, the study schedule requires 7 visits over 12 months. Usual care for this group would involve 4 visits to hospital, one of these is not required by the study participants and 3 are combined with study visits.

Infusion Protocol

Central to this project is the delivery of a standardised infusion protocol. This infusion will be an open label isoprenaline infusion in some parts of the project or will be a study drug infusion of either isoprenaline or placebo (normal saline) in other parts of the project. In all cases, each administration of an infusion will follow this protocol.

The infusion will be delivered to subjects in the supine position. Minimum monitoring of the participant at the time of the infusion will include non-invasive blood pressure monitoring at 1 minute intervals, continuous ECG monitoring, continuous monitoring of pulse oximetry and direct observation of the subject by a member of the study team. A blood sample to measure markers of response to the infusion may be taken during the infusion protocol, however such samples are an optional part of the consent process and do not constitute a core part of the study.

The study drug infusion will be drawn up in compliance with local policies regarding intravenous medications. Unblinded members of the nursing staff will identify whether the participant has been randomised to an isoprenaline infusion or to placebo. They will then draw up either 40 mls of normal saline (placebo infusion) into a 50 mls luer lock syringe or will make up the isoprenaline infusion in a 50 mls luer lock syringe. For the isoprenaline infusion, two ampules of isoprenaline sulphate, each containing 10 mls of 0.01 mg/ml solution will be added to 20 mls of normal saline. This will create 40 mls of 0.005mg/ml (5 micrograms/ml) solution containing a total of 0.2mg (200micrograms) isoprenaline sulphate.

The two infusions will be visually identical. The syringe will be labelled with the participant's candidate number, hospital number, name and date of birth but not with the syringe contents. All other cardiac electrophysiology laboratory staff and the study team will remain blinded. Unblinded nursing staff may attach and deliver the study drug infusion, however they should not be the nurse named to monitor the participant and they will not contribute to determination or recording of any study data. This process will only be required in the cardiac electrophysiology laboratory environment as all placebo controlled infusions will take place in this setting.

The minimum staff required to administer the infusion will include a registered nurse considered competent with administration of intravenous medications (as judged by the hospital trust at which the infusion is being delivered) and a licensed medical practitioner considered experienced in the field of cardiology and who is a certified Advanced Life Support provider.

Prior to administration of the study drug infusion, the study team will assess the participant regarding their potential suitability for use of antiarrhythmic medications in the event that atrial fibrillation which persists for greater than 10 minutes is induced.

The route of administration will depend on the situation in which the infusion is being administered. Where the infusion is being administered in a ward based environment, it is anticipated that the infusion will be delivered via a peripherally sited intravenous cannula usually sitting in an antecubital

fossa vein. Where the infusion is being given in the Cardiac Electrophysiology Laboratory, it will usually be administered via a central venous sheath sited in the femoral vein. Standard aspetic technique associated with the administration of intravenous drugs will be applied in all settings to comply with local policy.

The study drug infusion will be delivered following a modified version of the scheme previously published by Morady and colleagues(30). As their study suggested an accelerating rate of induction up to the maximum dose delivered (20mcg/min), to allow for the potential effect of our study using the sulphate form of the drug rather than the hydrochloride and given the documented favourable tolerability profile of the infusion, we have elected to add a further dose increment to our protocol. This remains within the FDA approved dosing regimen for the drug.

The protocol will be..

- Infusion started at 5mcg per minute for 2 minutes
- Increased to 10mcg per minute for 2 minutes
- Increased to 15mcg per minute for 2 minutes
- Increased to 20mcg per minute for 2 minutes
- Increased to 25mcg per minute for 2 minutes
- Infusion is then stopped and subject is monitored over a 10 minute washout phase

Subjects will either follow the protocol to its completion or will reach one of the pre-defined infusion endpoints at which point the infusion will be stopped. These endpoints as described previously are..

Outcome after which infusion would be stopped

- **Occurrence of atrial fibrillation lasting greater than 30 seconds during study drug infusion protocol**
- **Occurrence of an arrhythmia other than atrial fibrillation, sinus tachycardia or junctional rhythm lasting greater than 30 seconds during study drug infusion protocol**
- **Discontinuation of the study drug infusion**
    - **Severe tremulousness or anxiety**
    - **Complaints of severe chest tightness**
    - **Decrease in systolic blood pressure to consistently < 70mmHg**
    - **Electrocardiographic changes suggestive of myocardial ischaemia**

The diagnosis of atrial fibrillation will rest with the clinician acting as operator for the procedure aided by the attending cardiac physiologists.

If a subject experiences an arrhythmia lasting greater than 30 seconds and thus triggering the infusion to stop, they will be monitored for 10 minutes. It is anticipated from previous literature that in the majority of cases, subjects will have spontaneously reverted to normal sinus rhythm after this period of time. It is accepted however that in some cases an arrhythmia will persist beyond this point and in those cases, the supervising physician will restore sinus rhythm.

All subjects will be monitored for at least 30 minutes after an infusion administration.

At all infusions, the following data will be recorded

- Level of sedation at the start of the infusion (AVPU score)
- Total dose of sedation given in the 20 minutes prior to the start of the infusion

- The presence or absence of an episode of arrhythmia lasting greater than 30 seconds
    - The diagnosis for the arrhythmia seen
    - The time from initiation of the infusion to the start of the episode of arrhythmia lasting greater than 30 seconds
    - The time from the start of the episode of arrhythmia lasting greater than 30 seconds to the restoration of sinus rhythm
    - The method of restoration of sinus rhythm
- The presence or absence of one of the other reasons for stopping the infusion
    - The reason for stopping the infusion
- The time from the start of the infusion to its cessation for whatever reason
- The maximum rate of infusion delivered to the study subject
- Minute by minute heart rate and blood pressure measurements over the course of the infusion
- The presence of episodes of arrhythmia lasting less than 30 seconds
    - The diagnosis for each arrhythmia seen
    - The time from initiation of the infusion to the start of each episode of arrhythmia lasting less than 30 seconds
    - The time from initiation of the infusion to the end of each episode of arrhythmia lasting less than 30 seconds

Concomitant Drug Therapy

In line with the product literature regarding Isoprenaline, epinephrine (adrenaline) should not be administered simultaneously with the study drug infusion due to the risks of the combined actions of isoprenaline and epinephrine. Likewise potent inhalational anaesthetic agents such as halothane will be avoided during the delivery of the study drug infusion due to the potential for sensitisation of the myocardium to the effects of sympathomimetic agents.

Study Procedure - Protocol Part A

Subjects recruited to Part A of the study will be required to make a minimum of two and maximum of three visits to the hospital for that part of the study. They will be required to attend for administration of an open label, isoprenaline study infusion on two separate occasions at least two weeks apart. The paroxysmal atrial fibrillation group of subjects will be enrolled to this part of the study.

- **Part A - Contact 1 - Enrolment Visit**

Following a statement of interest to be involved in the study and satisfactory screening with regard to inclusion and exclusion criteria, the subject will be invited to attend for an enrolment visit. At this contact between the potential participant and investigators, the research team will offer further explanation of the study, answer any questions the recruit has and ensure that the individual still wishes to take part in the study and that they still conform to the inclusion / exclusion criteria. The participant will have been given or sent a copy of the relevant Participant Information Sheet prior to this contact. If suitable and willing, they will be enrolled into the study having given written informed consent to do so.

Once the participant is enrolled in the study, the investigators will take baseline demographics and medical information. Baseline demographics will include age, sex, marital status, occupation and ethnic group. Baseline medical information will include current medical diagnoses, duration of those diagnoses, duration of symptoms relating to any diagnoses, number of episodes of symptoms in the month prior, NYHA class, $CHADS_2$ score, CHA_2DS_2 VASc score, HAS BLED score, Framingham risk of atrial fibrillation score, alcohol consumption, smoking status and exercise / activity history.

At the end of the visit, he participant will be invited to schedule Visit 2, the "First Isoprenaline Infusion Administration Visit", at a time which is convenient for them. They will be asked to stop antiarrhythmic drugs 6 half-lives prior to the visit and be asked to starve for 6 hours prior to it also.

Schedule for participants involved in Protocol Part A

Study Procedure	NHS clinical review and / or subsequent telephone conversation	Visit 1 (Enrolment)	Visit 2 (First Isoprenaline Infusion Administration)	Visit 3 (Repeat Isoprenaline Infusion Administration)
Identification of possible suitable subject	X			
Subject given written information and approached about study	X			
Eligibility assessment		X		
Informed Consent		X		
Demographic data collection		X		
Medical history data collection		X		
Observations (to include pulse, BP, SpO_2, Weight)			X	x
12 lead ECG		X	X	X
Serum taken for analysis (Optional)		x	X	x
Open label Isoprenaline infusion administration			x	x
Total visit time	20 mins	20 mins	3 hours	3 hours
Study measurements / Consultation time	5 mins	20 mins	45 mins	45 mins

- **Part A – Visit 2 – First Isoprenaline Infusion Administration**

All subjects will have been asked to stop antiarrhythmic drugs 6 half-lives prior to this visit and have been asked to starve for 6 hours prior to their visit. This will be confirmed with the subject at this point.

If a participant is experiencing an episode of atrial fibrillation at the time of attending for either Visit 2 or 3 they will not be able to go ahead with the infusion administration. The study team will confirm that the participant wishes to remain involved in the study and if they do, they will be asked to re-attend on another occasion. If they re-attend, again in atrial fibrillation, they will be withdrawn from the study as it is not felt to be in their best interests to continue.

Measurements will be taken of basic physiological observations including pulse rate and rhythm, blood pressure from both arms, oxygen saturations, weight in kilograms and height in metres. All subjects will undergo 12 lead ECG recording. Where the optional consent is provided, blood samples will be taken.

Administration of the isoprenaline study infusion will take place with the subject in a bed in a ward based environment, likely on the Cardiac Investigation Unit at the Royal Bournemouth Hospital. The subject will be prepared by attaching monitoring equipment to include a non-invasive blood pressure cuff from which measurements will be recorded at one minute intervals from this point until the end of the study drug infusion, continuous monitoring of the ECG and so heart rate, and continuous monitoring of oxygen saturation level by pulse oximetry. A dedicated defibrillator device will be at the bedside in case of the need for cardioversion.

Intravenous access will be secured by an appropriately qualified member of the study team. This is anticipated to usually take the form of a peripheral intravenous cannula sited in an antecubital fossa vein.

The open label, isoprenaline study drug infusion will then be started and administered as per the drug infusion protocol already described. Outcomes will be recorded as described in that protocol and if atrial fibrillation persists for greater than 10 minutes sinus rhythm will be restored by medical intervention as outlined.

Participants will be monitored for at least 30 minutes after the end of the infusion protocol. At this point they will have their basic physiological observations repeated to include pulse rate and rhythm, blood pressure and oxygen saturations. Release from hospital will be at the discretion of the supervising clinician. It is anticipated that a small number of subjects will be required to remain under observation for a number of hours especially in the context of the need for sedation and cardioversion for subjects who remain in persistent arrhythmia following the infusion.

At release from this visit, subjects will be advised to restart any antiarrhythmic agents which they had withheld as part of the study protocol for this visit. Subjects will be reminded of the contact details for the study team should there be any questions or concerns from the participant following the visit.

- **Part A – Visit 3 – Repeat Isoprenaline Infusion Administration**

This visit will occur a minimum of 2 weeks after the First Isoprenaline Infusion Administration Visit. This time period allows return to baseline medication and symptom status. It is anticipated that for many subjects, the 2 visits will be significantly more than 2 weeks apart. The protocol for the visit is identical to the First Isoprenaline Infusion Administration visit.

Again, all subjects will have been asked to stop antiarrhythmic drugs 6 half-lives prior to this visit and have been asked to starve for 6 hours prior to their visit. This will be confirmed with the subject at this point.

Measurements will be taken of basic physiological observations including pulse rate and rhythm, blood pressure and oxygen saturations. All subjects will undergo 12 lead ECG recording. Where the optional consent is provided, blood samples will be taken.

Administration of the isoprenaline study infusion will take place with the subject in a bed in the same ward based environment. The subject will be prepared by attaching monitoring equipment to include a non-invasive blood pressure cuff from which measurements will be recorded at one minute intervals from this point until the end of the study drug infusion, continuous monitoring of the ECG and so heart rate, and continuous monitoring of oxygen saturation level by pulse oximetry. A dedicated defibrillator device will be at the bedside in case of the need for cardioversion.

Intravenous access will be secured by an appropriately qualified member of the study team. This is anticipated to usually take the form of a peripheral intravenous cannula sited in an antecubital fossa vein.

The open label, isoprenaline study drug infusion will then be started and administered as per the drug infusion protocol already described. Outcomes will be recorded as described in that protocol and if atrial fibrillation persists for greater than 10 minutes sinus rhythm will be restored by medical intervention as outlined.

Participants will be monitored for at least 30 minutes after the end of the infusion protocol. At this point they will have their basic physiological observations repeated to include pulse rate and rhythm, blood pressure and oxygen saturations. Release from hospital will be at the discretion of the supervising clinician. It is anticipated that a small number of subjects will be required to remain under observation for a number of hours especially in the context of the need for sedation and cardioversion for subjects who remain in persistent arrhythmia following the infusion.

At release from this visit, subjects will be advised to restart any antiarrhythmic agents which they had withheld as part of the study protocol for this visit. They will also be provided with contact information for the study team and advised that they should contact the team with any concerns or problems following their participation in the project. The conclusion of this visit represents the end of their participation in the study.

Study Procedure - Protocol Part B

This part of the protocol involves participants who will have been recruited as they are undergoing an ablation procedure on the NHS. The SVT ablation, atrial flutter ablation and the pulmonary vein isolation groups will be recruited to this part of the study.

The study infusion will be delivered in a double blinded, randomised manner. Each participant group will undergo 1 to 1 randomisation to receive either an isoprenaline infusion at the start and end of their ablation or the placebo infusion at the start and end.

Part B of the protocol will require a minimum of 4 encounters with the study team. For subjects who have given consent to undergo additional ECG monitoring, the study schedule requires 7 visits over the 12 months. Usual NHS care for all participants enrolled to Part B of the protocol would involve 4 visits to hospital, one of these may not be required by the study participants and 3 are combined with study visits.

Schedule for participants involved in Protocol Part B					
Study Procedure	NHS clinical review and / or subsequent telephone conversation	Visit 1 (Enrolment)	Visit 2 (Ablation Procedure)	Visit 3 (6 week F/U)	Visit 4 (3 month F/U)
Identification of possible suitable subject	X				
Subject given written information and approached about study	X				
Eligibility assessment		X			
Informed Consent		X			
Demographic data collection		X			
Medical history data collection		X			
Observations (to include pulse, BP, SpO_2, Weight)		x	X (UC)		
12 lead ECG		X	X (UC)	X	X (UC)
Questionnaires		X			X
Novacor fitting (7 day ambulatory ECG monitor)		X		X (UC)	
Serum taken for analysis (Optional)		X	X		X
Study Drug Infusion prior to Ablation			X		
Study Drug Infusion following Ablation			X		
Follow Up Interview (may be telephone based)					X
Consultation time	5 mins	45 mins	N/A	N/A	15 mins
Study measurements time	N/A	15 mins	60 mins	15 mins	40 mins
Visit part of usual NHS clinical requirements	x		x	x	x

- **Part B - Visit 1 - Enrolment Visit**

Following a statement of interest to be involved in the study and satisfactory screening with regard to inclusion and exclusion criteria, the subject will be invited for the enrolment visit. At this first formal meeting between the potential participant and investigators, the research team will ensure that the individual still wishes to take part in the study and that they still conform to the inclusion / exclusion criteria. If suitable and willing, they will be enrolled into the study having given written informed consent to do so.

At this enrolment visit the investigators will take baseline demographics and medical information and will perform some non-invasive measurements. Baseline demographics will include age, sex, marital status, occupation and ethnic group. Baseline medical information will include diagnosis for which patient is undergoing ablation procedure, duration of that diagnosis, duration of symptoms relating to diagnosis, number of episodes of symptoms in the month prior, NYHA class, $CHADS_2$ score, CHA_2DS_2 VASc score, HAS BLED score, Framingham risk of atrial fibrillation score, past cardiac history and the presence of any co-morbidities. All available previous 12 lead ECG and ambulatory ECG recordings will be reviewed and previous arrhythmia episodes will be documented. Usual alcohol consumption, smoking status and exercise / activity history will also be recorded.

Measurements taken of basic physiological observations will include pulse rate and rhythm, blood pressure, oxygen saturations, weight in kilograms and height in metres. At this first visit, blood pressure from both arms will be recorded. All subjects will undergo 12 lead ECG recording. Where the optional consent is provided, blood samples will be taken. Blood will be taken with the intention of storing it for future analysis. This analysis will not have been determined at the time of sampling and patients will be consented for this. The analysis may include genetic testing.

Subjects will complete a quality of life questionnaire which will be a modified combined version of the RAND 36 item health survey (a measure of general health status) and the Symptom Checklist—Frequency and Severity Scale (an instrument specific for cardiac arrhythmias).

At the end of the enrolment visit, each participant will be fitted with a Novacor R-Test ambulatory ECG monitor which, on this and each occasion they are required to wear one during the study, they will wear continuously for a period of 7 days. Where available, each subject will be offered an addressed, pre-paid envelope in which to return the device.

- **Part B - Visit 2 – Ablation Procedure**

The study participants will all undergo measurement of physiological observations including pulse rate, blood pressure and oxygen saturation levels as well as a 12 lead ECG recording on arrival on the day of their ablation procedure. All subjects will have been asked to stop antiarrhythmic drugs 6 half-lives prior to this visit and have been asked to starve for 6 hours prior to their visit. These would all be considered part of their usual care in that setting.

Once the subject has entered the cardiac electrophysiology laboratory they will have monitoring and specialist electrophysiology equipment attached as is usual prior to an ablation procedure. A dedicated defibrillation device will be available. The operator will then gain intravenous access via the femoral vein as is standard practice and insert the required number of venous sheaths. Where the optional consent is provided, blood samples may be taken via the sheaths that are sited.

Usual NHS Care around an ablation procedure

Procedure	NHS clinical review	Ablation	6 week visit	Extra Visit not in study	3 month F/U
NHS Clinical Review in Outpatient Department	X				X
Observations (to include pulse, BP, SpO_2, Weight)		X			
12 lead ECG	X	X			X
Novacor fitting (7 day ECG monitor)			X		
Novacor return (7 day ECG monitor)				X	
Ablation Procedure		X			
Consultation time	20 mins	30 mins	N/A	N/A	20 mins
Intervention time	20 mins	120 mins	20 mins	10 mins	N/A

Before catheters are inserted into the heart, the patient will be sedated so that they are asleep but responsive to voice. The level of sedation will be recorded along with the total dose at this point. The operator will then attach the study infusion to the side arm of one of the femoral sheaths. Heart rate and blood pressure will be recorded at this point and at one minute intervals from this point until the end of the study drug infusion. The pre-ablation study drug infusion will then be started and administered as per the drug infusion protocol already described.

If arrhythmia persists for greater than 10 minutes, it will be at the discretion of the attending clinician to decide whether to perform DC cardioversion, continue to wait or to proceed with the intended ablation procedure. If the participant is in persistent arrhythmia at the time of the start of the pre-ablation infusion (most likely to occur in the atrial flutter ablation group) then that infusion will not be performed and the operator will proceed to the ablation procedure. Such participants will receive the post-ablation infusion as per protocol.

After the pre-ablation study drug infusion, the attending clinician will then proceed to the clinically indicated ablation procedure. The diagnosis and endpoint for the ablation procedure will be

recorded. When the attending physician is confident they have achieved their endpoint for the procedure, efforts will be made to induce a similar level of sedation as that recorded at the time of the pre-ablation study drug infusion. It is accepted that this may not be possible in the real world setting.

Once efforts have been made to achieve a similar level of sedation, the post-ablation study drug infusion will be administered using the standard infusion protocol for the identical infusion delivered prior to ablation.

All patients will be expected to leave the cardiac electrophysiology laboratory with a heart rate approaching that seen at baseline (if in sinus rhythm at the start of the procedure) and in sinus rhythm. It is accepted that some patients will require DC cardioversion if atrial fibrillation occurring during the infusion protocol persists for greater than 10 minutes.

It is also anticipated that it may be difficult for participants to tell whether they are undergoing a part of the study protocol or a part of their NHS ablation procedure at any particular time once they have entered the Cardiac Electrophysiology Laboratory. This is due to the use of sedation and the similarity between the participant experience in each part of the procedure. The potential for confusion will be clearly explained in the patient information sheet.

- **Part B - Visit 3 – 6 week follow up**

As part of usual care, patients will attend for holter monitoring 6 weeks following an ablation. For study participants this will take the form of a 7 day Novacor R-Test monitor. Where available, subjects will be offered an addressed, pre-paid envelope in which to return the device rather than having to return to the hospital to drop it off as they would with standard NHS care. At the time of fitting for the holter monitor, each subject will undergo a 12 lead ECG recording.

- **Part B - Visit 4 – 3 month follow up**

Again as part of usual care following an ablation procedure, a patient would visit for a 12 lead ECG, clinical review in the outpatient department and discussion of the results of the holter recorder performed at 6 weeks. Participants in the study would visit as they usually would 3 months after their procedure and meet with the clinical team in the outpatient setting. A copy of the 12 lead ECG taken would form part of the study documentation.

In addition, at that visit to hospital, all participants in the study will undergo a brief follow up interview, repeat the quality of life questionnaire they completed previously and will have repeat blood samples drawn for genetic and biomarker evaluation (if they have consented to the option of having blood samples drawn). For subjects who are not undergoing additional monitoring as part of their involvement in the study, the conclusion of this visit represents the end of their participation in the study.

Study Procedure – Additional Monitoring Participants

Some participants recruited to Protocol Part B will be offered the chance to be involved in an extension to their clinical follow up as part of the study.

Participants recruited to undergoing additional ECG monitoring will be informed that they should seek out documentation of their heart rhythm with a 12 lead ECG at any time they feel they may be in an abnormal heart rhythm. This advice would be considered usual NHS practice. The study

subjects would be asked to have a copy of any such ECGs forwarded on to the study investigator team.

Participants recruited to undergoing additional ECG monitoring will also be asked to attend for the following further visits / tests

- **Visit 4 – 3 month follow up (additional monitoring)**

At the conclusion of the 3 month follow up study visit, those subjects recruited to additional monitoring will undergo fitting of a Novacor ambulatory ECG recorder fitted to be worn for 7 days. They will be offered a prepaid envelope in which to return the device.

- **Visit 5 and 6 – 6 and 9 month follow up**

At the 6 and 9 month visits subjects will have a follow up interview, undergo a recording of their 12 lead ECG and have a Novacor ambulatory ECG recorder fitted to be worn for 7 days.

- **Visit 8 – 1 year follow up**

All participants will be asked to attend at 1 year following their ablation procedure for a 12 lead ECG and fitting of a 7 day Novacor R-Test ambulatory ECG recorder. Again provision will be offered to return the device without a further visit to the hospital. In addition, at the time of their attendance at hospital, participants will undergo a further verbal follow up interview and further serum drawn for genetic and biomarker evaluation (if they have consented to the option of having blood samples drawn). The conclusion of this visit represents the end of their routine contact with the study team and return of the R-Test device represents the end of their participation in the study.

Serum Samples

The optional serum samples taken from those who volunteer during the study will be stored as anonymised samples in secure, restricted access, temperature and access monitored fridges or freezers within the Molecular Pathology Laboratory at the Royal Bournemouth Hospital. The pathology department is restricted access, restricted to named staff only. Only specific members of the molecular pathology research team have access to the fridges / freezers. Members of the research team wishing to store and access samples must undergo specific training and agree to adhere to SOPs. A log of all staff with access to the department is maintained.

The samples will be stored for future analysis. The analysis is expected to be looking for biomarkers of risk of atrial fibrillation or serum markers describing the mechanism of induction of atrial fibrillation by Isoprenaline. The markers will include both protein and genetic markers. Any future research using the anonymised samples will be prospectively submitted to an ethics committee for review before the end of this project, prior to such research being carried out.

Protocol Deviations

The investigators are authorised to provide alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest. The reasons for deviation from protocol should be recorded.

Reasons for Discontinuation of Subject's Participation in Study

A subject can voluntarily discontinue their involvement in the study at any stage without the need to give reason and without prejudicing further treatment. In addition, subjects will be removed from the study if they cease to meet the inclusion criteria after enrolment (eg participant in SVT block has electrophysiology study that demonstrates atrial tachycardia rather than ANRT or AVNRT) or if they develop one of the exclusion criteria (eg require treatment with amiodarone in the time between the 2 infusions required for Protocol Part A). Subjects will also be excluded from continuation in the study if they develop anaphylaxis, chest pain or ECG features of myocardial ischaemia during the infusion protocol.

Where subjects withdraw or are excluded from the study after enrolment but prior to their completion of the study schedule, a further subject will be recruited to their subject group to take their place. Any data collected before withdrawal may be included in the study results.

End of Study Actions

Having withdrawn from the study, been excluded from continued participation or having completed their final visit or ambulatory ECG recording, participants will have completed their involvement in the study. There are no specific interventions required at completion of the study schedule. All participants will be informed that they may still contact the study team should they have questions or concerns regarding their health or their involvement in the study.

Safety Assessment

Adverse Events

Definitions

- **Adverse Event (AE):** any untoward medical occurrence in a patient or clinical study subject.
- **Serious Adverse Event (SAE):** any untoward and unexpected medical occurrence or affect that:
    - **Results in death**
    - **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
    - **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
    - **Results in persistent or significant disability or incapacity**
    - **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

The MHRA Drug Analysis Print safety information on isoprenaline documents only 169 adverse drug reactions over 47 years of widespread clinical use in the UK. We would therefore consider all such events rare phenomena. The following list of adverse reactions is that described in the FDA approved product literature for Isuprel.

Source	Common	Uncommon	Rare
FDA Approved product literature for Isoprenaline	Nervousness Headache Dizziness Nausea Tachycardia Palpitations Hypertension Hypotension Tachyarrhythmias Dyspnoea Flushing of the skin Sweating Mild Tremors Pallor	Visual Blurring Weakness	Stokes Adams attacks Transient Heart block Pulmonary Oedema Ventricular Arrhythmias Angina

A number of the above reactions represent predictable, normal effects of isoprenaline or any similar sympathomimetic including endogenous adrenaline. They do not pose a risk to the health of the participant experiencing them and such experiences are described and quoted as being likely to occur in the Participant Information Sheets. As such we do not believe it to be useful to perform full adverse event reporting procedures for each occurrence. These events will be recorded on the case report form in all instances but will only be reported as an adverse event if it is felt that they represent a SAE or Suspected Unexpected Serious Adverse Reactions (SUSAR). The reactions which will be dealt with in this way are.

- Nervousness
- Tachycardia
- Palpitations
- Hypertension
- Hypotension
- Tachyarrhythmias
- Flushing of the skin
- Sweating
- Mild tremors
- Pallor

If any serious uncommon or rare adverse events occur the study will be suspended until the event is fully assessed by the Chief Investigator, Sponsor, Medicines and Healthcare Regulatory Agency (MHRA) and Research Ethics Committee (REC) as per the reporting procedure below.

It is noted that this study runs alongside standard NHS care for many participants which includes a cardiac catheter ablation procedure. It is anticipated from registry data regarding the rate of complication(53)(54) associated with such procedures will mean that some participants will experience them. Below is a list of serious adverse events which may occur during the study but

which the investigators would expect that, upon full review, would be linked with the ablation procedure, rather than the administration of the study drug protocol.

- Cardiac Tamponade or Perforation
- Vascular Access Complication
- Haematoma
- Pulmonary Vein Stenosis
- Atrio-Oesophageal Fistula formation

Un-blinding Procedure

If, in the context of a medical emergency, the clinical team caring for the participant require un-blinding as to the contents of the study drug infusion, this will occur immediately. The most simple method for this to occur is that the un-blinded nurse who drew up the study drug can be asked directly to inform the clinical team and / or investigators as to the contents of the infusion. It is anticipated that the nurse will remain be in the room for the duration of the infusion in all cases but will not take part in any of the subsequent study procedures or observations.

If the nurse is unsure as to the contents of the infusion for any reason, or if the nurse who drew up the study infusion is not present for any reason, the participant's study number can be correlated with the instructions in the randomisation folder from which the un-blinded nurses will determine which infusion to draw up. This document will be kept within the cardiac electrophysiology laboratory and so will be available immediately.

Un-blinding will always be reported as a serious adverse event.

Reporting procedure

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance. All project team members will be GCP trained.

All such events, whether expected or not, should be recorded in the CRF. In the event of an adverse event/reaction, a member of research team will:

- Review all documentation (e.g. hospital notes, laboratory and diagnostic reports) relevant to the event
- Record the event and relevant comments in the subject's medical notes (or source data where this is not the medical notes)
- Record the event in detail on a case record form to allow analysis at a later stage.

The Chief Investigator will keep the Sponsor, REC and Regulatory Authority informed of any relevant significant findings. At the conclusion of the study all adverse event/reactions, recorded during the study will be subject to statistical analysis and that analysis and subsequent conclusions included in the final study report.

For all adverse event/reactions the investigator will make an assessment of intensity, causality, expectedness and seriousness.

If it becomes apparent during monitoring of the study that there are multiple minor adverse events relating to the study drug then a discussion with the study team, sponsor and ethics committee will be mandatory and the study may have to be stopped prematurely.

Serious Adverse Events (SAE)

Immediately (within a maximum of 24 hours) after a member of the research team becoming aware of a serious adverse event the sponsor must be notified. The investigator (or delegated person) will make an initial report, in writing. Initial reports will be followed with a follow up report within 24 hours of the initial report. Written reports will be made by completing an SAE/SUSAR reporting form provided by the sponsor. The initial report will include as much information as is available at the time.

After the initial report the investigator will actively to follow up the subject. The investigator (or delegated person) will provide information missing from the initial report within five working days of the initial report to the regulatory bodies specified above.

Investigators (or delegated persons) will provide follow-up information, each time new information is available, using the Sponsor SAE forms until the SAE has resolved or a decision for no further follow up has been taken.

SUSARs will, where appropriate be reported to the MHRA in line with The Medicines for Human Use (Clinical Trials) Regulations 2004.

For all studies the Chief Investigator will inform all Principal Investigators of relevant information about SAEs that could adversely affect the safety of subjects.

The Chief Investigator will provide the REC and MHRA with copies of all reports.

At the end of the study the Chief Investigator will submit an end of study report to the Sponsor, MHRA and REC.

Direct Access to Source Data/Documents

The participants' medical notes will be used for the purposes of recording source data for the procedure. They will be stored in the hospital medical records department in line with standard NHS security and confidentiality. All data collected will be recorded on the anonymised data collection case report form. Paper copies of the study data will be kept in a file and transferred onto a secure NHS desktop computer in a locked office. The Data Protection Act and Caldicott Principles will be adhered to at all times.

Quality Control and Quality Assurance

The "Isoprenaline Infusion as a method of Induction of Atrial Fibrillation; A randomised controlled trial investigating the use of Isoprenaline to induce an episode of atrial fibrillation" study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirements

Regulatory review

Before commencing the investigation, the Chief Investigator will have obtained appropriate Research Ethics Committee review and been granted a favorable opinion. The study Sponsor will

have applied for a Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Authority (MHRA) and been granted a favorable opinion. This study will be performed at the Royal Bournemouth Hospital and John Radcliffe Hospital Sites. Both the Royal Bournemouth Hospital and the John Radcliffe Hospital will confirm that 'NHS permission' has been granted to conduct the study at that site.

The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Association General Assembly, Helsinki, 1964 and amended by the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. Any amendments to the protocol should be approved by the sponsor and have ethical, regulatory and Trust R&D approval before implementation.

Data Handling and Record Keeping

Confidentiality

Review of person identifiable data for the purpose of identification of potential study recruits will be performed only by a clinician or other healthcare provider working within the team directly responsible for the clinical care of that patient.

Enrolled participants will be allocated a unique code number that will be used on all research documentation to ensure confidentiality. No personal identifiable information will be recorded on any data collection documentation. Only authorised members of the research team will have access to this research data. All research data will be stored securely in adherence with the Data Protection Act 1998 and Trust Confidentiality Policy. Data will be stored for 10 years

Consent will always be obtained to allow authorised staff employed by the sponsor to review identifiable data to ensure the study is monitored / audited to assess compliance with the protocol.

Data Collection

An authorised member of the research team will collect details of past medical history and current medication from the case notes and enter the data on individual case report forms (CRF) for each participants. Each CRF will be fully anonymised, identified only by a unique study number that is allocated to each participant on randomisation.

Financing and Insurance

Indemnity

The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust holds negligent harm insurance policies which apply to this study. The Oxford University Hospitals NHS Trust holds negligent harm insurance policies which apply to this study.

Sponsor

The Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust will act as the Sponsor for this study.

Funding

Funding for the study will be managed by the Royal Bournemouth Hospital.

Funding has been secured from the following bodies

- Bournemouth and Christchurch Cardiac Research Fund

In addition we aim to apply for NIHR portfolio adoption status in order to access further research infrastructure.

Study Reporting and Publication Policy

As this is a randomised controlled trial, we will report its findings adhering to the CONSORT statement recommendations.

Results of the study will be presented at relevant clinical meetings and published in an appropriate peer reviewed journal. Participants will be informed that they may contact the research team to obtain a copy of the study outcome.

References

1. Arrowsmith JE. Shortage of isoproterenol (isoprenaline) hydrochloride. Br J Anaesth. 2002 Sep;89(3):528.

2. Gilman AG, Rall TW, Nies AS, Taylor P, editors. Goodman and Gilman's The pharmacological basis of therapeutics. 8th ed. New York: Pergamon Press; 1990. 1811 p.

3. Marathon Pharmaceuticals LLC. » Isuprel® - Approved Prescribing Information [Internet]. 2013 [cited 2014 Apr 1]. Available from: http://marathonpharma.com/products/isuprel/

4. Josephson ME. Clinical cardiac electrophysiology: techniques and interpretations. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.

5. Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, et al. ACC/AHA/ESC 2006 Guidelines for the Management of Patients With Atrial Fibrillation. J Am Coll Cardiol. 2006 Aug;48(4):e149–e246.

6. Atrial Fibrillation Association. Keeping your finger on the pulse; Why the UK must address the personal, clinical and economic impact of atrial fibrillation [Internet]. 2010 [cited 2014 Mar 20]. Available from: http://www.atrialfibrillation.org.uk/files/file/af-afa-keeping-your-finger-on-the-pulse-report.pdf

7. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide Epidemiology of Atrial Fibrillation: A Global Burden of Disease 2010 Study. Circulation [Internet]. 2013 Dec 17; Available from: http://circ.ahajournals.org/content/early/2013/12/16/CIRCULATIONAHA.113.005119.abstract

8. Calkins H, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen S-A, et al. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: Recommendations for Patient Selection, Procedural Techniques, Patient Management and Follow-up, Definitions, Endpoints, and Research Trial Design. Heart Rhythm. 2012 Apr;9(4):632–696.e21.

9. Morillo CA, Verma A, Connolly SJ, Kuck KH, Nair GM, Champagne J, et al. Radiofrequency ablation vs antiarrhythmic drugs as first-line treatment of paroxysmal atrial fibrillation (RAAFT-2): a randomized trial. JAMA J Am Med Assoc. 2014 Feb 19;311(7):692–700.

10. Oral H, Ozaydin M, Tada H, Chugh A, Scharf C, Hassan S, et al. Mechanistic significance of intermittent pulmonary vein tachycardia in patients with atrial fibrillation. J Cardiovasc Electrophysiol. 2002 Jul;13(7):645–50.

11. Haïssaguerre M, Sanders P, Hocini M, Hsu L-F, Shah DC, Scavée C, et al. Changes in atrial fibrillation cycle length and inducibility during catheter ablation and their relation to outcome. Circulation. 2004 Jun 22;109(24):3007–13.

12. Essebag V, Baldessin F, Reynolds MR, McClennen S, Shah J, Kwaku KF, et al. Non-inducibility post-pulmonary vein isolation achieving exit block predicts freedom from atrial fibrillation. Eur Heart J. 2005 Dec;26(23):2550–5.

13. Oral H, Chugh A, Lemola K, Cheung P, Hall B, Good E, et al. Noninducibility of Atrial Fibrillation as an End Point of Left Atrial Circumferential Ablation for Paroxysmal Atrial Fibrillation: A Randomized Study. Circulation. 2004 Nov 2;110(18):2797–801.

14. Jaïs P, Hocini M, Sanders P, Hsu L-F, Takahashi Y, Rotter M, et al. Long-term evaluation of atrial fibrillation ablation guided by noninducibility. Heart Rhythm Off J Heart Rhythm Soc. 2006 Feb 1;3(2):140–5.

15. Leong-Sit P, Robinson M, Zado ES, Callans DJ, Garcia F, Lin D, et al. Inducibility of atrial fibrillation and flutter following pulmonary vein ablation. J Cardiovasc Electrophysiol. 2013 Jun;24(6):617–23.

16. Chang S-L, Tai C-T, Lin Y-J, Wongcharoen W, Lo L-W, Tuan T-C, et al. The efficacy of inducibility and circumferential ablation with pulmonary vein isolation in patients with paroxysmal atrial fibrillation. J Cardiovasc Electrophysiol. 2007 Jun;18(6):607–11.

17. Huang W, Liu T, Shehata M, Zhang K, Yao Y, Niu G, et al. Inducibility of atrial fibrillation in the absence of atrial fibrillation: what does it mean to be normal? Heart Rhythm Off J Heart Rhythm Soc. 2011 Apr;8(4):489–92.

18. Kumar S, Kalman JM, Sutherland F, Spence SJ, Finch S, Sparks PB. Atrial fibrillation inducibility in the absence of structural heart disease or clinical atrial fibrillation: critical dependence on induction protocol, inducibility definition, and number of inductions. Circ Arrhythm Electrophysiol. 2012 Jun 1;5(3):531–6.

19. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med. 1998 Sep 3;339(10):659–66.

20. Tsai C-F, Tai C-T, Hsieh M-H, Lin W-S, Yu W-C, Ueng K-C, et al. Initiation of Atrial Fibrillation by Ectopic Beats Originating From the Superior Vena Cava Electrophysiological Characteristics and Results of Radiofrequency Ablation. Circulation. 2000 Jul 4;102(1):67–74.

21. Jai¨s P, Hai¨ssaguerre M, Shah DC, Chouairi S, Gencel L, Hocini M, et al. A Focal Source of Atrial Fibrillation Treated by Discrete Radiofrequency Ablation. Circulation. 1997 Feb 4;95(3):572–6.

22. Lin W-S, Tai C-T, Hsieh M-H, Tsai C-F, Lin Y-K, Tsao H-M, et al. Catheter Ablation of Paroxysmal Atrial Fibrillation Initiated by Non–Pulmonary Vein Ectopy. Circulation. 2003 Jul 1;107(25):3176–83.

23. Chen SA, Hsieh MH, Tai CT, Tsai CF, Prakash VS, Yu WC, et al. Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. Circulation. 1999 Nov 2;100(18):1879–86.

24. Chen P-S, Maruyama M, Lin S-F. Arrhythmogenic Foci and the Mechanisms of Atrial Fibrillation. Circ Arrhythm Electrophysiol. 2010 Feb 1;3(1):7–9.

25. Chen SA, Chiang CE, Yang CJ, Cheng CC, Wu TJ, Wang SP, et al. Sustained atrial tachycardia in adult patients. Electrophysiological characteristics, pharmacological response, possible mechanisms, and effects of radiofrequency ablation. Circulation. 1994 Sep 1;90(3):1262–78.

26. Hsieh MH, Chen SA, Tai CT, Tsai CF, Prakash VS, Yu WC, et al. Double multielectrode mapping catheters facilitate radiofrequency catheter ablation of focal atrial fibrillation originating from pulmonary veins. J Cardiovasc Electrophysiol. 1999 Feb;10(2):136–44.

27. Oral H, Scharf C, Chugh A, Hall B, Cheung P, Good E, et al. Catheter ablation for paroxysmal atrial fibrillation: segmental pulmonary vein ostial ablation versus left atrial ablation. Circulation. 2003 Nov 11;108(19):2355–60.

28. Proietti R, Santangeli P, Di Biase L, Joza J, Bernier ML, Wang Y, et al. Comparative effectiveness of wide antral versus ostial pulmonary vein isolation: a systematic review and meta-analysis. Circ Arrhythm Electrophysiol. 2014 Feb 1;7(1):39–45.

29. Jacobson J, Passman R. Atrial Fibrillation Induction with Isoproterenol: Teaching an Old Drug New Tricks. J Cardiovasc Electrophysiol. 2008 May 1;19(5):471–2.

30. Oral H, Crawford T, Frederick M, Gadeela N, Wimmer A, Dey S, et al. Inducibility of Paroxysmal Atrial Fibrillation by Isoproterenol and its Relation to the Mode of Onset of Atrial Fibrillation. J Cardiovasc Electrophysiol. 2008 May 1;19(5):466–70.

31. Crawford T, Chugh A, Good E, Yoshida K, Jongnarangsin K, Ebinger M, et al. Clinical Value of Noninducibility by High-Dose Isoproterenol Versus Rapid Atrial Pacing After Catheter Ablation of Paroxysmal Atrial Fibrillation. J Cardiovasc Electrophysiol. 2010 Jan;21(1):13–20.

32. Elayi CS, Di Biase L, Bai R, Burkhardt JD, Mohanty P, Santangeli P, et al. Administration of isoproterenol and adenosine to guide supplemental ablation after pulmonary vein antrum isolation. J Cardiovasc Electrophysiol. 2013 Nov;24(11):1199–206.

33. Ter Keurs HEDJ, Boyden PA. Calcium and Arrhythmogenesis. Physiol Rev. 2007 Apr 1;87(2):457–506.

34. Dewland TA, Vittinghoff E, Mandyam MC, Heckbert SR, Siscovick DS, Stein PK, et al. Atrial Ectopy as a Predictor of Incident Atrial Fibrillation A Cohort Study. Ann Intern Med. 2013 Dec 3;159(11):721–8.

35. Kolb C, Nürnberger S, Ndrepepa G, Zrenner B, Schömig A, Schmitt C. Modes of initiation of paroxysmal atrial fibrillation from analysis of spontaneously occurring episodes using a 12-lead Holter monitoring system. Am J Cardiol. 2001 Oct 15;88(8):853–7.

36. Roten L, Derval N, Jaïs P. Catheter Ablation for Persistent Atrial Fibrillation Elimination of Triggers Is Not Sufficient. Circ Arrhythm Electrophysiol. 2012 Dec 1;5(6):1224–32.

37. Choi E-K, Shen MJ, Han S, Kim D, Hwang S, Sayfo S, et al. Intrinsic Cardiac Nerve Activity and Paroxysmal Atrial Tachyarrhythmia in Ambulatory Dogs. Circulation. 2010 Jun 22;121(24):2615–23.

38. Patterson E, Po SS, Scherlag BJ, Lazzara R. Triggered firing in pulmonary veins initiated by in vitro autonomic nerve stimulation. Heart Rhythm. 2005 Jun;2(6):624–31.

39. Sharifov OF, Fedorov VV, Beloshapko GG, Glukhov AV, Yushmanova AV, Rosenshtraukh LV. Roles of adrenergic and cholinergic stimulation in spontaneous atrial fibrillation in dogs. J Am Coll Cardiol. 2004 Feb 4;43(3):483–90.

40. Bettoni M, Zimmermann M. Autonomic Tone Variations Before the Onset of Paroxysmal Atrial Fibrillation. Circulation. 2002 Jun 11;105(23):2753–9.

41. Zimmermann M, Kalusche D. Fluctuation in Autonomic Tone is a Major Determinant of Sustained Atrial Arrhythmias in Patients with Focal Ectopy Originating from the Pulmonary Veins. J Cardiovasc Electrophysiol. 2001 Mar 1;12(3):285–91.

42. Lim PB, Malcolme-Lawes LC, Stuber T, Wright I, Francis DP, Davies DW, et al. Intrinsic cardiac autonomic stimulation induces pulmonary vein ectopy and triggers atrial fibrillation in humans. J Cardiovasc Electrophysiol. 2011 Jun;22(6):638–46.

43. Pokushalov E, Turov A, Shugayev P, Artyomenko S, Romanov A, Shirokova N. Catheter Ablation of Left Atrial Ganglionated Plexi for Atrial Fibrillation. Asian Cardiovasc Thorac Ann. 2008 Jun 1;16(3):194–201.

44. Razavi M, Cheng J, Rasekh A, Yang D, Delapasse S, Ai T, et al. Slow Pathway Ablation Decreases Vulnerability to Pacing-Induced Atrial Fibrillation: Possible Role of Vagal Denervation. Pacing Clin Electrophysiol. 2006 Nov 1;29(11):1234–9.

45. Calvo D, Atienza F, Jalife J, Martínez-Alzamora N, Bravo L, Almendral J, et al. High-rate pacing-induced atrial fibrillation effectively reveals properties of spontaneously occurring paroxysmal atrial fibrillation in humans. Eur Eur Pacing Arrhythm Card Electrophysiol J Work Groups Card Pacing Arrhythm Card Cell Electrophysiol Eur Soc Cardiol. 2012 Nov;14(11):1560–6.

46. Lin D, Poku J, Beshai J, Lewkowiez L, Gerstenfeld EP, Dixit S, et al. High Dose Isoproterenol is Tolerated and Effective in Potentiating Triggers and Assessing the Efficacy of Atrial Fibrillation Ablation. Pacing Clin Electrophysiol. 2003 Apr 1;26(4p2):1001 (Abstract).

47. Fujita T, Ajisaka R, Matsumoto R, Iida K, Iida K, Sugishita Y, et al. Isoproterenol infusion stress two-dimensional echocardiography in diagnosis of coronary artery disease in elderly patients. Comparison with the other stress testing methods. Jpn Heart J. 1986 May;27(3):287–97.

48. Watanabe S, Ajisaka R, Masuoka T, Iida K, Sugishita Y, Ito I, et al. Isoproterenol stress thallium scintigraphy for detecting coronary artery disease. J Cardiol. 1989 Sep;19(3):657–65.

49. Mertes H, Sawada SG, Ryan T, Segar DS, Kovacs R, Foltz J, et al. Symptoms, adverse effects, and complications associated with dobutamine stress echocardiography. Experience in 1118 patients. Circulation. 1993 Jul 1;88(1):15–9.

50. Daoud EG, Bogun F, Goyal R, Harvey M, Man KC, Strickberger SA, et al. Effect of Atrial Fibrillation on Atrial Refractoriness in Humans. Circulation. 1996 Oct 1;94(7):1600–6.

51. Sparks PB, Jayaprakash S, Mond HG, Vohra JK, Grigg LE, Kalman JM. Left atrial mechanical function after brief duration atrial fibrillation. J Am Coll Cardiol. 1999 Feb;33(2):342–9.

52. Dimarco JP, Garan H, Ruskin JN. Complications in patients undergoing cardiac electrophysiologic procedures. Ann Intern Med. 1982 Oct;97(4):490–3.

53. Hunter RJ, McCready J, Diab I, Page SP, Finlay M, Richmond L, et al. Maintenance of sinus rhythm with an ablation strategy in patients with atrial fibrillation is associated with a lower risk of stroke and death. Heart. 2012 Jan 1;98(1):48–53.

54. Page SP, Herring N, Hunter RJ, Withycombe E, Lovell M, Wali G, et al. Periprocedural Stroke Risk in Patients Undergoing Catheter Ablation for Atrial Fibrillation on Uninterrupted Warfarin. J Cardiovasc Electrophysiol. 2014 Apr 1;n/a–n/a.

Appendices

- Appendix 1
  - IMP SmPC (FDA approved product literature)
- Appendix 2
  - PiS
- Appendix 3
  - Consent forms
- Appendix 4
  - Letter to GP