Protocol Title:	Comparison of (R) and (S) Propafenone for Prevention of Atrial Fibrillation Induction
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Comparison of *(R)* and *(S)* Propafenone for Prevention of Atrial Fibrillation Induction

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1.0 BACKGROUND

1A. The need for more mechanism-based therapy for atrial fibrillation: AF is the most common sustained cardiac arrhythmia affecting 2-5 million adults in the U.S. at an estimated cost of over 26 million dollars per day. With the aging of the population, the projected number of people affected with AF is estimated to be 12 million by 2050. The AF epidemic is further complicated by the lack of effective therapies. Despite recent advances in catheter-based and surgical treatments for AF, antiarrhythmic drugs (AAD) remain the mainstay of treatment for most patients with symptomatic AF. However, response in an individual patient is highly variable and carries risks of proarrhythmia and extracardiac toxicity. The poor efficacy of AAD is due to the poor understanding of the underlying pathophysiology and failure to target therapy to underlying mechanisms. The current strategy for a trial of AAD therapy focuses primarily not on efficacy, but on the side-effect profile of available membrane active drugs. There is little evidence-based data to assist the physician in selecting the most appropriate AAD for a particular patient. Over the last decade, genetic studies have provided important insights into the molecular mechanisms of AF, and our data suggest that genetic factors also contribute to variable drug actions. Given that the current success rate for AADs in AF is ~50% over a 6-12 month period, continued research aimed at refining AAD therapy such that underlying mechanisms are targeted is a major research priority in this field and our study will contribute to achieving this goal.

1B. Role of "leaky" RyR2 Ca release channels in AF pathophysiology: To better understand the molecular pathophysiology of AF, it is important to place it in the context of common arrhythmia mechanisms such as focal ectopic activity and reentry. Ectopic activity can be triggered by early afterdepolarizations (EADs), delayed afterdepolarizations (DADs), or enhanced automaticity. Rapid focal activity in the pulmonary veins (PVs) can not only initiate AF but sustain it. High frequency firing drivers or rotors or a single localized reentrant circuit can also give rise to the irregular atrial discharge that is typical for AF. Atrial fibrillatory activity may also be caused by multiple fixed and functional circuits that are time and space dependent. While the cellular mechanisms causing paroxysmal AF in humans are clearly multifactorial, experimental evidence from our group as well as others suggest that in mouse models Ca leak mediated by RyR2 Ca release channels is one important mechanism responsible for triggering of this form of the arrhythmia. Since Ca leak has been demonstrated in atrial tissue from patients with persistent and paroxysmal AF, but the causal relationship to human AF remains controversial, the overarching goal of this application is to test the hypothesis that leaky RyR2 channels confer increased susceptibility to paroxysmal AF that can be targeted therapeutically.

1C. Are "leaky" RyR2 channels a common downstream consequence of genetic AF risk variants? Recent insight into underlying AF mechanisms has been advanced by the identification of common and rare genetic variants associated with AF. Linkage analyses and candidate gene approaches have identified mutations in a variety of genes encoding cardiac ion channels, gap junction proteins, and signaling molecules. In contrast, genome-wide association studies (GWAS) have identified and validated 9 common AF susceptibility alleles, with several loci on chromosome (chr) 4q25 having the strongest association with AF (relative risk 1.39-1.72). Although the mechanism for this association remains uncertain, the locus is adjacent to the paired-like homeodomain transcription factor 2 (*PITX2*) gene which is critical for cardiac development, and reduced Pitx2 expression generates AF risk in mice. Given how commonly leaky RyR2 channels are found in studies of human AF, and that individuals carrying 4q25 risk alleles respond better to Class IC drugs flecainide and propafenone, both of which also inhibit RyR2 channels, this raises the *hypothesis* that *PITX2* variants and possibly other rare AF-linked variants may initiate a cascade of cellular signaling events that ultimately leads to deranged RyR2 gating, which then contributes to AF by increasing Ca leak, generation of DADs and triggered activity. Hence, such derangement of Ca handling may constitute a common electrophysiological mechanism by which common genetic variants increase susceptibility to paroxysmal AF. To investigate this hypothesis, we will conduct a proof-of-concept clinical trial whereby the role of Ca leak for paroxysmal AF will be tested in humans by comparing AF suppression in the clinical EP laboratory. *(R)*- and *(S)*-propafenone have varying degrees of RyR2 channel inhibition and will therefore be used as tools to provide the first evaluation of whether RyR2 Ca leak contributes to AF inducibility in humans.

2.0 Rationale and Specific Aims

The overarching aim of our study is to test the hypothesis that RyR2 blockade is beneficial for preventing AF induction in humans with paroxysmal AF. We have received an NIH R01 award to evaluate the efficacy of RyR2 channel blockade to prevent inducible AF or atrial flutter (AFL) in patients undergoing an AF ablation. We will test the role of *RyR2* channel blockade by comparing drugs with variable *RyR2* action: *(R)*-propafenone, (a combined RyR2 and Na channel blocker), and *(S)*-propafenone (largely devoid of *RyR2* inhibition).

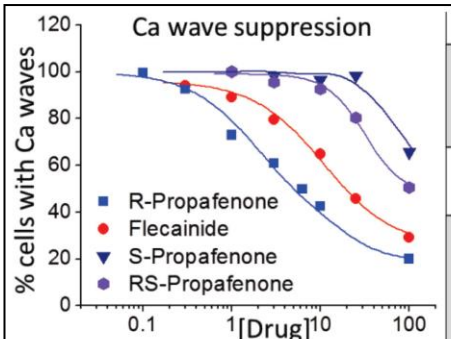
Specific Aim: To test the hypothesis that *(R)*-propafenone is superior to *(S)*-propafenone in suppressing induction of AF/AFL by a rapid atrial pacing protocol.

Rationale: Recent human studies have shown that paroxysmal forms of AF are associated with increased Ca leak from intracellular Ca stores and RyR2 dysfunction. But, it remains controversial if this abnormal Ca leakiness is mechanistically important for AF risk in humans. In mice, enhanced diastolic Ca release mediated by loss of the Ca-binding protein Casq2 can cause AF by promoting regional Ca elevations and DADs. Furthermore, inhibition of spontaneous Ca release with (*R*)-propafenone prevented AF by reducing the rate of DADs and triggered atrial activity, whereas Na channel blockers without RyR2 blocking properties (S-propafenone and lidocaine) were much less effective. These data suggest that RyR2 blocking properties are important for therapeutic efficacy against AF caused by leaky RyR2. The general approach for this study will utilize a rapid atrial pacing protocol to induce AF in subjects undergoing AF ablation. Subjects referred for AF ablation will be randomized to either *(R)*-propafenone, *(S)*-propafenone, or placebo (vehicle) in a double blind, randomized fashion. A single 10-minute infusion of intravenous *(R)*- or *(S)*-propafenone or placebo (vehicle) will be given at the start of the ablation procedure before a rapid atrial pacing protocol is performed to test inducibility of AF.

3.0 Animal Studies and Previous Human Studies

3.1: (R)-propafenone is the most potent inhibitor of spontaneous Ca release among clinically-approved AADs. We recently screened clinically available AAD for activity against spontaneous Ca release due to leaky RyR2 channels using permeabilized ventricular myocytes from Casq2-/- mice. In this assay, cell membrane permeabilization excludes any confounding drug effects on cell membrane ion channels (*i.e.*, Na channels) on Ca storage and release. We found that *(R)*-propafenone was the most potent inhibitor of spontaneous Ca waves (Fig. 1). The *(S)*-enantiomer of propafenone, which has significantly less activity against RyR2 channels, was essentially ineffective against Ca waves (Fig. 1). This is an intriguing finding, because *(R)*-propafenone inhibits the metabolism and elimination of *(S)*- propafenone, leading to much higher levels of *(S)*-propafenone compared to *(R)*-propafenone during chronic administration in patients, which may limit the efficacy of racemic propafenone currently used to treat AF patients.

3.2: (R)- and (S)-propafenone as tools to determine the role of RyR2 channel block for preventing AF induction. If leaky RyR2 channels render atria susceptible to AF induction, as suggested by mouse models with mutant RyR2 or loss of Casq2, then agents with RyR2 channel blocking properties should have superior efficacy compared to agents that do not. Consistent with its higher potency suppressing Ca release *in vitro* (Fig. 1), we recently reported that *(R)*-propafenone exhibited superior efficacy compared to *(S)*-propafenone and lidocaine for suppressing spontaneous Ca release, DADs and pacing-induced AF in Casq2-/- mice (Fig. 2). Furthermore, *(R)*-propafenone was significantly more effective than Na channel blockers without RyR2 blocking properties (lidocaine, S-propafenone) in preventing CPVT in Casq2-/- mice, and in suppressing spontaneous Ca elevations and DADs in Langendorff-perfused rabbit ventricles. Unlike other experimental RyR2 blockers, *(R)*- and *(S)*-propafenone are already in clinical use as



	Drug	IC_{50} µM
Ia	Quinidine	52±1.5
	Procainamide	>100
	Disopyramide	>100
Ib	Lidocaine	57±2.7
	Mexiletine	>100
Ic	**R-Propafen.**	**2.0±0.2**
	Flecainide	12±0.6
	Encainide	28±1.9
	S-Propafen.	54±18
II	Carvedilol	10±2.5
III	Sotalol	>100

Figure 1: Suppression of Ca wave activity by AADs.

the racemic, and therefore will be used to test our hypothesis that leaky RyR2 channels confer AF risk that can be targeted therapeutically in humans.

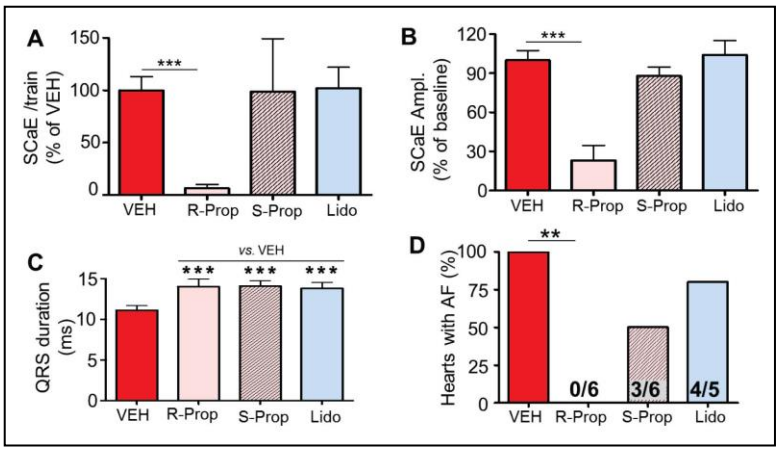


Figure 2: Effect of R-propafenone (R-Prop), S-propafenone (S-Prop) and lidocaine on spontaneous Ca elevations (A, B), QRS (C) and AF induction (D) in intact Casq2-/- hearts.

3.3 Increased AF risk due to chr4q25 SNPs near PITX2. While the link between 4q25 SNPs and altered PITX2 expression in humans remains controversial, mouse models support a causal role of reduced Pitx2 expression for increased AF susceptibility. The initial report by the Martin lab attributed increased AF susceptibility in heterozygous Pitx2+/- mice to failure to suppress a left-sided pacemaker. Pitx2c, the major cardiac isoform, suppresses sinoatrial node (SAN) specific gene expression in the left atrium (LA) by binding to and repressing Shox2. Shox2 is essential for SAN development, and Pitx2+/- mice show increased expression in the LA. This is intriguing; because in mice and humans Pitx2c is expressed after birth only in the LA and in the PVs. Together with a recent study demonstrating that Pitx2 regulates expression of ion channels (including RyR2) in the adult mouse LA, these data suggest that Pitx2 plays a role beyond regulating SAN gene expression in the adult LA. However, published studies have not investigated whether RyR2 Ca leak contributes to AF in Pitx2 mice. Furthermore, due to restricted expression of Pitx2, an effect on the RyR may be localized to LA and PV region and we therefore will determine the origin of AF in this model as shown in Fig. 3.

Chromosome 4q25 SNPs modulate differential response to AADs in patients with AF. Here, we examined whether symptomatic response to AAD is modulated by the three common AF risk loci on chr4q25 (near *PITX2*), 16q22 (in *ZFHX3*), and 1q21 (in *KCNN3*). We studied 478 (discovery cohort) and 198 (validation cohort), age and gender matched Caucasian patients with AF. Clinical variables such as age, hypertension, and lone AF failed to predict response to AADs. However, a 4q25 WT SNP was associated with successful symptom control (OR 2.97, 95% CI 1.42-6.21, P=0.003). Furthermore, individuals who carried a variant 4q25 SNP responded better to class I (flecainide or propafenone) than class III (>95% sotalol) AADs in both the discovery and validation cohorts (Table 1). These findings provide additional rationale for our study as they suggest that common AF risk variants predict differential response to AAD.

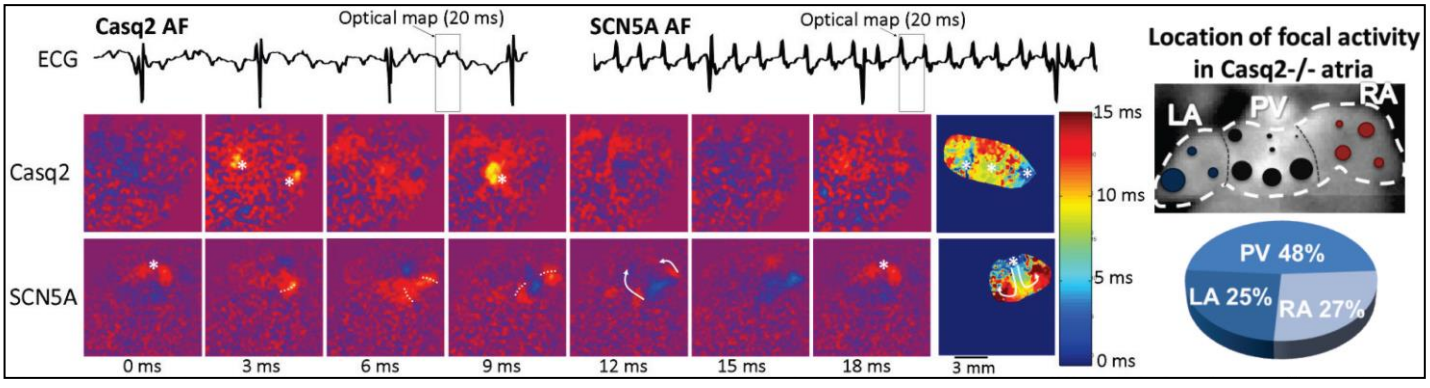


Figure 3: Posterior atrial activation mapping during AF. Right panel: Anatomical origin of focal AF in Casq2-/- (n=7)

3.3 (R)-propafenone prevents AF induction in Pitx2+/- heterozygous mice. Pitx2+/- mice were kindly provided by Dr. James Martin, Baylor Univ. Consistent with literature reports, AF inducibility at baseline was 67% in *Pitx2+/-* mice using atrial burst pacing (Fig. 3). Administration of *(R)*-propafenone (5 mg/kg i.p.) completely prevented AF in *Pitx2+/-* mice (Fig. 4).

Table 1. Differential response to AADs based on 4q25 SNP genotype.[52]

Discovery Cohort			
Drug class	Wild type (n=309)	Minor allele carriers (n=90)	P-value
Class I	87 (28%)	37 (41%)	0.02
Class III	222 (72%)	53 (59%)	0.02
Validation Cohort			
Drug class	Wild type (n=97)	Minor allele carriers (n=46)	*P*
Class I	37 (39%)	30 (67%)	0.01
Class III	60 (61%)	16 (34%)	0.02

3.4 The Vanderbilt AF Ablation Registry (VAFAR). To demonstrate the feasibility of performing a clinical trial in patients undergoing AF ablation, we present data from a clinical and genetic bio-repository (VAFAR) that was established by Dr. M. Benjamin Shoemaker (Co-investigator) in 2011. VAFAR has prospectively enrolled over 900 patients undergoing AF ablation. Uniform protocols for enrollment, blood collection, storage, DNA extraction, intra-procedural electrophysiologic data collection, and clinical follow-up are in place.

3.5 VAFAR demonstrates that 4q25 risk allele carriers have impaired response to AF ablation. In 2013, we examined atrial tachyarrhythmia recurrence (AF/AFL, or atrial tachycardia [AT]) following 378 catheter-based AF ablations in VAFAR). The final cohort consisted of 311 patients (median age 60 years [IQR 52-66]; 47% paroxysmal; 10% lone AF). The overall recurrence rate over 18-months follow-up was 53%. Using an additive genetic model, a graded gene-dose response was detected based on the number of 4q25 risk alleles (P=0.037) such that heterozygous risk allele carriers suffered a 21% earlier recurrence of AT/AF (survival time ratio 0.79 [95% CI: 0.62-0.99]), and homozygous risk allele carriers a 39% earlier recurrence (survival time ratio 0.61 [0.37-1.0]). These findings suggest that common 4q25 AF risk alleles modulate response to ablation therapy in patients with predominantly paroxysmal AF.

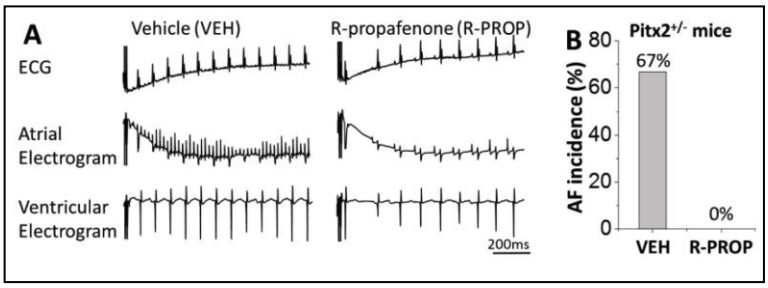


Figure 4: R-propafenone prevents AF induction in *Pitx2+/-* mice.

4.0 Inclusion/Exclusion Criteria

Table 2: Eligibility Criteria

Inclusion	Exclusion
atrial fibrillation	long-standing persistent AF
≥ 18 years of age	presents in AF/AFL the morning of the ablation procedure
undergoing AF ablation	the presence of any of the following in a patient without a permanent pacemaker or ICD: a) sick sinus syndrome indicated by the inability to previously tolerate an antiarrhythmic drug due to bradycardia b) sinus bradycardia with HR <50 bpm at the time of study drug administration c) right bundle branch block, left bundle branch block, or bifascicular block d) PR > 280 ms, or history of 2nd or 3rd degree AV block
	concomitant use of CYP3A4 inhibitors and CYP2D6 inhibitors
	previous surgical or catheter ablation for AF or MAZE procedure
	amiodarone use within 3 months prior to enrollment
	AAD (other than amiodarone) within 5 half-lives prior to AF ablation
	expected life span < 1 year
	creatinine clearance <30 ml/min
	reversible cause of AF (ie. thyrotoxicosis)
	unrevascularized coronary artery disease
	Canadian Class IV angina
	left ventricular ejection fraction <40%
	New York Heart Association Class III or IV symptoms

	previous heart transplantation
	planned heart transplantation or ventricular assist device
	cardiac/thoracic surgery ≤ 6 months prior to enrollment
	severe asthma or COPD
	Breastfeeding

5.0 Enrollment/Randomization

Recruitment and informed consent: Eligible subjects will be identified through StarPanel. A member of the study team will request an introduction from the clinical staff, if the patient agrees. Following the introduction, the team member will approach the patient prior to AF ablation. Information describing the study, why the research is being done and what will be learned will be provided. The risks as described above will be delineated. The benefits will be described as general scientific knowledge, along with potential treatment advances. No direct immediate benefit to the subjects is anticipated. Informed consent will be documented with the subject's signature using an IRB-approved consent form for this protocol.

Randomization: This will be a double-blind, randomized study. Eligible subjects will be randomized according to a permuted block scheme with a block size of balancing interval, varying randomly according to the outcome of a computer-generated random number. This ensures that the cumulative number of assignments to each treatment (R-propafenone, S-propafenone, or placebo/vehicle) will be in balance after each block of assignments had been made. A statistician will design the randomization table and enable the randomization tool within REDCap. After a patient enrolls for the study, the study nurse will determine the treatment assignment using the randomization tool in REDCap. The day prior to the ablation procedure, the study nurse will notify IDS to prepare the study drug. The morning of the AF ablation procedure, the study nurse will pick up the study drug from the IDS.

An initial set of 6 patients received (R)- or (S)-propafenone at a rate of 1mg/kg to establish subject safety. This was open-label and not randomized. The data was reviewed with the DSMB on Dec 1, 2016. The DSMB recommended continuing the study with the 1mg/kg dose.

The study drug will be administered via a peripheral i.v. at a rate of 1mg/kg (maximum of 100mg) over 10 minutes. This will occur following induction of general anesthesia at the start of the procedure (Table 3). Dr. Shoemaker will remain blinded to the treatment assignment as he is responsible for performing the study pacing protocol, electrogram interpretation, and interval measurement.

Table 3. Time Table for Study Protocol During AF Ablation

Time Elapsed (hrs:mins)	Standard of Care Activities	Research-only Activities
0:00→0:45	General anesthesia + TEE	
0:45→0:55	Sterile Prep and Drape	
0:55→1:00	1. Operator Scrubs 2. Official Time-out	Study Drug Infusion Starts (0:55)
1:00→1:10	Venous Sheaths Placed	Study Drug Infusion Ends (1:05)
1:10→1:20	CS Catheter Placed	
1:20→1:25		1. Blood Sample Drawn + TEE 2. EP Study (AVNERP, AERP) 3. Rapid Atrial Pacing Protocol 4. 1° Endpoint Determined 5. End of Research Protocol
1:25→end	AF ablation proceeds according to standard of care procedures	

6.0 Study Procedures

Encounter 1: *Enrollment and Informed Consent*

- Performed by study nurse in VU outpatient clinic or inpatient setting

Encounter 2: *Day of AF Ablation*

- Ablation will be scheduled by the patient's physician according to clinical availability and is often offset by 1-60+ days from time of enrollment. The scheduling of the AF ablation procedure and post-procedure care are determined by the availability of the operator and patient according to standard of care practices.
- AF ablation will be performed according to standard of care practice by the patient's physician. Table 3 summarizes an estimated time frame for the clinical activities and simultaneous performance of the study protocol
- After the patient arrives in the operating room, they will be undergo standard of care preparation for surgery including sterile drape and administration of general anesthesia using endotracheal intubation and mechanical ventilation.
- A transesophageal echocardiogram (TEE) is performed as standard clinical practice by the cardiac anesthesiolgy attending physician immediately following intubation. This is performed to exclude the presence of a left atrial appendage thrombus and to document the presence of any pericardial fluid at baseline. The TEE is then used later in the procedure to assist in visualization of the interatrial septum for transseptal puncture. As part of the research protocol TEE pictures of the LV will be recorded to allow for offline measurement of LV contractility.
- "Time out" will be performed at the start of the case by the primary operator. Immediately following time-out, the study drug infusion will be started (1 mg/kg over 10 minutes; maximum dose 100 mg).
- 15 minutes following completion of the study drug infusion, blood will be collected from central venous sheaths that are placed at the beginning of the case. Blood will be drawn from the sheaths immediately before the AF induction protocol. This will be to enable measurement of serum propafenone levels at the time of AF induction testing. Blood will be delivered to the core laboratory for Cardiovascular Translational and Clinical Research for DNA extraction and serum/plasma storage.
- Next, repeat TEE recordings of the LV will be performed.
- The AF ablation procedure will be performed as per standard of care by the primary operator and clinical team. Dr. Shoemaker and a study nurse will perform the study protocol.
- A standard electrophysiologic (EP) study will be performed at the start of the case after intracardiac catheter placement (including a decapolar CS catheter). The standard clinical EP study includes

measurement of AV block cycle length, AV node effective refractory period (ERP), atrial ERP, and ventricular block cycle length. Diastolic pacing threshold will be determined, and AERP will be tested at a pacing output of 2 times the diastolic threshold at 2 ms (standard of care). Following measurement of these conduction intervals, the pacing protocol to test for AF inducibility will be performed as follows:

- *Stimulation Protocol #1:*
  - A standardized protocol will be used to induce AF or atrial flutter (AFL).
  - This protocol is similar to common clinically used protocols to induce supraventricular tachyarrhythmias during performance of clinical EP studies.
  - The LA will be paced from the most distal CS electrode that can consistently capture the atrium. The CS catheter will be placed in standard clinical position.
  - 15 beat bursts of 20mA amplitude and pulse width of 2 msec will be used
  - We will wait 10 seconds between bursts and decrement by 10msec for each burst
  - Step 1: starting cycle length will be 250 msec and decrement down to 180 msec or loss of 1:1 atrial capture (AERP). If there is no 1:1 capture at 250ms, the starting cycle length will be 10ms greater than the coupling interval of the atrial ERP at a drive train of 600 ms.
  - Step 2 will be used if there is no sustained AF/AFL during Step 1, and it will consist of 5 atrial pacing bursts of 15 seconds at the lowest cycle length of which 1:1 atrial capture was achieved.
  - The parameters for this AF induction pacing protocol can be programmed into the cardiac stimulator system (EP-4 Cardiac Stimulator, St. Jude Medical Inc., St. Paul, MN) and performed automatically. **The time required to perform Step 1 of the research pacing protocol is ≤ 96 seconds, and Step 2 (conditional on step 1 being non-inducible) is ≤ 125 seconds**.
  - Sustained AF/AFL will be defined by a duration of >30 seconds.
  - Patients who develop sustained AF/AFL will either: 1) spontaneously convert prior to ablation; 2) convert during the ablation procedure (majority of patients); or 3) undergo direct current cardioversion (DCCV) at the end of the case. DCCV during AF ablation procedures is common and will be performed according to standard clinical practice at the discretion of the attending physician.
  - Some patients will develop AF/AFL during the standard clinical EP study. If it sustains >30 seconds it will be counted toward the primary endpoint, and the research AF induction pacing protocol will not be performed.

- Standard-of-care monitoring following an AF ablation is inpatient 23-hour continuous cardiac telemetry observation with specialized cardiac-trained nursing care. Cardiac-trained nurse practioners remain in the hospital overnight and communicate with the electrophysiology fellow on call at night for any patient care issues. Intravenous access is maintained post-procedure during their hospitalization.

7.0 Risks

Patients undergoing AF ablation are at risk for complications by nature of the procedure. These risks are inherent to the patient population studied here. The risks of obtaining blood samples from existing venous lines and determination of serum propafenone levels are minimal. There are risks associated with inducing atrial fibrillation and D/C cardioversion, however these are commonplace during AF ablation and add little to the risk inherent to this study population. There are risks associated with propafenone which include: new or worsened arrhythmias, unmasking Brugada syndrome, new or worsened congestive heart failure, conduction disturbances, altered pacemaker thresholds, agranulocytosis, exacerbation of myasthenia gravis, elevated ANA titers, and impaired spermatogenesis. Proarrhythmic effects may be more pronounced in patients with patients with liver or kidney disease, or patients taking other QT-prolonging drugs or inhibitors of CYP2D6, CYP3A4, and CYP1A2 isoenzymes. The majority of these risks are associated with chronic use, and are less applicable to the single dose regimen used for this study. Furthermore, the potential arrhythmic adverse effects are easily treated in the setting of the EP lab. There may be unknown risks of R- and S- propafenone, although these specific enantiomers have been previously used in clinical studies, both orally and as a single IV dose as we propose.

Major side effects of propafenone include bradycardia and proarrhythmia. We will monitor for these side effects with continuous cardiac telemetry. Patients are admitted for 23-hour observation following atrial fibrillation ablation. Propafenone will no longer be biologically active prior to discharge in the majority of

patients. In a small subset of patients who are poor metabolizers, duration of action may extend to 32 hours, however the peak effect will have been observed prior to discharge. At the discretion of the attending cardiac electrophysiologist, the decision will be made to withhold discharge in the case of unresolved symptomatic bradycardia or ventricular ectopy believed to compromise patient safety.

One risk of donating samples for genetic research may be the release of information that could link the patient to the stored samples and/or the results of the tests run on those samples. The release of this information could cause problems with insurance or future employment. We have mechanisms in place to protect against such risks.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Data and Safety Monitoring Plan

Oversight of the data and safety monitoring plan will be provided by a Data and Safety Monitoring Board (DSMB). The DSMB will meet at least twice a year and review data on adverse events, adverse drug reactions, data quality, and study recruitment. DSMB reports will be sent to the IRB at least yearly.

[redacted], who will serve as Chair, is Professor of Pediatrics and has over 20 years of experience in clinical research. The remainder of the DSMB is comprised of [redacted] (University of Maryland), [redacted] (University of Calgary), [redacted] (Vanderbilt, Department of Bioethics), and [redacted] an Associate Professor in the Department of Biostatistics and will serve as the statistical member of the DSMB. He is Assistant Director [redacted]. He has also served on the DSMB of 8 clinical trials since 2004.

Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others:

Definitions of adverse events: an adverse event (AE) is "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment".

Adverse events include:

- Worsening (change in nature, severity or frequency) of conditions present at the onset of the trial
- Patient / subject deterioration due to the primary illness
- Intercurrent illnesses
- Drug interactions
- Events related or possibly related to concomitant medications
- Abnormal laboratory values or changes of vital signs, as well as significant shifts from baseline within the range of normal, which the Investigator considers clinically significant.

Unexpected Adverse Drug Reaction: an unexpected Adverse Drug Reaction is “an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator’s Brochure for an unapproved investigational medicinal product)”. Definitions of serious adverse events or serious adverse drug reaction: during clinical investigations, adverse events may occur which, if suspected to be drug-related (adverse drug reactions), must be significant enough to lead to important changes in the way the medicinal product is developed (e.g., change in dose, population, needed monitoring, consent forms). This is particularly true for reactions, which, in their most severe forms, threaten life or function.

A serious adverse event/experience (SAE) or reaction is any untoward medical occurrence that at any dose:

1. results in death
2. is life-threatening
3. requires inpatient hospitalization or prolongation of existing hospitalization
4. results in persistent or significant disability/ incapacity (as per reporter’s opinion)

5. is a congenital anomaly/birth defect
6. is another medically important condition
7. The term "life-threatening" in the definition of "serious" refers to an event in which the patient is 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.

Important medical conditions that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Definition of severity of adverse events:

Mild: Causing no limitation of usual activities; the subject / patient may experience slight discomfort.

Moderate: Causing some limitation of usual activities; the subject / patient may experience annoying discomfort.

Severe: Causing inability to carry out usual activities; the subject / patient may experience intolerable comfort or pain.

Definition of adverse event causality:

The Investigator will determine causality of each adverse event by using the classification criteria: unlikely, likely, or not assessable.

Unlikely: The AE is considered by the Investigator to be due to a pre-existing condition, a known manifestation of the target disease, a recurrent condition, or is likely explained by environmental or diagnostic therapeutic factors or was pre-existing and did not deteriorate.

Likely: The AE occurred during or after administration of the study treatment or a pre-existing event worsened within an appropriate period of time, and at least one of the following criteria is applicable:
- the event could not be explained by the clinical condition or history of the subject, environmental or toxic factors, or other diagnostic or therapeutic measure;
- was an expected ADR associated with study treatment or a class-labeled drug effect;
- AE subsided or disappeared after withdrawal or dose reduction of study treatment; or
- AE recurred after re-exposure to study treatment.

Not assessable: There is insufficient or conflicting evidence for classifying the causality of the AE as likely or unlikely. Lack of information may apply for this situation.

Note: AEs with causality 'likely' or 'not assessable' are considered to be 'possibly drug-related.'

Adverse event reporting

Any adverse events (AEs) will be reported to the PI within 72 hours of notification of the event. The PI will notify the DSMB of any major adverse events. Any unanticipated problems involving risk to the participants or others will be discussed with the PI and DSMB. Non-serious AEs and incidences of noncompliance with the protocol will be reported to the IRB at the time of annual review.

Serious Adverse Events (SAEs) will be reported according to the following procedure:

The occurrence of serious adverse events will be reported to the Investigator within 24 hours after notification of their occurrence. The Investigator will report SAEs to the DSMB and Vanderbilt Institutional Review Board within 7 days of the Investigator's notification of the event.

In an unanticipated event of prolonged side effect, requiring prolongation of hospital stay, patients will be retained in the hospital until side effects have resolved. For minor side effects, where inpatient care is deemed unnecessary, follow- up will be maintained via phone or as outpatient if necessary. Patient and their families will be given the PI's contact number for reporting any other effects of medication following discharge.

Any newly discovered information which may affect the subject or their caregiver's decision to continue to participate in the study will be passed on to them as soon as possible. This may also result in a change to the consent form and review by the IRB.

9.0 Study Withdrawal/Discontinuation

Participants may withdraw from the study at any time by informing the study staff verbally or in writing. If an individual withdraws their consent, we will withdraw the participant. Contact information for the PI and study staff will be made available to the participant upon enrollment in the consent document. Any remaining biological samples and data will be destroyed. Any data or biological samples that have been used for research prior to their withdrawal request will not be withdrawn and destroyed.

A participant may be withdrawn from the study by the PI if any of the following occurs:

i. A procedural complication occurs prior to completion of the study protocol that requires the AF ablation procedure to be aborted, or precludes collection of study data.

ii. The patient becomes hemodynamically unstable for any other reason that requires the AF ablation procedure to be cancelled prior to completion of the study protocol, or precludes the collection of study data

iii. The primary operator determines it is in the patient's best interest to forego completion of the study protocol

iv. The patient spontaneously converts to AF or atrial tachycardia/flutter prior to completion of study protocol

10.0 Statistical Considerations

We will test the hypothesis that *(R)*-propafenone is superior to *(S)*-propafenone and *(S)*-propafenone is superior to (P)-placebo at suppressing AF in patients undergoing an AF ablation. Our primary analysis will use multivariable regression to test whether our primary determinant (*R*-propafenone versus *S*-propafenone) and (*S*-propafenone versus placebo) significantly reduces the primary endpoint of inducibility of AF after study drug infusion (yes/no). Inducibility of AF will expressed as an ordinal variable based on the stage of the induction protocol that AF was induced (Stage 0, Stage 1, Stage 2). Patients who had only AFL induced will not be included in the primary analysis, but will be included in a secondary analysis. Adjustment will be made for age, sex, and other established AF risk factors. To avoid over-fitting of multivariable regression, a 10:1 ratio for degrees of freedom per the less frequent outcome event will be used in our modeling. The sample size of 243 (108 for *R*-propafenone, 108 for *S*-propafenone, and 27 for placebo), was selected to power an exploratory pharmacogenomics aims that analyzes common variants at the 4q25 AF risk allele. A blinded interim analysis reviewed by the DSMB in March 2019, demonstrated a difference was emerging between the treatment groups for the new primary ordinal endpoint with a P-value of 0.075, thus continued enrollment was recommended.

11.0 Privacy/Confidentiality Issues

The conduct of genetic studies raises specific issues with respect to protection of human subjects. We describe here mechanisms in place at Vanderbilt through IRB policy to protect against such risks; these apply to all studies described below. All records are retained on password-protected computers accessible only to members of the study team. Computers containing these records are only connected to networks if they include appropriate firewalls and security measures. Deidentified records and DNA samples may be shared with other investigators who have IRB-approved protocols and who agree to comply with the protections provided at this institution. These research materials are transferred only by secure methods. The identity of any individuals and their families are not to be revealed in any publication without their written informed consent.

12.0 Follow-up and Record Retention

The expected duration of this study is estimated to be 4 years. The study results will be retained for at least six years after the study is completed. At that time, the research information, with the exception of genetic information, not already in the medical record will be destroyed. Genetic information will be kept for an undetermined period of time for future gene research.