A randomized controlled trial of RyR2 inhibition with dantrolene and susceptibility to ventricular arrhythmias in patients with structural heart disease

Principal Investigator:

William G. Stevenson, MD
Professor of Medicine
Arrhythmia Section
Division of Cardiovascular Medicine
Vanderbilt University Medical Center

Co-Principal Investigator:

M. Benjamin Shoemaker, MD, MSCI
Assistant Professor of Medicine
Division of Cardiovascular Medicine, Arrhythmia Section
Vanderbilt University Medical Center

Table of Contents:

1.0 BACKGROUND

1.1 Structural heart disease causes calcium leak from the cardiac ryanodine receptor 2 channel (RyR2)

This is a randomized controlled trial that aims to study whether RyR2 inhibition with dantrolene reduces susceptibility to ventricular arrhythmias and improves hemodynamics in patients with structural heart disease. **Figure 1** is the concept diagram of our proposed trial. The idea to be tested is that structural heart disease results in post-translational modifications to RyR2, which is the intracellular Ca channel, causing Ca to leave the sarcoplasmic reticulum and raising the concentration of intracellular Ca. This causes arrhythmias through delayed after depolarizations (DADs) that can trigger ventricular tachycardia (VT) and ventricular fibrillation (VF) and also are believed to contribute to changes in refractoriness and conduction that promote scar-related reentrant VT. Intracellular Ca overload also reduces cardiac Ca cycling which reduces cardiac contractility. Both conditions promote VT and VF which are the major causes of sudden cardiac death (SCD) in patients with structural heart disease.

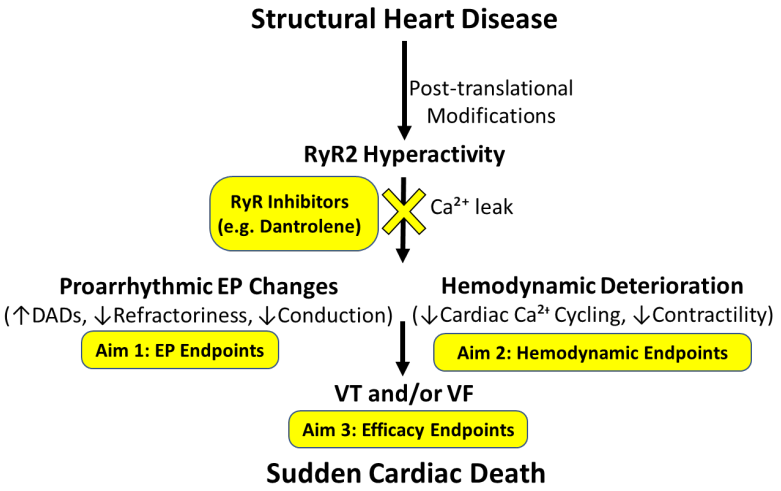

Figure 1: Concept Diagram for a Future Phase II Clinical Trial.

1.2 Ventricular arrhythmias due to structural heart disease are a major cause of sudden cardiac death

An estimated 150,000 to 450,000 individuals suffer SCD per year in the U.S, and approximately 90% are estimated to have underlying coronary artery and/or structural heart disease (**Figure 2**).[1-4] Recent reports estimate VT or VF are detected as the initial rhythm in ~20% of SCD cases, however when there is a shorter time between collapse and initial rhythm detection this estimate increases to 75-80%.[2,4] Along with improved strategies to identify at-risk individuals and increase the rate of successful resuscitation, new therapies are desperately needed to prevent the recurrence of VT/VF. Implantable cardioverter defibrillators (ICDs) can successfully terminate VT/VF, but do not prevent it. ICD shocks are associated with increased mortality, heart failure, post-traumatic stress disorder, and healthcare utilization.[5-10] Furthermore, once VT occurs, it is likely to recur, thus therapies to prevent recurrent VT/VF (e.g. antiarrhythmic drugs [AADs]) are of great importance.[11-13]

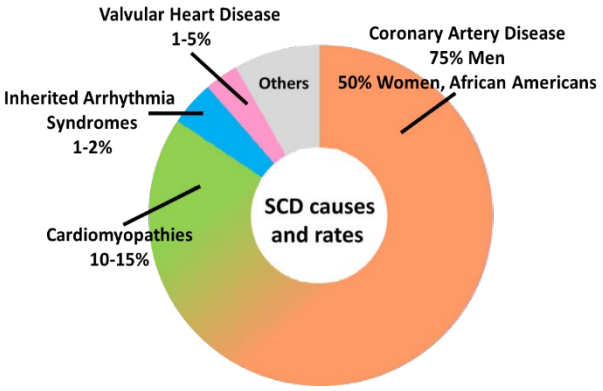

Figure 2: Structural heart disease due to CAD, cardiomyopathy, and valvular heart disease is a major cause of SCD**.** Image: Hayashi et al. (2015)

1.3 New pharmacologic therapies are needed to prevent VT/VF in structural heart disease.

In 2018 there are fewer AADs available for clinical use than 20 years ago.[14] Discouraging the development of new agents are the problems associated with AADs that target ion channels on the cell surface. They possess a narrow therapeutic window with higher drug levels leading to pro-arrhythmia that is worse in structural heart disease. Currently available AADs target the cardiac sodium (Na) current, rapidly-activating potassium (K) current, beta-adrenergic stimulation, or multiple currents (e.g. amiodarone) and all have significant limitations. Class IC agents that predominantly block Na channels, (e.g. flecainide, propafenone) increase mortality in patients with structural heart disease and are relatively contraindicated in this population (CAST).[15] K channel blockers prolong the QT interval, can cause polymorphic VT and have disappointing efficacy for VT. The risk of drug-induced torsades-de-pointes, a life-threatening VT, is

increased in structural heart disease due to down regulated K channels and abnormal calcium (Ca) homeostasis.[16,17] Amiodarone is the most effective AAD for preventing recurrent VT/VF, but 10% of participants treated with amiodarone still have recurrent VT within one year and ~20% discontinue it due to potentially severe non-cardiac toxicities.[12] Furthermore, intolerance to amiodarone is more common at the doses used for refractory VT/VF.[13]

1.4 Mechanisms promoting monomorphic and polymorphic VT in structural heart disease.

In structural heart disease, monomorphic VT is most often due to reentry in regions of scar comprised of myocyte bundles and fibrosis. As Dr. Stevenson and others showed in early landmark studies, the arrhythmia substrate is often complex, such that numerous reentrant circuits are present giving rise to multiple different monomorphic VTs (pleomorphism) or polymorphic VTs.[18-23] In addition to reentry, automaticity and triggered activity are potentially important mechanisms contributing to VT in structural heart disease as they can provide the triggers necessary to initiate reentry, or in some cases may be the dominant mechanism.[24-26] A major mechanism for triggered activity are DADs due to intracellular Ca overload. Abnormal Ca handling is well recognized in models of heart failure and hypertrophy. The diminished cell-to-cell coupling associated with fibrosis in areas of scar may also increase susceptibly to triggered automaticity resulting in an automatic VT or initiating a reentrant VT.[24-26] Spontaneous Ca leak also inactivates Na channels which would be predicted to slow conduction velocity[27] and activates SK channels which shortens refractoriness[28-32], both of which may extend the excitable gap to promote scar-related reentry. Conditions that increase intracellular Ca are common in heart failure, especially β-adrenergic stimulation and increased heart rate. Relevant to our proposal is that dantrolene at clinically achievable doses has been found to reduce spontaneous Ca leak and DADs in ventricular cardiomyocytes isolated from failing human hearts without changing actional potential duration or reducing contractility.[33]

A major knowledge gap we seek to address is the clinical potential of selectively targeting intracellular Ca to treat VT/VF in structural heart disease. Currently available AADs that reduce intracellular Ca are also potent Na channel blockers (e.g. flecainide, propafenone). Na channel blockade reduces conduction velocity and promotes scar-related reentry, thereby negating the benefit of RyR2 inhibition for reducing triggered activity in structural heart disease. Dantrolene inhibits RyR2-mediated Ca leak but does not block the Na channel.[27] By reducing spontaneous Ca leak, dantrolene may reduce Ca mediated Na channel inactivation thereby reversing slowed conduction in abnormal tissues. It also may reduce SK channel activation thereby reversing shortened refractoriness in abnormal tissues. Accordingly, an overarching goal of our work is to investigate the ability of RyR2 inhibition with dantrolene to speed conduction and prolong refractoriness in *diseased (low voltage) tissue*, and to assess whether it has a similar effect in *higher voltage tissue* that may have less fibrosis. Taken together, we seek to test the idea that selective RyR2 inhibition with dantrolene will reduce VT/VF in structural heart disease by reversing proarrhythmic changes in conduction and refractoriness that promote scar-related reentry.

1.5 Antiarrhythmic drugs and VT ablation: complementary approaches for refractory VT.

Presently ICDs are the major protection for prevention of SCD in patients with structural heart disease. Over 90,000 are implanted in the U.S. annually and within 2 years of implant up to 30% of patients will experience an ICD shock for an arrhythmia.[34] ICD shocks are associated with increased risk for heart failure and mortality despite the presence of the ICD and are associated with increased health care resource utilization.[5-7] Shocks also reduce quality of life and cause post-traumatic stress disorder.[8-10] Available

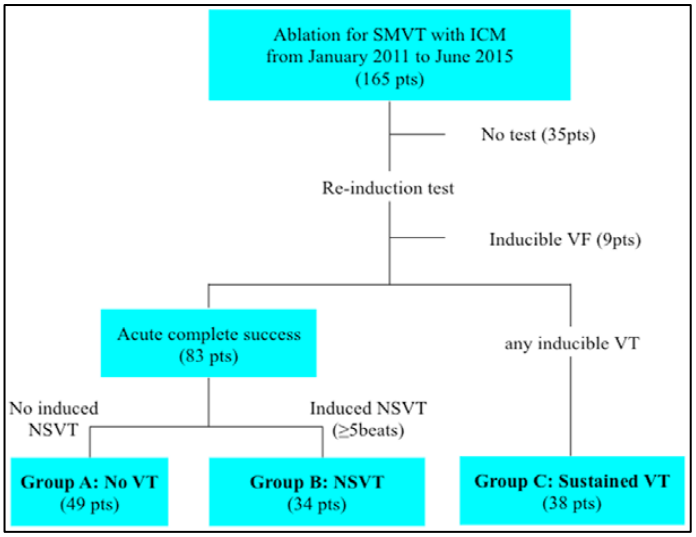

Figure 3: Ventricular arrhythmias induced using programmed stimulation following VT ablation. Image: Fujii, Stevenson and colleagues (2017).

AADs can reduce VT but have limited efficacy and significant toxicity[12], therefore ablation is an alternative therapy used to prevent VT.

The efficacy of ablation is also limited. In multicenter trials approximately ½ of patients experience a recurrence of VT during follow-up, although the VT frequency is reduced for many. VT ablation is superior to escalating AAD therapy in patients with a prior myocardial infarction who have VT during AAD therapy, however, many continue to require AADs following ablation. [11,13,35] The acute effect of catheter ablation is typically assessed using programmed stimulation to induce VT at the end of the procedure. **Figure 3** presents the breakdown of ventricular arrhythmias that were acutely inducible following VT ablation by Dr. Stevenson and colleagues (N=165 patients).[36] These data support the design of a clinical trial proposing to study ventricular arrhythmias that remain inducible in patients following a VT ablation procedure. Monomorphic VT that lasted >30 seconds was induced in 38 patients, VF was induced in 9 patients, and non-sustained VT (NSVT) was induced in 34 patients. These arrhythmias are not ablated because an ablation target site could not be found or they do not match a VT that had been observed clinically (e.g. NSVT, VF, monomorphic VT that is much faster than spontaneously occurring VT), and therefore predict a lower risk of VT recurrence.[37] However, a major finding of this study was that inducible NSVT was a marker for VT recurrence. Similarly, inducible polymorphic VT/VF, and rapid monomorphic VTs are associated with an increased risk of spontaneous VT in other studies.[37,38] Thus inducibility of these arrhythmias indicates the continued presence of an arrhythmia substrate which lends itself to the electrophysiologic assessment of therapy, particularly in paired, blinded assessments. In our proposal we selected a duration of 10 seconds as our definition for an inducible ventricular arrhythmia, as this has been used in other studies of drug assessment and reduces the likelihood of VT/VF requiring cardioversion.[39]

1.6 The effect of dantrolene on muscle strength and respiratory mechanics.

A side effect of dantrolene is mild muscular weakness[40,41]. The impact of dantrolene, specifically Ryanodex, on respiratory mechanics, however, is unclear. Dantrolene is typically given to patients with malignant hyperthermia who are intubated, who have been given neuromuscular blocking drugs, and who are under general anesthesia, since the triggers for malignant hyperthermia require general anesthesia or paralytic administration. We seek to measure the effect of the administration of dantrolene/Ryanodex on incidence of interventions to support respiration (bag/mask ventilation, CPAP, BiPAP, LMA placement, tracheal intubation) in the perioperative period. We will also compare additional markers of respiratory mechanics and strength (secondary endpoints) between groups including negative inspiratory force, dynamometer measured grip strength, accelerometer measured twitch strength and train-of-four, ventilation (respiratory rate, tidal volume, minute ventilation), markers of gas exchange (arterial pH, pCO2, pO2, base excess), and electrolytes.

2.0 RATIONALE AND SPECIFIC AIMS

Sudden cardiac death (SCD) accounts for up to 15-20% of all deaths per year in the U.S. and ventricular tachycardia (VT) and ventricular fibrillation (VF) from structural heart disease are major causes.[2,42] To prevent these deaths, approximately 90,000 implantable cardioverter defibrillators (ICDs) are placed annually in the U.S. Up to 30% of these patients will have VT/VF terminated by the ICD within 2 years, often by a shock.[34,43] Unfortunately, ICDs terminate VT/VF after it occurs, *but do not prevent it*. The occurrence of VT/VF is associated with increased risk of heart failure hospitalizations, and death, and once VT occurs, it is likely to recur.[11-13]

For decades chronic therapy with antiarrhythmic drugs (AADs) has been the major option to prevent recurrence of VT/VF. Currently available AADs target the cardiac sodium (Na) current, rapidly-activating potassium (K) current, beta-adrenergic stimulation, or multiple currents (e.g. amiodarone). Through clinical trials (CAST[15]) and clinical experience, it was recognized that Na channel blockers increased mortality in patients with structural heart disease through scar-related VT. Similarly, QT-related proarrhythmia is a risk with K channel blockers. Amiodarone is the most effective AAD for preventing recurrent VT/VF, but 10% of participants treated still have recurrent VT within one year and ~20% discontinue it due to pulmonary toxicity,

thyroid toxicity and symptomatic bradycardia (OPTIC Trial).[12] Taken together, a major unmet need is the development of a new class of AADs to prevent VT/VF in structural heart disease.

Research by our group and others has focused on the role of diastolic calcium (Ca) leak via the RyR2 channel as a cause of delayed afterdepolarizations (DADs) and polymorphic VT in rare genetic disorders such as catecholaminergic polymorphic VT (CPVT).[44,45] This led to the discovery that drugs which stabilize the RyR2 channel eliminate DADs and polymorphic VT in CPVT; this includes not only the experimental probe drug ent-verticilide but also clinically used agents such as flecainide and its derivatives and dantrolene, which is being studied here.[46,47] The recognition that structural heart disease results in post-translational modification of RyR2 and diastolic Ca leak is consistent with the idea that Ca leak is also involved in generating VT/VF in more common diseases.[48-51] Accordingly, the **overarching hypothesis** to be tested is that RyR2 hyperactivity in patients with structural heart disease drives proarrhythmic changes in refractoriness and conduction, and decreases cardiac contractility, which promotes VT/VF (**Figure 1**).

Despite the success of flecainide to treat CPVT, it is relatively contraindicated in patients with structural heart disease because of its Na-channel inhibition. Dantrolene, a currently available drug that inhibits RyR2, but has no Na or K channel activity, will be used as a tool to study RyR2 modulation. Here, we propose a randomized controlled trial of dantrolene versus placebo in patients with structural heart disease referred for VT ablation to evaluate electrophysiologic, hemodynamic, and arrhythmia prevention endpoints. Dantrolene's inhibition of RyR1 will also be studied to define its effect on muscle and respiratory strength in this clinical population, which will be important if dantrolene is to be considered for repurposing as an antiarrhythmic drug. Accordingly, we propose the following Specific Aims:

Aim 1: **To conduct a randomized, placebo-controlled trial of dantrolene to study the effect of RyR inhibition on cardiac electrophysiology, hemodynamics, arrhythmia inducibility, muscle strength, and respiratory mechanics in patients with structural heart disease referred for VT ablation.**

Aim 2: To explore the pharmacokinetic/pharmacodynamic relationship of I.V. dantrolene and its short-term effect on cardiac electrophysiology, hemodynamics, and muscle and respiratory strength.

3.0 ANIMAL STUDIES AND PREVIOUS HUMAN STUDIES

3.1 Experimental evidence: RyR2 inhibitors treat VT/VF and improve hemodynamics

Experimental evidence from animal models of VF and scar-related VT demonstrates the efficacy of RyR2 inhibition with dantrolene to treat and prevent VT/VF.[52] In a swine model, treatment with I.V. dantrolene administered during VF decreased the number of shocks required for successful defibrillation, the time to return of spontaneous circulation (defined as systolic blood pressure >60 mmHg), and the number of episodes of refibrillation.[52] Dantrolene (2 mg/kg) was administered during CPR 3 minutes prior to attempted defibrillation, suggesting the myocardial uptake and pharmacodynamic effect of dantrolene on cardiac electrophysiology is rapid. Refibrillation was initiated by a premature ventricular complex (PVC) in 96% of cases and the proportion of animals refibrillating was 71% (5/7) in the control group compared to 27% (3/11) in the dantrolene group, supporting the ability of dantrolene to reduce spontaneous ventricular ectopy.

In preliminary data generated by Dr. Bjorn Knollmann (unpublished), mouse infarct models were generated by coronary ligation which resulted in a chronic transmural infarct involving ~50% of the LV. A wildtype model and a transgenic heterozygous calsequestrin-2 (*Casq2*+/-) knockout model were used. Heterozygous *Casq2* mice have hyperactive RyR2 Ca release, but do not exhibit catecholaminergic polymorphic VT (CPVT) as seen in homozygous *Casq2* mice. 3-4 weeks after coronary ligation, mice underwent programmed stimulation using transesophageal pacing. Monomorphic VT was inducible. Treatment with dantrolene (Ryanodex) or ent-verticilide (an investigational RyR2 specific inhibitor) significantly reduced inducible VT and ventricular ectopy (premature ventricular complexes) compared to vehicle (placebo) (**Figure 4**). These data support the idea that RyR2 inhibition with dantrolene reduces scar-related monomorphic VT in experimental models and supports our plans to advance this research into clinical investigation.

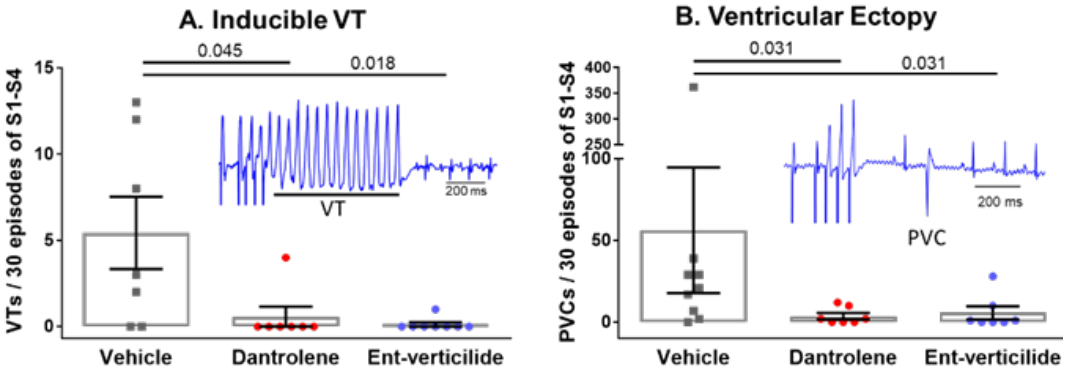

Figure 4: RyR2 inhibition with dantrolene (Ryanodex) and ent-verticilide reduces inducible VT and ventricular ectopy compared to vehicle (placebo) in a Casq2+/- chronic infarct mouse model (unpublished data courtesy of Bjorn Knollmann, MD/PhD).

3.2 Single dose and chronic oral exposure to dantrolene in humans

In an unpublished Phase 1 study conducted to support the FDA application for Ryanodex (approved in 2014), 30 healthy volunteers were given a single I.V. dose of Ryanodex (1.0, 1.75, 2.0, 2.25, and 2.5 mg/kg). Adverse reactions were dose dependent and the most common were flushing (27% of subjects) and somnolence (13%). All adverse events were classified as mild/moderate and spontaneously resolved without sequelae. There were no severe or serious adverse events and no dose limiting toxicity. There were no significant changes from baseline in vital signs or ECG assessments. Recently, the experience of patients prescribed chronic oral dantrolene for the treatment of muscle spasticity was reported.[53] In 20 patients with a mean of 10.9 years of follow-up on oral dantrolene there were no major cardiac events, no QTc or QRS prolongation, and no change in cardiac function on serial echocardiograms.

3.3 A pilot study to demonstrate the feasibility of I.V. dantrolene to study the effects of RyR inhibition in patients with structural heart disease undergoing VT ablation

In preparation for our randomized controlled trial of dantrolene, an open label pilot study was recently completed. Five participants were enrolled and dantrolene (1 mg/kg I.V) was given. The study protocol was successfully performed. Evidence of a pharmacodynamic effect was observed as muscle strength measured by twitch height was found to decrease by 60%. Cardiac electrophysiology and hemodynamic measurements were feasible. No serious adverse events (SAEs) occurred. Two adverse events (AEs) occurred. One participant experienced a possibly drug-related exacerbation of pre-existing sinus node dysfunction, which required a temporary period of ventricular pacing and an increase in vasopressor dose, which resolved by the time the participant left the EP lab. As a result of this AE, the requirement of a permanent pacemaker or implantable cardioverter defibrillator was added to the inclusion criteria. The other AE was not related to the study drug and was an oropharnygeal laceration that likely occurred during laryngeal mask airway (LMA) placement prior to study drug administration. It required intubation overnight for airway protection. The results of the pilot study were reviewed by the data safety monitoring board on April 15th, 2020, which recommended proceeding with the RCT phase of the study and remaining at the 1 mg/kg dose of dantrolene.

4.0 INCLUSION/EXCLUSION CRITERIA (TABLE 1)

Inclusion
≥18 years of age
Able to give written, informed consent
Referred for catheter-based VT ablation or PVC ablation
Structural heart disease (LVEF < 50%, history of prior myocardial infarction, cardiac sarcoid or valvular heart disease)
Permanent pacemaker or implantable cardioverter defibrillator
Exclusion
Mechanical ventricular support (e.g. LVAD, ECMO)
NYHA class IV heart failure
LVEF <20%
Severe renal insufficiency (GFR<30 mL/min)
Morbid obesity (BMI≥ 40 kg/m^2)
Chronic liver disease (Child Pugh class A-C)
Current use of calcium channel blockers
Neuromuscular disorder (e.g. muscular dystrophy)
Chronic obstructive pulmonary disease or restrictive lung disease requiring oxygen therapy or history of intubation
Pregnant or nursing
History of dysphagia

Table 1. Eligibility Criteria

5.0 ENROLLMENT AND RANDOMIZATION

Recruitment and informed consent: Eligible subjects will be identified by either: 1) Arrhythmia Clinic nurses or physicians will contact a member of the study team if they are scheduling an eligible subject for VT ablation or PVC ablation, or 2) the study coordinator will review the EP Lab schedule for VT and PVC ablation cases and screen their medical record for eligibility. The study coordinator will then request the clinical staff to obtain permission from the patient to contact them about research. If the patient agrees, a member of the study team will approach the patient in person in Arrhythmia Clinic, Vanderbilt University Hospital, or by phone prior to ablation. Information describing the study, why the research is being done and what will be learned will be provided. The risks as described in this protocol 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. The patient will be given either a hard or electronic copy of the informed consent document. At least 24 hours will be given for the patient to consider participation after which the study coordinator will recontact the patient. If the patient desires to participate, informed consent will be documented with the subject's signature using the IRB-approved consent form for this protocol. This will occur in Arrhythmia Clinic or Vanderbilt University Hospital. If participation is being discussed with the patient by phone, a tentative agreement to participate will be obtained and the study coordinator will meet the patient on the morning of the procedure at Vanderbilt University Hospital to sign the informed consent document.

Randomization: 84 participants will be randomly assigned in a 2:1 ratio to treatment (dantrolene) or control (placebo). A computer-generated randomization list will be prepared by the study statistician using the stratified permuted block randomization, where block size varies randomly from 4 or 6 to ensure overall balance across treatment arms. Randomization will be stratified by 1) amiodarone use within the past 21 days defined as chronic oral use or >5 grams cumulative dose including ongoing use and 2) planned PVC vs. VT ablation. The study statistician will generate the allocation schedule but will remain blinded to the treatment assignment.

Dropouts and replacement: To ensure adequate statistical power, we will account for up to a 20% rate of patient withdrawal from the study prior to randomization (for reasons such as non-study related procedural complications, hemodynamic instability, prolonged case duration), therefore up to 105 participants may need to be enrolled for 84 participants to complete the study.

Study Drugs: The investigational compound is dantrolene sodium (RYANODEX, Eagle Pharmaceuticals Inc, Woodcliff Lake, NJ). Ryanodex is supplied in a single dose vial by the manufacturer and it is reconstituted with 5 mL of sterile water prior to use. Each vial contains 250 mg of Ryanodex. Based on results from the pilot study, Ryanodex will be administered as an I.V. push over 3 minutes at a dose of 1 mg/kg. Ryanodex was tested in a Phase I study and safely tolerated in doses ranging from 1 mg/kg to 2.5 mg/kg. It is approved for use in adults with malignant hyperthermia at a recommended dose of 2.5 mg/kg. The calculated dose is based on measured body weight with a maximum dose of 250 mg used in our trial.

6.0 STUDY PROCEDURES

6.1 VT or PVC Ablation will be Performed According to Standard-of-Care Clinical Practice

Duration (mins)	STANDARD CLINICAL PROTOCOL	RESEARCH PROTOCOL	
		EP team	Anesthesia team
---	Pre-procedure holding room		Pre-drug muscle strength (grip and NIF)
60-140*	Clinical ablation procedure completed		
5	Post-ablation EPS & inducibility	Pre-drug EPS & inducibility	NIF, tidal volume, minute ventilation, ABG
5	Post-ablation ICE survey	LVERP & conduction time testing	
5	Post-ablation hemodynamics	Pre-drug hemodynamics	
3		Study drug given	
10		PK/PD blood sampling with measurement of ECG intervals and Fick cardiac output	NIF, tidal volume, minute ventilation, ABG
5			
5			
5		LV ERP & conduction time testing	
2		Post-drug hemodynamics	
5		Post-drug inducibility testing	
---	Procedure completed		
5	Post-procedure care unit		2-hour post-drug muscle strength (grip and NIF) and ABG

Table 2. List of peri-procedural clinical and research activities. NIF: negative inspiratory f
ICE: intracardiac echocardiogram; LVERP: left ventricular effective refractory perio
pharmacokinetics/pharmacodynamics.

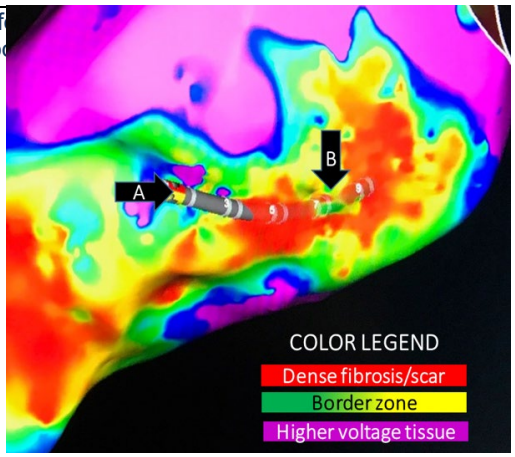

Figure 5: A voltage map of the LV endocardial surface. From a patient undergoing VT ablation at Vanderbilt who met our proposed eligibility criteria, the pacing catheter is positioned in the scar border zone. Pacing for conduction time and ERP measurements was performed from the distal bipolar electrodes (A) and a more proximal electrode (B).

This is a description of the clinical activities for VT or PVC ablation. **Table 2** provides an overview of the timeline for clinical and research activities. VUMC is a high-volume PVC and VT ablation center that in 2018 performed greater than 250 VT ablations. Dr. Stevenson directs the VT ablation research program at VUMC and has been a pioneer in the field. He, along with other international experts, authored the 2019 Heart Rhythm Society's expert consensus statement on catheter ablation for VT. [54] VT or PVC ablation will be performed according to our standard clinical practice. When feasible, amiodarone is held for 48 hours prior to ablation. Other oral AADs will be held 24 to 48 hours prior to the procedure and continuous I.V. AADs will be held 4 to 6 hours prior to the beginning of the procedure. Sedation during PVC or VT ablation and study protocol will be per usual care and remain at the attending anesthesiologist's discretion. Typically, PVC or VT ablations are done under monitored anesthesia care (MAC) with varying levels of sedation from moderate to deep sedation, although general anesthesia with or without a secured airway can occur. Propofol and dexmedetomidine infusions are typically used to achieve the desired level of anesthesia. The procedure will be performed with sterile preparation. The left ventricle will be accessed via either retrograde aortic or trans-septal access. Sheaths are placed in the femoral veins and artery for central vascular access. Hemodynamics are monitored with a radial or femoral arterial line, and a PA catheter. Standard EP catheters include: 1) a quadripolar catheter placed in the right ventricle, 2) a multipolar mapping array, and 3) an irrigated-tip radiofrequency ablation catheter. An intracardiac echocardiogram (ICE) catheter is also placed. A pre-ablation survey with an intracardiac echocardiogram (ICE) is performed to inspect the heart for baseline abnormalities (e.g. thrombus, pericardial effusion). A 3D mapping system is used to create an electroanatomic voltage map where low voltage areas (bipolar electrograms <1.5 mV) identify regions of fibrosis/scar to help localize the potential VT substrate. An EP study is performed, which includes programmed ventricular stimulation to induce VT. The programmed ventricular stimulation protocol is pacing at a fixed rate for 8 beats at a cycle length of 400-600 ms followed by early extrastimuli. In most cases, the clinical VT, which resembles spontaneous VT recorded by an ICD or on ECG, can be induced. Non-clinical ventricular arrhythmias are also often encountered which are usually monomorphic VTs that differ from the spontaneously observed VT (typically having a faster rate than the clinical VT) or polymorphic VT. The clinical VT and nonclinical monomorphic VTs that have a cycle length slower or only 20 ms shorter than clinical VT are targeted for ablation starting in low voltage areas. The reentry circuit may be identified by mapping during VT (activation mapping). More frequently ablation targets are identified during sinus rhythm as areas in the scar where pacing replicates the VT morphology (pace mapping), or electrograms have characteristics of abnormal conduction consistent with reentry substrate. When it is suspected that ablation has eliminated the targeted VTs, programmed ventricular stimulation is repeated. If any sustained monomorphic VTs are induced, additional ablation may be performed if there are additional regions that can be identified for those VTs. Acute procedural success is defined as 1) absence of any inducible sustained monomorphic VT, or 2) modification of the arrhythmia substrate if the clinical VT is no longer inducible, but other VTs are induced. Acute procedural failure is defined as continued inducibility of the clinical VT. Following completion of ablation, post-ablation hemodynamics are recorded, and an ICE survey is performed. For stable patients undergoing elective VT ablation, such as our study population, post-anesthesia recovery is performed in the EP Lab and the patient leaves awake, extubated, and hemodynamically stable.

6.2 List of Encounters Detailing the Clinical and Research Activities

Encounter 1: *Enrollment and Informed Consent*

- Performed by the study team in the Vanderbilt Arrhythmia Clinic or Vanderbilt University Hospital.

Encounter 2: *Time of Ablation*

- The participant will arrive the morning of ablation to the EP pre-op holding room and undergo standard preparations for ablation according to the clinical protocols (e.g. labs, vitals, exam, procedural consent).
- The participant will undergo baseline muscle (grip) and respiratory (NIF) strength testing in the pre-op area.
- The participant is then transported to the operating room (EP lab) where standard of care ablation is performed under the direction of the clinical team. See section 6.1 for full details.
- Research activities will begin after completion of the ablation portion of the ablation procedure. Research activities occur concurrently with clinical activities and are performed by a dedicated physician and nurse team to avoid extending overall case time.
- The clinical post-ablation EP study with programmed ventricular stimulation will serve as the baseline (pre-dantrolene) assessment of cardiac electrophysiology parameters (conduction and refractoriness) and ventricular arrhythmia inducibility. Data analyses are performed offline following the case to avoid adding procedural time. See sections 6.3 to 6.6 for full details.
- Hemodynamic monitoring is routinely performed during ablation with an arterial line and pulmonary artery (PA) catheter (aka. Swann-Ganz Catheter). See sections 6.7 to 6.10 for full details. The pre-dantrolene arterial blood pressure mean right atrial, PA systolic, PA diastolic, mean PA pressure, and pulmonary artery wedge pressures will be recorded.
- Blood samples will also be drawn for calculation of a Fick cardiac output and for the pharmacokinetic (PK)/pharmacodynamic (PD) analysis. See section 6.12 for full details of the PK/PD analysis. A 2 mL sample from the femoral venous, arterial, and distal PA catheter will be collected.
- I.V. dantrolene will be administered over 3 minutes. Peak drug levels occur rapidly allowing the data collection protocol to proceed without delay.
- The post-dantrolene hemodynamics will be recorded.
- The post-dantrolene EP study with programmed ventricular stimulation will be repeated.
- A 2 mL sample from the femoral venous, arterial, and distal PA catheter will be collected at 5, 10, 15, and 20 minutes post-dantrolene. The total amount of blood collected during the ablation is 30 mL.
- Respiratory mechanics (tidal volume, rate, minute ventilation) and an arterial blood gas (ABG) will be measured at 0, 10, and 20 minutes, and need for additional respiratory support will be assessed continuously as is standard care (see Section 6.13).
- The clinical and research activities will be complete, and the participant will leave the operating room awake, extubated, and hemodynamically stable.
- The participant will undergo baseline muscle (grip) and respiratory (NIF) strength testing in the post-op recovery area and an ABG will be measured 120 minutes after study drug was initiated

Encounter 3: *Post-ablation Day 0-2.*

- Following ablation, the participant will be returned to the EP holding room. It is clinical standard-of-care for patients to remain in the hospital at least overnight for monitoring. They will be under nursing observation and on continuous cardiac telemetry and oximetry. They will be on strict bedrest until post-operative Day #1 and they will be ambulated with assistance the morning after the procedure.
- 2 mL blood samples will be drawn at intervals of 4 to 12 hours for up to 48-hours during the post-ablation period. No more than 15 mL total will be drawn. We will use indwelling catheters whenever possible for blood collections. All patients will have indwelling catheters throughout the duration of study, and we do not anticipate needing to perform phlebotomy with a fresh stick during the study. See section 6.12 for full details.

- The research protocol is complete when the participant is discharged from the hospital.

6.3 EP Catheter Placement and Pacing Protocol

The scar and its border zone (defined by peak to peak bipolar voltage of 1 to 2 mV) will have been defined during the clinical procedure (**Figure 5**). Following ablation a 10-pole catheter with 2-8-2 mm interelectrode spacing is positioned in a ventricular area remote from the scar (peak to peak bipolar electrogram voltage > 2 mV) ensuring that electrograms of adequate quality for measurement are present on at least two of the electrode pairs, and that the pacing threshold on one of these pairs is less than 10 ma. The position of this catheter is marked on the 3D-electroanatomic map so that it can be positioned in the same location when the pacing protocol is repeated following administration of the study drug. Pacing threshold is determined, and stimulation is performed at 2-times the threshold. An electrode that has stable capture with pacing is selected for pacing and bipolar signals are recorded from the other electrodes. Pacing is performed at a basic cycle length (CL) of 600 ms (100 beats per minute [bpm]) for 8 beats with the addition of a single extrastimulus followed by a 3 second pause. The initial coupling interval of the extrastimulus is 400 ms. It is then decremented by 10 ms down to loss of capture on two consecutive attempts to define the effective refractory period. The 10-pole catheter is then repositioned to the scar borderzone. The above steps are then repeated at this location. The electrograms from the EP study are exported for offline measurement and analysis. A conduction curve will be plotted to define the conduction time (primary endpoint) and conduction restitution (secondary endpoint).

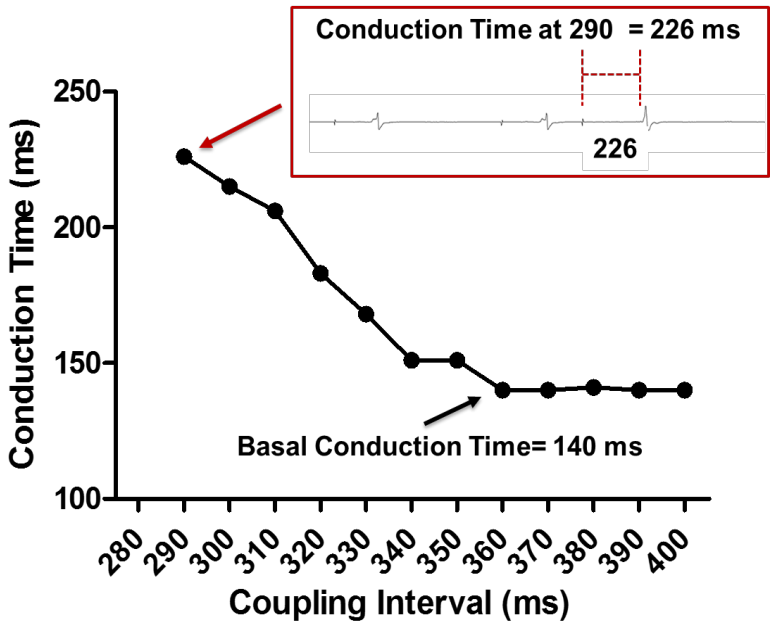

Figure 7: A **Conduction Curve** for the patient and catheter position in Figure 5. (Inserted panel shows data from the point at the red arrow). At the coupling interval of 290 ms the time from the stimulation artifact to the electrogram at a proximal electrode is 226 ms and is plotted on the conduction curve. The basal conduction time is 140 ms.

6.4 Refractoriness

Ventricular Effective Refractory Period (VERP) is defined as the longest stimulus coupling interval which fails to capture the ventricle twice (**Figure 6**). The drive train for ERP testing will be 600 ms.

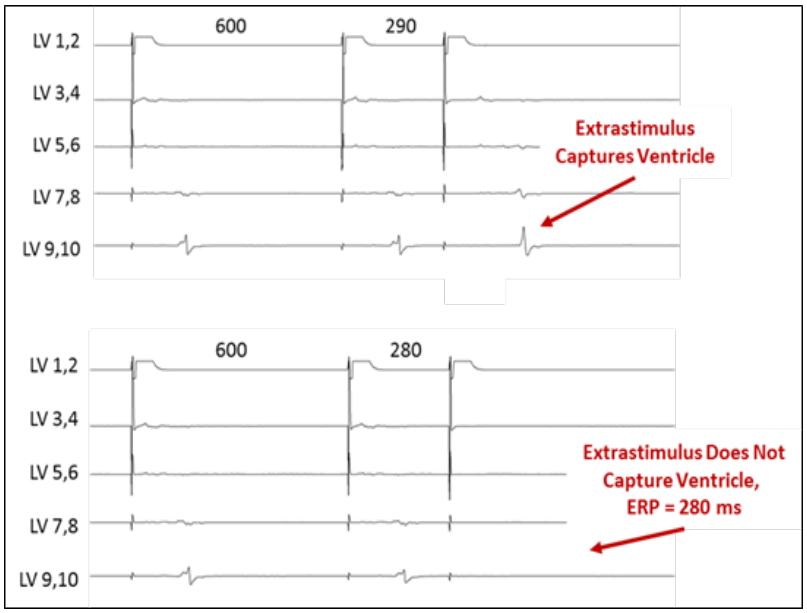

Figure 6: Ventricular effective refractory period (ERP) is displayed for the same patient and catheter position shown in Figure 5. Pacing is from the distal electrode (labeled A in Fig. 5).

6.5 Conduction

All measurements of conduction are performed off-line following completion of the procedure and therefore do not prolong case time. They are performed by members of the investigator team who are blinded to drug administration. *Conduction Time* is measured as the time from the: 1) stimulus artifact to the local electrogram on the electrode most distant from the site of pacing that has a consistent reliable electrogram (**Figure 7**). The dominant peak of the bipolar electrogram will be used for measurement of conduction times. If multiple similar amplitude

signals are present, as may be encounted in or near scar, the latest sharp peak will be used for measurement. Multiple measurements for conduction time will be obtained and are specific to a given location (scar border zone or remote higher voltage site). *Conduction Restitution*[55]: from plots of conduction time versus coupling intervals the following parameters are determined and compared pre-post dantrolene administration: 1) The paced conduction time at basic cycle length; 2) Onset of the paced conduction time increase (the longest coupling interval at which the paced conduction time exceeded that of the basic cycle length by 5 ms and continued to increase); and 3) The maximum paced activation time.

6.6 Ventricular Arrhythmia Induction Protocol

A standardized induction protocol is performed using programmed ventricular stimulation from the RV apex. The protocol is based on a prior study evaluating a range of induction protocols used to test inducibility before and after AAD administration in the EP lab.[39] Sedation and vasopressor infusion is maintained at constant levels throughout the study protocol and, any changes warranted for clinical care will be documented. The pacing cycle length is 8 beats followed by extrastimuli (ES) and a 3 second rest period. The basic cycle length of the pacing drive train (S1 – S1) is 600 ms (100 bpm). The first extrastimulus (S2) is introduced at 300 ms and decremented by 10 ms to VERP or 200 ms. S2 is then set to a coupling interval (S1 – S2) of VERP (or 200 ms) + 20 ms and a second extrastimulus (S3) added at a coupling interval of S1-S2 + 50 ms and scanned to refractoriness or a minimum coupling interval of 200 ms. The second extrasimulus is then set to its VERP + 20 ms and a third extrastimulus (S4) added at an initial coupling interval (S3 – S4) of S2 – S3 + 50 ms and scanned in to refractorness or 200 ms. Thus up to 3 extrasimuli are added and the minimum coupling interval will not be shorter than 200 ms. The induction protocol is stopped when: 1) the primary endpoint is met, or 2) the induction protocol has been completed. The primary endpoint is an episode of sustained VT that lasts ≥10 seconds or two episodes of non-sustained ventricular arrhythmia defined as VT or VF that lasts 3-9 seconds.

6.7 Pulmonary Artery (Swan-Ganz) Catheter Placement

The participant is supine and under deep sedation or general anesthesia during the procedure. Due to the creation of a complex electroanatomic map, it is critical that patients do not move during an ablation procedure. This is also ideal for comparative hemodynamic assessments. The transducer is placed midway between the level of the anterior and posterior surfaces of the patient's chest and zeroed to atmospheric pressure. The balloon-tipped PA catheter is placed via a femoral venous sheath and advanced to the right atrium (RA). A combination of pressure wave form interpretation and fluoroscopy is used to guide the PA catheter through the RV to the distal PA. The PA catheter remains in the same position during the pre and post-drug hemodynamic measurements.

6.8 Pressure Measurement

Recordings are obtained to measure the following pressures: mean RA, PA systolic, PA diastolic, and mean PA. All pressures are recorded as the average of 3 measurements obtained at end expiration.

6.9 Arterial Blood Pressure Measurement

As part of an ablation procedure, invasive arterial blood pressure is monitored via an arterial pressure catheter (femoral and/or radial artery). As clinical endpoints, systolic, diastolic, mean and pulse pressures are recorded immediately prior to study drug administration, and then every 2 minutes after drug administration during the research protocol (except during pacing maneuvers). Changes in vasopressor infusion rate and boluses are also recorded.

6.10 Cardiac Output, RV Function, and Vascular Resistance Assessment

Cardiac output is measured using the Fick method. A pre-study drug cardiac output is recorded, and then cardiac output is sampled 4 times post-study drug for the purposes of Aim 2 and for PK/PD analysis. Indices of RV function are calculated including RV stroke volume index and PA pulsatility index. Systemic and pulmonary vascular resistance are also calculated. Note, if the pre-study drug mixed venous oxygen saturation (SvO2) is <50%, the research protocol will be stopped, and the study drug will not be administered.

6.11 ECG Intervals

ECG intervals (RR, PR, QRS, QT) are recorded from a continuous 12-lead tracing obtained prior to study drug administration and then for 20 minutes after study drug. Measurements are made offline at a sweep speed of 100 mm/s. Measurements are made every minute during the post-study drug period. Measurements are recorded as the median of 3 beats.

6.12 Pharmacokinetic (PK)-Pharmacodynamic (PD) Analysis

PK/PD analysis will be performed by Scott Akers (Lipscomb University). Ryanodex is a new formulation of dantrolene (nanoparticle suspension for injection) with enhanced aqueous solubility designed for rapid I.V administration. The FDA determined that our proposal is exempted from requirements for an Investigational New Drug application (see accompanying letter from FDA). The PK of Ryanodex have been investigated in healthy volunteers at a dose range of 1 to 2.5 mg/kg and demonstrated a dose-proportional increase in dantrolene exposure with a peak concentration observed 1 minute after drug administration. Mean PK parameter estimates are independent of dose with a mean half-life of 10 hours, volume of distribution of 36.4 L, and systemic clearance of 2.5 L/hr. One major determinant of the short-term effects of many cardiac drugs is the concentration of drug within the myocardial tissue. Previous *in-vivo* studies evaluating the myocardial uptake of drugs in humans using a transcoronary blood sampling technique have demonstrated that lipophilic drugs like dantrolene exhibit rapid uptake into myocardial tissue with peak myocardial tissue content (1-4% of dose administered) being achieved within 0.5-5 minutes after I.V. bolus.[56,57] To define the PK/PD relationship of dantrolene and its short-term effect on the specific hemodynamic (PA wedge pressure, Fick cardiac output) and EP parameters (electrocardiogram intervals [RR, PR, QRS, QT]), femoral venous, arterial, and PA distal blood samples will be collected simultaneously from indwelling sheaths in all subjects at the pre-dantrolene timepoint and at 5, 10, 15, and 20 minutes post-dantrolene. Arterial drug concentrations will be assumed to represent dantrolene concentrations present in the ascending aorta and reflect concentrations entering the coronary circulation. As such, the temporal relationship between dantrolene concentrations (arterial and venous) and peak hemodynamic and EP effects will be evaluated using PK/PD modeling approaches to determine direct response correlations or a potential time lag in response.

Following the PK/PD phase of the study, PK sampling will be performed by collecting up to seven blood samples per subject that are drawn at variable time intervals. Blood samples will be 2 mL each and no more than a total of 15 mL of blood will be drawn post-operatively. PK will be analyzed using both an intensive and sparse (population) PK approach.[58] Dantrolene plasma concentrations in all blood samples will be determined using liquid chromatography coupled with tandem mass spectrometry (LCMS-MS) with an assay threshold of 5 ng/ml. Plasma concentration-time data from individual subjects will be imported into Phoenix WinNonlin® 8.0 software (Certara USA, Inc., Princeton, NJ) to estimate the PK parameters of dantrolene from population PK approaches.

6.13 Measurement of Respiratory Mechanics and Ventilation

To enhance the safety of the study protocol and enable research on the effect of dantrolene on respiratory mechanics, the study team will include cardiac anesthesiologists (Drs. Siegrist, Mantinan, Billings, Pretorius, and Shotwell) and will specifically study the effect of dantrolene on respiratory mechanics. Prior to the procedure, a baseline measurement of respiratory muscle strength will be obtained with a negative inspiratory force test (NIF), which is a quick, non-invasive bedside breathing test. During the procedure, to monitor adequacy of ventilation, maintain safety, and measure the effect of dantrolene administration on ventilation, the anesthesiologist will monitor respiration oxygenation as is standard care for all patients during procedures and surgeries. If the patient is already under general anesthesia with controlled ventilation (either with laryngeal mask airway [LMA] or endotracheal tube [ETT]), attempts will be made to have the patient spontaneously breathing with neuromuscular blockers reversed. These measurements include respiratory rate, minute ventilation, tidal volume, as well as arterial blood gas measurements (pH/pO2/pCO2/BE/Na/K/Cl/Ca). These measurements will be recorded prior to study drug administration and at 10 and 20 minutes after study drug administration. Finally, two hours and again at 24 hours after study drug administration, a NIF will be repeated to assess whether the respiratory muscle strength has returned to baseline. An arterial blood gas will also be repeated at 2 hours after study drug administration (sample obtained from the radial arterial line).

6.14 Measurement of Muscle Strength

To monitor the effect of dantrolene on muscle strength, a baseline assessment of handgrip strength will be obtained prior to the procedure using a dynamometer. Finally, two hours and again at 24 hours after study drug administration, an assessment of grip strength using the dynamometer will be repeated to assess whether the respiratory muscle strength has returned to baseline.

7.0 RISKS

Patients with structural heart disease and PVCs or VT who are undergoing ablation are at risk for complications by nature of their underlying disease process and the procedure. These risks are inherent to the patient population studied here. The risks of obtaining blood samples from phlebotomy of peripheral veins and existing venous lines are minimal. There are risks associated with inducing ventricular arrhythmias, however the pacing maneuvers used in this protocol are commonplace during ablation and have a long history of being used both clinically and for research purposes.[59,60] There are risks associated with dantrolene (Ryanodex). A comprehensive list of adverse reactions from the Ryanodex brochure include skeletal muscle weakness, dyspnea, respiratory muscle weakness, dysphagia, somnolence, lightheadedness, tissue necrosis (only if extravasated into surrounding tissue), nausea, thrombophlebitis, urticaria, and anaphylaxis. Drug interactions include calcium channel blockers, therefore patients using calcium channel blockers are excluded from the trial. Precautions also include muscle relaxants, antipsychotic and antianxiety agents as they may potentiate weakness, somnolence, and dizziness with Ryanodex. The risk of adverse reactions are dose-dependent and we propose here to use the lowest approved dose of Ryanodex (1 mg/kg). Furthermore, a one-time dose of I.V. Ryanodex will be given in the EP lab while under the care of both the Cardiac EP and Anesthesiology teams. Collaborating investigators on this study include cardiac anesthesiologists who will be monitoring respiratory status and measuring respiratory mechanics, ventilation, and muscle strength which will add additional safety precautions to the protocol. According to standard clinical protocol following ablation, patients are on strict bedrest until post-operative Day #1 with continuous cardiac and respiratory telemetry. Participants will be ambulated with assistance for the first time on post-operative Day #1. This minimizes the risks associated with the potential for skeletal and respiratory muscle weakness and lightheadedness. Furthermore, the eligibility criteria for this trial will exclude participants with a history of skeletal muscle disorders (e.g. muscular dystrophy), pulmonary disease requiring supplemental oxygen or prior intubation, morbid obesity, or a history of dysphagia. According to the Ryanodex brochure, patients must not drive 48 hours after Ryanodex administration. However, the vast majority of patients undergoing ablation are on pre-existing driving restrictions because they have experienced recent ICD shocks and/or ventricular arrhythmias.

Table 3. Anticipated risks associated with dantrolene, PVC or VT ablation procedures, and the study protocol

Risks from Study Drug (Ryanodex)	Risks from ablation procedure	
Flushing	Acute myocardial infarction	Heart block
Somnolence	Anemia	Hematoma
Dysphonia	Arterial dissection	Hypotension
Dysphagia	Atrial fibrillation/flutter/tachcyardia	Hypoxia-volume overload
Nausea	Back pain	Heparin-induced thrombocytopenia and disseminated intravascular coagulation
Feeling abnormal	Bleeding	ICD lead malfunction/ICD shock
Headache	Cardiac or valvular injury	Incessant VT/Recurrent VT/VT storm
Vomiting	Cardiac insufficiency	Mitral valve regurgitation
Vision blurred	Cardiac perforation	Pleural effusion
Pain in extremity	Cardiogenic shock	Pneumonia
Muscular weakness/asthenia	Cerebrovascular accident	Pericarditis
Bradycardia (AV block, sinus node dysfunction)	Transient ischemic attack	Pulmonary edema
Tachycardia	Cardiac ischemia	Respiratory distress/insufficiency
Infusion site pain/necrosis if extravasated	Chest pain	Pseudoaneurysm
Dizziness/lightheadedness	Congestive heart failure	Urinary retention
Dyspnea, respiratory muscle weakness	Death	
Urticaria, skin erythema	Electromechanical dissociation	
Thrombophlebitis	Epistaxis secondary to anticoagulation	
Anaphlaxis	Headache	

8.0 REPORTING OF ADVERSE EVENTS AND UNANTICIPATED PROBLEMS INVOLVING RISK TO PARTICIPANTS AND OTHERS

8.1 Data and Safety Monitoring Plan

The Data Safety Monitoring Board (DSMB) reviews safety data, study progress, and data quality. The DSMB is chaired by Dr. Prince Kannankeril, MD (Professor of Pediatrics, Division of Pediatric Cardiology, Vanderbilt Children's Hospital) who is a clinical investigator and practicing cardiac electrophysiologist. Other members are Dr. Gregory Michaud, MD (Professor of Medicine, Director of the Division of Cardiac Electrophysiology, VUMC) who is a clinical researcher and has expertise in complex ablation including VT ablation; Dr. Chris Hughes who is an Anesthesiologist and clinical researcher (Associate Professor of Anesthesiology); and G. Dan Ayers, MS (Senior Associate, Department of Biostatistics, VUMC) who has extensive prior experience as a DSMB statistician. The DSMB will review all serious adverse events (AEs). Any serious AE will be reported to the DSMB and IRB within 7 days from the investigators' awareness of the event. Any untoward medical event will be classified as an AE, regardless of its causal relationship with the study. The DSMB reviewed the results of the pilot study and provided input for the Phase II trial and recommended to proceed.

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

Adverse event reporting will utilize the NIH Common Criteria for Adverse Event Reporting (CTCAE version 5.0, published November 2017). It defines an adverse event (AE) as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure.

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:

Grades: refer to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL).

- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

- Grade 4 Life-threatening consequences; urgent intervention indicated.

- Grade 5 Death related to AE.

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 the Vanderbilt University Medical Center 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 AND DISCONTINUATION

A participant 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. The participant, for any reason, does not undergo VT or PVC ablation.

ii. Prior to administration of dantrolene, the participant experiences a complication during the ablation procedure and/or is not considered by the clinical or research team to be clinically stable enough to undergo the research protocol

iii. Prior to administration of dantrolene, if any of the following criteria are met on the pre-study drug assessment, the research protocol will be stopped, and the study drug will not be administered:

   a. Mixed venous oxygen saturation (SvO2) is <50%

   b. Criteria for RV failure (RA pressure >15 mmHg and CI <2.0 L/min/m^2)

   c. Criteria for severe pulmonary hypertension (PA systolic pressure ≥60 mmHg)

iv. The participant experienced an adverse event related to the study drug/protocol.

10.0 STATISTICAL CONSIDERATIONS

Statistical Considerations: The statistical analysis plan was formulated and will be performed by Dr. Fei Ye, Co-investigator and Associate Professor of Biostatistics. Analysis Sets: 1) Full analysis set (FAS)**:** All randomized patients. The FAS will be used in the analysis of all efficacy endpoints. Participants will be included in FAS according to the treatment to which they are randomized. 2) Safety analysis set (SAS)**:** All randomized participants who have received dantrolene. The SAS will be used in the analyses of all safety endpoints. Participants will be analyzed according to the treatment they received.

General Statistical Strategies: Baseline characteristics will be summarized descriptively by treatment group and by stratification factors. Categorical data will be presented as frequencies and percentages and analyzed with Fisher's exact test and chi-squared test. For continuous data, summary statistics will be presented. For single time-point data, between-group differences will be assessed with t-test or ANOVA. Nonparametric counterparts, Wilcoxon rank sum test and Kruskal-Wallis test, will be used when assumptions for parametric methods are not met. For correlated data (e.g., repeated measurements) will be analyzed with mixed-effect models (LMM). Multiple comparison issues will be corrected using the Bonferroni's. Analyses will be performed using R (latest version).

Modified "Intention-to-Treat" (mITT) Set: all randomized patients who underwent VT/PVC ablation and received any study treatment (dantrolene or placebo) will be analyzed according to the study arm they were originally assigned, regardless of what treatment they received. We chose this population because eligibility is defined with respect to ablation, and all trial endpoints are postoperative outcomes.

EP Endpoints: The primary hypothesis in Aim 1 tests whether RyR2 block with dantrolene significantly prolongs refractoriness and speeds conduction within low voltage areas adjacent to dense fibrosis and scar but has no significant effect within remote areas of relatively higher voltage.

Primary Analysis (Aim 1): All FAS will be included in the primary analysis of conduction time and ERP. The primary analysis will be restricted to measurements performed in the LV endocardium.

Conduction Time: is a continuous measurement. To most accurately measure conduction time, it is the mean value of multiple measurements along the horizontal aspect of the conduction curve (**Figure 7**). Conduction time is analyzed separately for the low voltage and higher voltage areas. Multivariable linear regression models will be built. Simple linear regression models will be used to test whether treatment with dantrolene is associated with a statistically significant decrease in mean post-drug conduction time when adjusted for mean pre-drug conduction time. Multivariable linear regression models will include additional adjustment for age, sex, mean bipolar voltage, amiodarone exposure (yes/no), change in beta-agonist exposure, and time from start of drug to post-drug EPS. Mean bipolar voltage will be the mean of the peak electrogram measured on the recording electrode for each of the extrastimuli.

Effective Refractory Period: is a continuous measurement. The ERP is measured at within the low voltage area and higher voltage areas at a drive train of 600 ms. It is measured pre-study drug and post-study drug. Multivariable linear regression models will be built. Separate models will be built for the low and higher voltage areas. Simple linear regression will test whether treatment with dantrolene is associated with a statistically significant increase in mean post-drug ERP when adjusted for mean pre-drug ERP. Multivariable analysis will be conducted using the same methods as described for conduction time.

Secondary Analyses Aim 1: activation time and conduction restitution are additional metrics for conduction. They are analyzed with the same statistical approach as described for conduction time.

Sample Size and Power Aim 1: The primary endpoints are conduction time and ERP. Pre- and post-drug difference in average conduction time and ERP will be compared between the dantrolene and the placebo group. We expect the conduction time to decrease and ERP to increase substantially after drug in the treatment arm, while no change is expected in the placebo arm. With 56 patients treated with dantrolene and 28 placebos, there will be 80% power to detect a 0.6xSD difference in the average conduction time and the average ERP change between the two study arms.

Hemodynamic Endpoints: The primary hypothesis in Aim 2 tests whether RyR2 inhibition increases cardiac output and decreases PA diastolic pressure in patients with structural heart disease. The primary endpoints are cardiac index and mean PA diastolic pressure. Secondary endpoints are RA and PA pressures, RV stroke volume index, PA pulsatility index, and systemic and pulmonary vascular resistances.

Primary Analysis (Aim 2): Our primary analyses use multivariable linear regression to test whether our primary determinant (dantrolene vs. placebo) is associated with: 1) post-drug mean cardiac index and 2) post-drug mean PA diastolic pressure. Adjustment is made for pre-study drug measurements. Additional adjustment in the multivariable model includes: age, sex, LV ejection fraction (continuous), change in beta-agonist exposure, ventricular arrhythmia induced (yes/no), and time from start of study drug to post-drug hemodynamic measurements. Mixed-effect models with linear regression will be built for the repeated measurements to account for the correlation structure in the data, where patient ID will be treated as a random effect. Fixed effect factors include treatment arm, measurement time (1,2,3, etc.), and all covariates. The cardiac index and PA diastolic pressure adjusted for pre-drug measurements is presented for both treatment arms, together with corresponding 95% confidence intervals (CIs) and p-values are graphically interpreted using boxplots, partial effect plots, residual plots, etc.

Secondary Analysis (Aim 2): Secondary endpoints are RA and PA pressures, PA pulsatility index, and systemic and pulmonary vascular resistances. They are analyzed with the same statistical approach as described for output and PA diastolic pressure.

Sample Size and Power Aim 2**:** The pre- and post-drug difference in average cardiac index and PA diastolic pressure will be compared between the dantrolene and the placebo group. We expect the cardiac output to increase and PA diastolic pressure to decrease substantially after drug in the treatment arm, while no change is expected in the placebo arm. However, to allow for unexpected effects (e.g. negative inotropy), two-sided tests will be performed. With 56 patients treated with dantrolene and 28 with placebo, the study will provide 80% power to detect a 0.6xSD difference in the average cardiac index and the average PA diastolic pressure change

between the two study arms. For instance, if the pre-drug PA diastolic pressure measurements have a SD of 4 mmHg, we will have 80% power to detect a difference of +/- 2.4 mmHg in PA diastolic pressure. If the pre-drug average PA diastolic pressure is 12 mmHg, this means a 20% change in the treatment arm is detectable assuming there is no change in the control arm. Each primary endpoint will be tested at the two-sided 5% significance level.

Inducibility Endpoints: The primary hypothesis in Aim 3 tests whether RyR2 inhibition with dantrolene reduces the susceptibility to ventricular arrhythmias. Susceptibility to ventricular arrhythmias is measured by testing the inducibility of VT/VF using programmed ventricular stimulation.

Primary Analysis Aim 3:

Inducibility of Ventricular Arrhythmias: Ventricular arrhythmias are defined as monomorphic or polymorphic VT, or VF. The primary endpoint is either a sustained hemodynamically stable ventricular arrhythmia of ≥10 seconds duration or an unstable ventricular arrhythmia requiring termination (with pacing or defibrillation), or two episodes of non-sustained ventricular arrhythmia defined as an episode lasting 3-9 seconds. To maximize statistical power, the primary analysis utilizes an ordinal endpoint that measures the relative inducibility based on the stage of the induction protocol and the arrhythmia induced (**Table 5**). Multivariable regression models are built. Univariable logistic regression modeling will be used to test whether treatment with dantrolene is associated with the category of inducibility (ordinal endpoint) measured post-study drug with adjustment for the stage of inducibility measured pre-study drug. Multivariable modeling includes additional adjustment for age (continuous), sex, amiodarone exposure (yes/no), change in beta-agonist exposure, and time from start of drug to post-drug stimulation protocol. Models will be evaluated for goodness-of-fit, and internally validated for calibration/discrimination with bootstrap.

Table 5. Ordinal Inducibility Endpoint

	Category	
Least inducible	0	No sustained VT/VF or NSVT
↓	1	1 episodes of NSVT with S4
↓	2	1 episodes of NSVT with S3
↓	3	1 episodes of NSVT with S2
↓	4	2 episodes of NSVT with S4*
↓	5	2 episodes of NSVT with S3*
↓	6	2 episodes of NSVT with S2*
↓	7	Sustained VT/VF with S4
Most inducible	8	Sustained VT/VF with S3
	9	Sustained VT/VF with S2

*the category assigned for 2 episodes of NSVT is based on the extrastimulus used to induce the second episode of NSVT.

Secondary Analyses Aim 3: A binary endpoint of inducibility (yes/no) is analyzed using logistic regression with the same covariate adjustment described for the primary analysis. Another secondary analysis tests the idea that RyR2 inhibition with dantrolene may alter inducible ventricular arrhythmias due to changes in conduction and refractoriness and hemodynamic improvement. An ordinal variable defining the cardiac rhythm stability class is analyzed (**Table 6**) using multivariable ordinal logistic regression. A secondary analysis will explore all the ventricular arrhythmia susceptibility endpoints for an interaction between cardiomyopathy type (ischemic versus non-ischemic) and treatment with dantrolene. Definitions: 1) Stable monomorphic VT is mean arterial pressure at 10 seconds (MAP_{10}) or immediately prior to termination of >50 mmHg; 2) Unstable VT is MAP_{10} or prior to termination of <50 mmHg; 3) Pleormorphic VT is more than one QRS morphology for 3 or more consecutive beats during the same VT episode; and 4) Polymorphic VT is a continually changing QRS morphology.[54] Sample Size and Power Aim 3: *Primary Endpoint*: The sample size selected for this trial was calculated to adequately power the analysis of the inducibility endpoint in Aim 3 which is an ordinal variable (Table 5). With N=84 patients (randomized to placebo and treatment in 1:2 ratio) with an expected 50% of patients in the placebo arm non-inducible, and 10% in each of the other categories, the study will achieve 80% power to detect an odds ratio (OR) of 0.3 at the two-sided significance level of 5%. With an anticipated 20% withdrawal rate prior to study drug, up to a total number of 105 patients will be enrolled in the study to ensure statistical power. *Secondary Endpoints*: 1) The binary status of inducibility (yes/no). With an effective sample size of 84, the study provides 80% power to detect an OR of 0.25, and a 70% power to detect an OR of 0.3 at the two-sided significance level of 5%.

Table 6. Secondary analysis of rhythm stability

	Category	
Least Stable	1	Ventricular Fibrillation
	2	Polymorphic VT
↓	3	Pleomorphic VT
↓	4	Unstable Monomorphic VT
Most Stable	5	Stable Monomorphic VT
	6	No Inducible VT/VF

Assessment of Safety (Aim 3)**:** An interim analysis will be performed by the DSMB when 50% of the target enrollment is reached to test for an association between dantrolene or placebo and significant adverse events/harm. In order to preserve statistical power for the secondary analyses, the interim analysis will not include early stopping rules for efficacy. Although none of our Phase I pre-clinical or clinical data have raised

safety concerns, and no adverse events have been observed, we planned the assessment of safety, which will be based on the frequency of adverse events. Other safety data such as blood pressure will be considered. All adverse events recorded during the study will be summarized. See Ethical Aspects of the Proposed Research for DSMB.

Respiratory Mechanics and Ventilation Endpoints: To monitor adequacy of ventilation, maintain safety, and measure the effect of dantrolene administration on ventilation, the anesthesiologist will monitor respiration and oxygenation as is standard of care for all patients during procedures and surgeries. The primary endpoint of the Respiratory Mechanics substudy is the incidence of the addition of respiratory support, including bag/mask ventilation, CPAP, BiPAP, LMA, or tracheal intubation, from the time of study drug administration until 120 minutes after study drug administration. Placement of airway support devices is at the discretion of the attending anesthesiologist and will be based on standard safety parameters such as oxygen saturation, adequacy of ventilation, and airway obstruction. Secondary endpoints include additional markers of respiratory mechanics and strength including negative inspiratory force, dynamometer measured grip strength, accelerometer (respiratory rate, tidal volume, minute ventilation), markers of gas exchange (arterial pH, pCO2, pO2, base excess), and electrolytes. Tidal volume, respiratory rate, and minute ventilation parameters will be continuously collected (recorded every minute) as available. We will summarize these data prior to study drug administration and at 10 and 20-minutes after dantrolene administration.

Prior to the ablation procedure and administration of anesthesia, a baseline negative inspiratory force (NIF) will be collected x3 in the supine position. A repeat NIF measurement will be again collected in the PACU 2 hours post dantrolene administration and then again at 24 hours. We will summarize NIF data prior to and following dantrolene administration.

Arterial blood gas (ABG) sampling will be performed at four time points: intraoperatively just prior to dantrolene administration, 10- and 20-minutes post dantrolene administration, and 2 hours post dantrolene administration in the PACU. ABG sampling will allow sampling of respiratory mechanic parameters including pH, pCO2, pO2, and base deficit/excess. We will summarize ABG data at these four timepoints.

Muscle Strength Endpoints: We will assess grip strength as measured with a handheld dynamometer x3 prior to the ablation procedure and administration of anesthesia and 2 hours following dantrolene administration and then again at 24 hours. We will summarize these data.

11.0 PRIVACY AND CONFIDENTIALITY ISSUES

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 6 months. The study results will be retained for at least six years after the study is completed. At that time, the research information not already in the medical record will be destroyed.

13.0 REFERENCES

1. Kong MH, Fonarow GC, Peterson ED, et al. Systematic review of the incidence of sudden cardiac death in the United States. Journal of the American College of Cardiology 2011;57:794-801.
2. Hayashi M, Shimizu W, Albert CM. The spectrum of epidemiology underlying sudden cardiac death. Circulation research 2015;116:1887-906.
3. Zipes DP, Camm AJ, Borggrefe M, et al. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death--executive summary: A report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death) Developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. European heart journal 2006;27:2099-140.

4. Benjamin EJ, Virani SS, Callaway CW, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation 2018;137:e67-e492.
5. Reynolds MR, Josephson ME. MADIT II (second Multicenter Automated Defibrillator Implantation Trial) debate: risk stratification, costs, and public policy. Circulation 2003;108:1779-83.
6. Poole JE, Johnson GW, Hellkamp AS, et al. Prognostic importance of defibrillator shocks in patients with heart failure. The New England journal of medicine 2008;359:1009-17.
7. Turakhia MP, Zweibel S, Swain AL, Mollenkopf SA, Reynolds MR. Healthcare Utilization and Expenditures Associated With Appropriate and Inappropriate Implantable Defibrillator Shocks. Circ Cardiovasc Qual Outcomes 2017;10.
8. Passman R, Subacius H, Ruo B, et al. Implantable cardioverter defibrillators and quality of life: results from the defibrillators in nonischemic cardiomyopathy treatment evaluation study. Arch Intern Med 2007;167:2226-32.
9. Irvine J, Dorian P, Baker B, et al. Quality of life in the Canadian Implantable Defibrillator Study (CIDS). American heart journal 2002;144:282-9.
10. Sears SF, Hauf JD, Kirian K, Hazelton G, Conti JB. Posttraumatic stress and the implantable cardioverter-defibrillator patient: what the electrophysiologist needs to know. Circulation Arrhythmia and electrophysiology 2011;4:242-50.
11. Kuck KH, Schaumann A, Eckardt L, et al. Catheter ablation of stable ventricular tachycardia before defibrillator implantation in patients with coronary heart disease (VTACH): a multicentre randomised controlled trial. Lancet 2010;375:31-40.
12. Connolly SJ, Dorian P, Roberts RS, et al. Comparison of beta-blockers, amiodarone plus beta-blockers, or sotalol for prevention of shocks from implantable cardioverter defibrillators: the OPTIC Study: a randomized trial. Jama 2006;295:165-71.
13. Sapp JL, Wells GA, Parkash R, et al. Ventricular Tachycardia Ablation versus Escalation of Antiarrhythmic Drugs. The New England journal of medicine 2016;375:111-21.
14. R. BLKBH-D. Goodman and Gilman's the pharmacological basis of therapeutics. Thirteenth ed. New York: McGraw Hill Medical; 2018.
15. Echt DS, Liebson PR, Mitchell LB, et al. Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial. The New England journal of medicine 1991;324:781-8.
16. Tsuji Y, Opthof T, Kamiya K, et al. Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. Cardiovascular research 2000;48:300-9.
17. Frommeyer G, Eckardt L. Drug-induced proarrhythmia: risk factors and electrophysiological mechanisms. Nat Rev Cardiol 2016;13:36-47.
18. Stevenson WG, Khan H, Sager P, et al. Identification of reentry circuit sites during catheter mapping and radiofrequency ablation of ventricular tachycardia late after myocardial infarction. Circulation 1993;88:1647-70.
19. Dillon SM, Allessie MA, Ursell PC, Wit AL. Influences of anisotropic tissue structure on reentrant circuits in the epicardial border zone of subacute canine infarcts. Circulation research 1988;63:182-206.
20. Downar E, Harris L, Mickleborough LL, Shaikh N, Parson ID. Endocardial mapping of ventricular tachycardia in the intact human ventricle: evidence for reentrant mechanisms. Journal of the American College of Cardiology 1988;11:783-91.
21. Downar E, Kimber S, Harris L, et al. Endocardial mapping of ventricular tachycardia in the intact human heart. II. Evidence for multiuse reentry in a functional sheet of surviving myocardium. Journal of the American College of Cardiology 1992;20:869-78.
22. Kleber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. Physiol Rev 2004;84:431-88.
23. de Bakker JM, van Capelle FJ, Janse MJ, et al. Reentry as a cause of ventricular tachycardia in patients with chronic ischemic heart disease: electrophysiologic and anatomic correlation. Circulation 1988;77:589-606.
24. Lopera G, Stevenson WG, Soejima K, et al. Identification and ablation of three types of ventricular tachycardia involving the his-purkinje system in patients with heart disease. Journal of cardiovascular electrophysiology 2004;15:52-8.
25. Huelsing DJ, Spitzer KW, Pollard AE. Spontaneous activity induced in rabbit Purkinje myocytes during coupling to a depolarized model cell. Cardiovascular research 2003;59:620-7.

26. Spitzer KW, Pollard AE, Yang L, Zaniboni M, Cordeiro JM, Huelsing DJ. Cell-to-cell electrical interactions during early and late repolarization. Journal of cardiovascular electrophysiology 2006;17 Suppl 1:S8-S14.
27. Singer DH, Lazzara R, Hoffman BF. Interrelationship between automaticity and conduction in Purkinje fibers. Circulation research 1967;21:537-58.
28. Chang PC, Chen PS. SK channels and ventricular arrhythmias in heart failure. Trends Cardiovasc Med 2015;25:508-14.
29. Chang PC, Turker I, Lopshire JC, et al. Heterogeneous upregulation of apamin-sensitive potassium currents in failing human ventricles. J Am Heart Assoc 2013;2:e004713.
30. Bonilla IM, Long VP, 3rd, Vargas-Pinto P, et al. Calcium-activated potassium current modulates ventricular repolarization in chronic heart failure. PLoS One 2014;9:e108824.
31. Chua SK, Chang PC, Maruyama M, et al. Small-conductance calcium-activated potassium channel and recurrent ventricular fibrillation in failing rabbit ventricles. Circulation research 2011;108:971-9.
32. Terentyev D, Rochira JA, Terentyeva R, Roder K, Koren G, Li W. Sarcoplasmic reticulum Ca(2)(+) release is both necessary and sufficient for SK channel activation in ventricular myocytes. Am J Physiol Heart Circ Physiol 2014;306:H738-46.
33. Hartmann N, Pabel S, Herting J, et al. Antiarrhythmic effects of dantrolene in human diseased cardiomyocytes. Heart rhythm : the official journal of the Heart Rhythm Society 2017;14:412-9.
34. Saxon LA, Hayes DL, Gilliam FR, et al. Long-term outcome after ICD and CRT implantation and influence of remote device follow-up: the ALTITUDE survival study. Circulation 2010;122:2359-67.
35. Stevenson WG, Wilber DJ, Natale A, et al. Irrigated radiofrequency catheter ablation guided by electroanatomic mapping for recurrent ventricular tachycardia after myocardial infarction: the multicenter thermocool ventricular tachycardia ablation trial. Circulation 2008;118:2773-82.
36. Fujii A, Nagashima K, Kumar S, et al. Significance of Inducible Nonsustained Ventricular Tachycardias After Catheter Ablation for Ventricular Tachycardia in Ischemic Cardiomyopathy. Circulation Arrhythmia and electrophysiology 2017;10.
37. Ghanbari H, Baser K, Yokokawa M, et al. Noninducibility in postinfarction ventricular tachycardia as an end point for ventricular tachycardia ablation and its effects on outcomes: a meta-analysis. Circulation Arrhythmia and electrophysiology 2014;7:677-83.
38. Buxton AE, Lee KL, Fisher JD, Josephson ME, Prystowsky EN, Hafley G. A randomized study of the prevention of sudden death in patients with coronary artery disease. Multicenter Unsustained Tachycardia Trial Investigators. The New England journal of medicine 1999;341:1882-90.
39. Cooper MJ, Koo CC, Skinner MP, et al. Comparison of immediate versus day to day variability of ventricular tachycardia induction by programmed stimulation. Journal of the American College of Cardiology 1989;13:1599-607.
40. Brandom BW, Larach MG, Chen MS, Young MC. Complications associated with the administration of dantrolene 1987 to 2006: a report from the North American Malignant Hyperthermia Registry of the Malignant Hyperthermia Association of the United States. Anesth Analg 2011;112:1115-23.
41. Flewellen EH, Nelson TE, Jones WP, Arens JF, Wagner DL. Dantrolene dose response in awake man: implications for management of malignant hyperthermia. Anesthesiology 1983;59:275-80.
42. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. Circulation 2001;104:2158-63.
43. Powell BD, Saxon LA, Boehmer JP, et al. Survival after shock therapy in implantable cardioverter-defibrillator and cardiac resynchronization therapy-defibrillator recipients according to rhythm shocked. The ALTITUDE survival by rhythm study. Journal of the American College of Cardiology 2013;62:1674-9.
44. Liu N, Colombi B, Memmi M, et al. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. Circulation research 2006;99:292-8.
45. Knollmann BC, Chopra N, Hlaing T, et al. Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca2+ release, and catecholaminergic polymorphic ventricular tachycardia. J Clin Invest 2006;116:2510-20.
46. Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med 2009;15:380-3.
47. Kobayashi S, Yano M, Uchinoumi H, et al. Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RyR2(R2474S/+) knock-in mouse model. Circ J 2010;74:2579-84.

48. Kubalova Z, Terentyev D, Viatchenko-Karpinski S, et al. Abnormal intrastore calcium signaling in chronic heart failure. Proc Natl Acad Sci U S A 2005;102:14104-9.
49. Marx SO, Reiken S, Hisamatsu Y, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 2000;101:365-76.
50. Belevych A, Kubalova Z, Terentyev D, Hamlin RL, Carnes CA, Gyorke S. Enhanced ryanodine receptor-mediated calcium leak determines reduced sarcoplasmic reticulum calcium content in chronic canine heart failure. Biophys J 2007;93:4083-92.
51. Wehrens XH, Lehnart SE, Reiken S, Vest JA, Wronska A, Marks AR. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. Proc Natl Acad Sci U S A 2006;103:511-8.
52. Zamiri N, Masse S, Ramadeen A, et al. Dantrolene improves survival after ventricular fibrillation by mitigating impaired calcium handling in animal models. Circulation 2014;129:875-85.
53. Bokhari MM, Spears DA, Lai PF, et al. Safety of Chronic Cardiac Ryanodine Receptor Modulation: A 10-Year Experience. JACC Clin Electrophysiol 2018;4:1480-1.
54. Aliot EM, Stevenson WG, Almendral-Garrote JM, et al. EHRA/HRS Expert Consensus on Catheter Ablation of Ventricular Arrhythmias: developed in a partnership with the European Heart Rhythm Association (EHRA), a Registered Branch of the European Society of Cardiology (ESC), and the Heart Rhythm Society (HRS); in collaboration with the American College of Cardiology (ACC) and the American Heart Association (AHA). Heart rhythm : the official journal of the Heart Rhythm Society 2009;6:886-933.
55. Postema PG, van Dessel PF, de Bakker JM, et al. Slow and discontinuous conduction conspire in Brugada syndrome: a right ventricular mapping and stimulation study. Circulation Arrhythmia and electrophysiology 2008;1:379-86.
56. Ritchie RH, Sallustio BC, Hii JT, Horowitz JD. Short-term myocardial uptake of d- and l-sotalol in humans: relation to hemodynamic and electrophysiologic effects. Journal of cardiovascular pharmacology 1998;31:876-84.
57. Weiss M. Functional characterization of drug uptake and metabolism in the heart. Expert Opin Drug Metab Toxicol 2011;7:1295-306.
58. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometrics Syst Pharmacol 2013;2:e38.
59. Mason JW. A comparison of seven antiarrhythmic drugs in patients with ventricular tachyarrhythmias. Electrophysiologic Study versus Electrocardiographic Monitoring Investigators. The New England journal of medicine 1993;329:452-8.
60. Wood M, Stambler B, Ellenbogen K. Recent insights in programmed electrical stimulation for the management of sustained ventricular arrhythmias. Curr Opin Cardiol 1994;9:3-11.