CLINICAL PROTOCOL

VEIN OF MARSHALL ETHANOL INFUSION FOR PERSISTENT ATRIAL FIBRILLATION

(IND 115,060)

(A MULTICENTER TRIAL)

Version 7.2

February 15, 2016

Clinical Trials.gov NCT 01898221

NIH-NHLBI R01HL115003-02

IND Sponsor

Houston Methodist Research Institute (HMRI)

6565 Fannin Street

Houston, TX 77030

Principal Investigator

Miguel Valderrábano, MD

Houston Methodist Hospital, Houston TX

Coordinating site

Houston Methodist Research Institute (HMRI)

Houston TX

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PROTOCOL SYNOPSIS

Title	Vein of Marshall Ethanol Infusion for Persistent Atrial Fibrillation
IND Sponsor	Houston Methodist Research Institute (HMRI) 6565 Fannin Street Houston, TX 77030
Name of Product	Dehydrated Alcohol Injection, USP
Clinical Phase	III
Patient Population	Patients with documented, persistent atrial fibrillation (AF that persists beyond 7 days) that have failed to respond to at least one class of antiarrhythmic drugs (due to failure or intolerance), and who are otherwise deemed candidates for radiofrequency ablation of AF.
Objectives	**VENUS-AF**. **V**ein of Marshall **E**thanol i**N**fusion in **U**ntreated per**S**istent **A**trial **F**ibrillation: ***To assess the role of VOM ethanol infusion in catheter ablation of persistent AF***. **MARS-AF.** Vein of **M**arshall **A**lcohol in **R**epeat ablation of per**S**istent **A**trial **F**ibrillation: ***To assess the impact of VOM ethanol infusion after a failed conventional ablation of persistent AF.***
Trial Design	Subjects who meet inclusion criteria will be randomized to either a conventional PVAI or PVAI with VOM procedure. Subjects will return for follow-up evaluations at 1, 3, 6, 9, and 12 months. One-month continuous cardiac event monitoring will be performed at 6 months and at 12 months. Studies performed at follow-up visits may include EKG, physical exam, QOL questionnaires, echocardiography, laboratory studies, anticoagulation therapy, and management of adverse events and AF recurrences. Patient and co-investigator performing follow-up of electrocardiographic data will be blinded to the type of procedure. Operator is not blinded.
Sample Size	405 total subjects, VENUS: 180 (VOM-PV) + 156 (PVAI) = 336 MARS: 37 (VOM-PV) + 32 (PVAI) = 69
Primary Endpoints ***De Novo (VENUS-AF) and Previously Failed Ablation (MARS-AF)***	***Efficacy:*** Freedom from symptomatic AF or atrial tachycardia (AT) AND reduction of AF/AT to less than 30 seconds in a continuous monitor at 6 and 12 months after a single procedure. **Safety**: Acute procedural complications and total mortality

Secondary Endpoints ***De Novo (VENUS-AF) and Previously Failed Ablation Study (MARS-AF)***	1. Freedom from AF/AT after >1 procedure. 2. Freedom from AF/AT on antiarrhythmic drugs. 3. AF burden (% time) on continuous monitoring at 6 and 12 months. 4. Procedural parameters: total procedure, fluoroscopy, total RF ablation time (first procedure), and total extent of ablated LA tissue. 5. Clinical/partial success: less than 25% AF burden on a continuous event monitor at 6 and 12 months from ablation procedure. 6. Sub-acute procedural complications (within 30 days). 7. Recurrence as persistent or paroxysmal AF, or flutter after 1 or 2 procedures. 8. LA function on Doppler echocardiography (LA strain[114ab]) at 12 months. 9. Incidence and mechanisms of atrial flutters. 10. Cardiovascular hospitalizations and *11.* QOL as determined by AFEQT questionnaire.
Inclusion Criteria	***VENUS-AF*** 1. Patients between the ages of 21 and 85 years undergoing their first ablation of AF. 2. Diagnosed with symptomatic persistent or long-standing persistent AF, defined as: ▪ AF not spontaneously converting to sinus rhythm, persisting for >7 days 3. Resistant or intolerant to at least one class I, II, or III antiarrhythmic drug (AAD) 4. Patients deemed candidates for RF ablation of AF 5. Able and willing to comply with pre-, post-, and follow-up requirements. ***MARS-AF*** ***Inclusion criteria*** 1. Patients between the ages of 21 and 85 years 2. Prior ablation for symptomatic persistent AF: ▪ Recurrence as symptomatic AF or AT at least 3 months after ablation 3. Resistant or intolerant to one class I, II, or III AAD prior to index ablation 4. Patients deemed candidates for RF ablation of AF 5. Able and willing to comply with pre-, post-, and follow-up requirements.

Exclusion Criteria- **VENUS-AF** **MARS-AF**	1. Left atrial thrombus by pre-procedural imaging. 2. LA diameter greater than 65 mm on long axis parasternal view, or left atrial volume more than 200 cc. 3. Left ventricular ejection fraction < 30%. 4. Cardiac surgery within the previous 180 days. 5. Expecting cardiac transplantation or other cardiac surgery within 180 days. 6. Coronary PTCA/stenting within the previous 90 days. 7. Documented history of a thrombo-embolic event within the previous 90 days. 8. Diagnosed atrial myxoma. 9. Significant restrictive, constrictive, or chronic obstructive pulmonary disease with chronic symptoms. 10. Significant congenital anomaly or medical problem that in the opinion of the investigator would preclude enrollment 11. Women who are pregnant or who plan to become pregnant during the study. 12. Acute illness or active infection at time of index procedure documented by pain, fever, drainage, positive culture and/or leukocytosis (WBC > 11.000 per mm^3) for which antibiotics have been or will be prescribed. 13. Creatinine > 2. 5 mg/dl (or > 221 µmol/L, except for patients in dialysis). 14. Unstable angina. 15. Myocardial infarction within the previous 60 days. 16. History of blood clotting or bleeding abnormalities. 17. Contraindication to anticoagulation. 18. Life expectancy less than 1 year. 19. Uncontrolled heart failure 20. Presence of an intramural thrombus, tumor, or other abnormality that precludes catheter introduction or positioning. 21. Presence of a condition that precludes vascular access. 22. INR greater than 3.5 within 24 hours of procedure- for patients taking warfarin. 23. Cannot be removed from antiarrhythmic drugs for reasons other than AF. 24. Unwilling or unable to provide informed consent.

1.0 INTRODUCTION

Atrial fibrillation (AF)* is the most common sustained arrhythmia in adults, and is a leading cause of stroke, disability and increased mortality.[1] Catheter ablation has become an increasingly accepted form of rhythm control and –other than surgery- is the only treatment form that can potentially cure AF. The ablation procedural strategy –pulmonary vein (PV) antral isolation (PVAI)- is best suited for paroxysmal AF,[2] in which ectopic beats arising from the PVs were shown to initiate AF.[3] However, it is unclear whether this mechanistic rationale applies to persistent AF,[4, 5] in which the role of the cardiac autonomic system, particularly the intrinsic cardiac ganglia, is being increasingly recognized as a modulator of atrial physiology leading to AF.[6, 7] The success of PVAI is significantly lower in persistent AF.[8] Expanding the ablation lesions to include larger areas of the atrial anatomy -such as the left atrial (LA) roof, coronary sinus (CS), LA appendage, septum, posterior wall, superior vena cava, and others- has improved outcomes, but also led to increases in procedural complexity and duration, need of repeat procedures,[9-12] and complications such as atrial flutters, particularly perimitral flutter (PMF).[13] Little mechanistic evidence supports this approach, which does not specifically address the intrinsic cardiac ganglia. Given that persistent AF has far greater prevalence and is a greater cause of stroke, disability and mortality than paroxysmal AF,[14] strategies to improve outcomes of catheter ablation of persistent AF are much needed.

We have developed a technique to perform rapid ablation of targeted atrial tissues in AF using ethanol infusion in the vein of Marshall (VOM).[15, 16] A previous R21 project has generated sufficient human data to support the safety –no safety issues were identified- and mechanistic utility of this technique by showing: 1) Effective, rapid and safe tissue ablation of LA tissue neighboring the LA ridge and left inferior PV; 2) Facilitation of cure of PMF by ablating most of the mitral isthmus; and 3) Regional LA vagal denervation. The broad, long term objective is to improve the outcomes of catheter ablation of persistent AF using the VOM as a target and a route to deliver ablative therapies.

2.0 SIGNIFICANCE

2.1 AF AS A CLINICAL AND HEALTH CARE PROBLEM

AF is the most common arrhythmia in the United States,[1] and is associated with significant morbidity and mortality, including up to 5-fold increased risk of stroke,[17, 18] 2-fold increased risk of dementia,[19-21] a 3-fold increased risk of heart failure[18] and a 40 to 90% increased risk of overall mortality.[22] Although the risk of stroke is comparable in persistent and paroxysmal AF,[23] the prevalence of persistent AF increases dramatically with increasing age,[24, 25] and thus is an overall more significant cause of morbidity and mortality. In the United States, there are currently an estimated 3.0 million adults with AF,[26] and this number is expected to double in the

* Abbreviations used: 3D: 3-dimensional; AF: atrial fibrillation; CFAE: complex fractionated atrial electrograms; CS: coronary sinus; LA: left atrium; PMF: perimitral flutter; PV: pulmonary vein; PVAI: PV antral isolation; RF: radiofrequency; VOM: vein of Marshall; VOM-PV: combined VOM ethanol infusion plus PVAI

next 25 years.[27] Hospitalizations with a primary diagnosis of AF are close to half a million per year,[28] which generates a tremendous economic burden on the health care system. When compared to health care costs of non-AF control subjects, patients with AF have greater annual healthcare costs (up to $8,705 total annual incremental cost). On the basis of current prevalence data, it is estimated that AF leads to a national incremental health care cost of up to $26 billion.[29]

2.2 INADEQUACY OF PHARMACOLOGICAL TREATMENT OPTIONS FOR PERSISTENT AF

Management strategies are directed at heart rate control and stroke prevention –mere palliation- or at rhythm control. It has been shown that rhythm control strategies using antiarrhythmic drugs offer no benefit in elderly patients[30] or patients with heart failure.[31] Most of the lack of benefit of such rhythm control strategy is thought to be due to the adverse effects and suboptimal efficacy of antiarrhythmic drugs, that can potentially augment mortality.[32] Indeed, preservation of normal sinus rhythm is associated with decreased mortality.[32] Dronedarone, the only antiarrhythmic drug shown to improve outcomes in nonpermanent AF compared to placebo,[33] has been shown to double mortality, stroke and hospitalization for heart failure in the PALLAS study in patients with permanent AF (prematurely terminated: www.clinicaltrials.gov and www.theheart.org/article/1264551.do). Thus, antiarrhythmic drugs remain suboptimal at best for the treatment of AF.

2.3 SHORTCOMINGS OF CATHETER ABLATION OF PERSISTENT AF

Weak mechanistic rationale. Isolation of the PVs[2] and adjacent LA (PV antrum)[34, 35] is the accepted procedural endpoint, based on the mechanistic concept that atrial extrasystoles arising from the PVs initiate *paroxysmal* AF.[3] Other, non-PV triggers have been demonstrated.[36] The link between PV extrasystoles and AF is clear in paroxysmal AF, but not in persistent AF, in which the mechanisms of AF seem to be related more to a chronic atrial substrate than to acute triggers.[4] Indeed, intramural reentry in the posterior LA seems to be particularly relevant in chronic models of AF.[37] In persistent AF, the procedure has evolved, rather simplistically, to include additional lesions -besides isolation of the PVs,[11, 38-40] variably placed in the posterior wall,[34] LA roof,[41, 42] and towards the mitral annulus,[43] the superior vena cava,[44] left atrial appendage,[45, 46] and other areas where complex fractionated atrial electrograms (CFAE) may be mapped.[13, 47] This brute force approach of simply destroying more tissue has yielded additional success, but new procedural targets with solid mechanistic bases are needed.

Suboptimal success and need for repeat procedures. Despite the additional tissue destruction, ablation success in persistent AF is with much lower than in paroxysmal AF,[48] with single procedure success reported as low as 27%,[40] 36%,[49]or 49%,[50] but up to 61%[13] or 67%,[51] depending on study heterogeneities in: definitions of persistent AF and of recurrence of AF, the type of AF monitoring, and ablation technique and operator experience. In order to achieve overall acceptable success rates, (which can reach up to 79%-94%),[13, 40, 51] there is a consistent need for repeat procedures (sometimes up to 4) and the concomitant use of antiarrhythmic drugs. The rate of repeat procedures in experienced centers can reach up to 70 to 80%.[9-12]

PMF after catheter ablation of persistent AF. Clinical failures of a first ablation procedure are

caused, in a significant portion of patients, by atrial flutters,[52-54] rather than recurrent AF, and recurrence as flutter portends a greater chance of success in a second procedure.[55] Such atrial flutters may be caused by perimitral reentry in up to 33-60% of the patients.[52, 54-56] Catheter ablation of PMF involves the creation of a linear lesion from the mitral annulus to the left inferior PV (the so-called mitral isthmus).[43, 57] Achieving a complete ablation (defined by bidirectional conduction block across the ablation line) can be very difficult,[10, 43, 58] with success rates reported as 32%,[59] 64%,[60] or 71%.[61] It sometimes requires ablation inside the CS,[54, 56] in close proximity to the circumflex coronary artery, which could be damaged.[60] Of note, incomplete ablation of the mitral isthmus is proarrhythmogenic,[62, 63] increasing the risk of recurrent flutter by up to 4 times.[62]

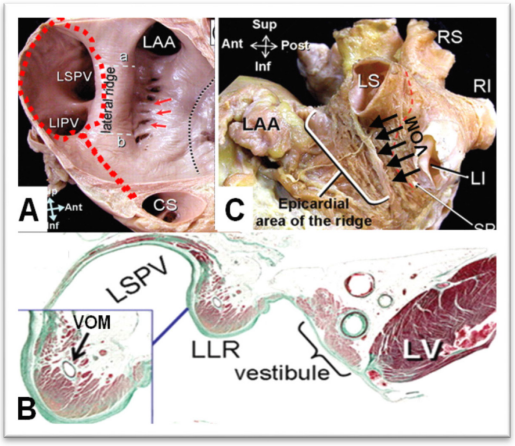
3.0 INNOVATION

The basis of this application is an entirely novel technique that was developed in our laboratory from its original conception, to its validation in animals,[15] to the demonstration of safety and feasibility in humans.[16] Ethanol is used in hypertrophic cardiomyopathy,[64] and in ventricular tachycardias that do not respond to conventional RF ablation.[65] When delivered in the VOM, we have shown that ethanol can help ablate neighboring atrial tissues, all of which are routinely targeted during conventional ablation.[15] Supported by an R21 grant that started in July 2010, significant human pilot data have been acquired that lend further support to the mechanistic rationale, safety, and potential clinical utility of this technique.

3.1 TARGETING THE INTRINSIC CARDIAC GANGLIA VIA THE VOM

The role of autonomic regulation in AF is highly relevant.[66] The cardiac autonomic system (Figure 1) can be divided into *extrinsic cardiac nerves* –vagus nerves and sympathetic chain-, and an *intrinsic cardiac ganglia* (a complex atrial epicardial network of ganglionated plexi with vagal and sympathetic nerves, including the ligament of Marshall). The intrinsic cardiac ganglia contain parasympathetic ganglia and its sympathetic nerves are only postganglionic.[67] These ganglia are not simple relay stations, but process multiple inputs from vagal efferent neurons, extrinsic sympathetic neurons, vagal afferent neurons, and sensory neurons.[67-73] Acetylcholine release by postganglionic neurons exerts effects on myocytes via muscarinic receptors and I_{KAch} channels, which shorten the action potential, allowing myocytes to sustain rapid activation rates (shorten refractoriness) and favoring the formation of rotors in AF.[75] Sympathetic innervation (norepinephrine) leads to enhanced automaticity, increased intracellular calcium and favors afterdepolarizations[76-78] that create extrasystoles that can initiate AF,[77] and destabilize rotors.[75, 79] Thus, a synergistic pro-AF effect can occur if both parasympathetic influences (shortening the action potential and refractoriness) and sympathetic influences (leading to extrasystoles via after depolarizations) activate simultaneously. Indeed, combined simultaneous sympathetic and parasympathetic discharges lead to AF.[6] Sympathovagal (stellate ganglion and vagus nerve) cryoablation of the *extrinsic cardiac nerves* eliminates paroxysmal AF episodes in a rapid atrial pacing model, but does not prevent the ultimate development of persistent AF.[6] The intrinsic cardiac autonomic system shows enhanced activity preceding AF, independent of the extrinsic system, that can play a role in developing persistent AF.[7]

Figure 1. Lateral LA and VOM



A, Cut open left atrium with left PVs and lateral ridge. Red dotted line indicates location of commonly placed ablation lesions. **B,** Microscopic view of the lateral ridge, showing the VOM (inset).**C,** Epicardial view of the lateral ridge, with VOM. *Modified from ref. 87.*

Translating this information into a modification of the ablation procedure to enhance its efficacy has proven difficult. Ablation of intrinsic autonomic ganglia has been proposed,[80] but the strategy has been RF ablation of the LA at locations where ganglia were identified as sites where bradycardic reflexes are triggered during high-frequency stimulation. Disappointingly, this approach has not been shown to add significant clinical benefit beyond PVAI.[81-83] Identification of vagal ganglia by finding bradycardic reflexes has not been shown to be more effective than simply using a standardized anatomic approach,[84] or to decrease AF inducibility.[83] Possible reasons for the failure of vagal ganglia RF ablation to impact procedural outcomes include: inaccurate ganglia localization, inadequate elimination of vagal innervation, given their epicardial location, and inadequate elimination of sympathetic innervation (not localizable by high-frequency stimulation).

The ligament of Marshall is the embryologic remnant of the left cardinal vein (superior vena cava), which, as it becomes atretic during development,[85] remains open as the VOM.[86] This vein drains in the CS and runs posteriorly and superiorly in the epicardial surface of the LA, towards the anterior aspect of the left-sided PVs, as part of a thick pectinate muscle that separates the veins from the LA appendage (left atrial ridge).[87] (See Figure 2).The VOM has been robustly shown to contain parasympathetic[88] and sympathetic[89] innervation,[90] and is *part of the intrinsic cardiac ganglia*.[91] The ligament of Marshall has been solidly implicated in arrhythmogenesis. As a *source of ectopic rhythms*, Scherlag, et al [92] demonstrated an ectopic rhythm arising from the ligament area upon left cardiac sympathetic nerve stimulation. Doshi, *et.al.* demonstrated the role of the ligament of Marshall in adrenergic atrial tachycardia.[93] Hwang *et al* demonstrated ectopic beats from the VOM leading to AF,[86, 94] as confirmed by others.[36, 95-99]

Focal ectopy arising in the VOM triggering AF has been demonstrated clinically[36, 86] and in experimental models of *persistent* AF.[100]

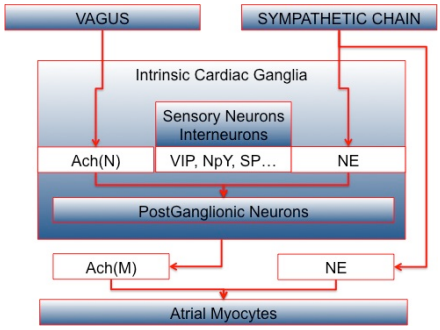


Figure 2. **Autonomic cardiac nerves**

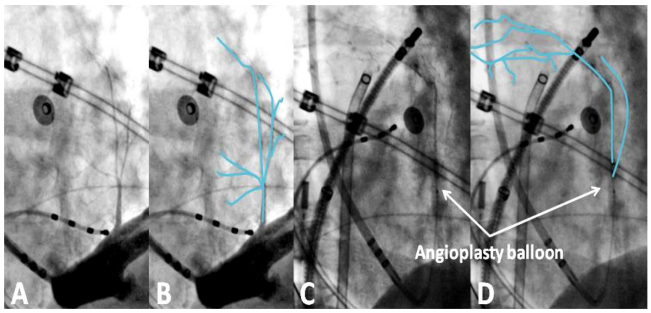
Inputs from the vagus (cholinergic nicotinic, Ach(N)), the sympathetic chain (using norepinephrine, NE) and from sensory neurons and interneurons (other neuromodulators, see text) are processed by intrinsic cardiac ganglia. Atrial myocytes receive output from postganglionic neurons via cholinergic muscarinic (Ach (M) receptors), and from sympathetic postganglionic adrenergic innervation.

High-frequency stimulation in the ligament of Marshall (without exciting the atrial myocardium) leads to induction of AF, and this induction is inhibited by both esmolol and atropine, suggesting autonomic mediation.[101] The VOM is present and can be cannulated in ~85% of patients,[94] and our data confirm that it is a *direct vascular route to the intrinsic cardiac ganglia that could be therapeutically utilized.*

3.2 VOM ETHANOL INFUSION: TECHNIQUE

We have refined the technique over the past 3 years. We enter the CS with a sheath advanced from the right internal jugular vein. A sub-selector catheter with a ~90° angle at the tip (typically, a left internal mammary artery angioplasty guide catheter) is advanced through the CS sheath with its tip pointing superiorly and posteriorly. Contrast injections through the sub-selector catheter help identify the VOM and direct the catheter tip to the VOM ostium. Then, an angioplasty wire is inserted into the VOM, over which an angioplasty balloon is advanced distally into the VOM. Contrast injections through the angioplasty balloon help delineate the size and branching patterns of the VOM. Ethanol injections are then delivered (up to four injections of 1 cc over 2 minutes each), each at different levels of the VOM –from distal in the VOM, where the first injection is delivered, the balloon is retracted ~1 cm after each injection until the balloon reaches the VOM ostium or 4 injections are given. Figure 3 shows an example.

Figure 3. VOM cannulation technique and LA venous plexus.



A, Contrast injection in the CS lumen through the sub-selector catheter with its tip close to the VOM, showing the VOM take-off and branching patterns outlined in **B**. **C**, selective venogram via an angioplasty balloon in a VOM branch. Collaterals fill LA veins in the LA roof (outlined in **D**).

In our experience to date, we have been able to perform successful cannulation of the VOM and to complete the protocol of ethanol infusion in 89 of a total of 106 patients (85%). Our success rates in the last half of the patients versus the first half have been higher (90% vs. 73%, $p<0.05$), suggesting that success is not only determined by anatomical factors (e.g., size and tortuosity of the VOM), but also by operator experience.

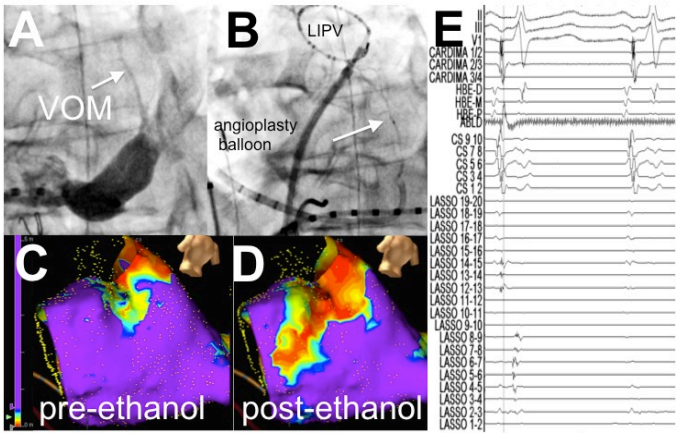
3.3 UNVEILING OF AN LA VENOUS PLEXUS

Our initial experience has confirmed that the VOM is a true atrial vein, communicating via capillaries with the LA myocardium, rather than a simple residual lumen of the ligament of Marshall, and thus the VOM is a viable route to deliver therapeutic agents in the LA. Additionally, with occlusive VOM venograms, we have found a heretofore-undescribed epicardial atrial venous plexus filled via collaterals.

3.4 VOM ETHANOL INFUSION: TISSUE ABLATION AND LEFT PV DISCONNECTION

The obvious effect of ethanol infusion is rapid ablation of atrial tissues in the vicinity of the VOM. Such areas are standard targets of ablation in persistent AF, and encompass the lateral ridge of the left atrium (which due its thickness can be difficult to ablate, see Figure 2), extending variably to areas around the left PVs, and towards the mitral annulus, including a large portion of the mitral isthmus. In our total experience of up to 89 cases, ethanol infusion can lead to isolation of the left inferior PV in up to 74% of the cases, and isolation of the left superior PV in 44% and generates an area of ablated tissue of 9.7±4.8 cm^2. Figure 4 shows an example.

Figure 4. **Tissue ablation by VOM ethanol infusion**

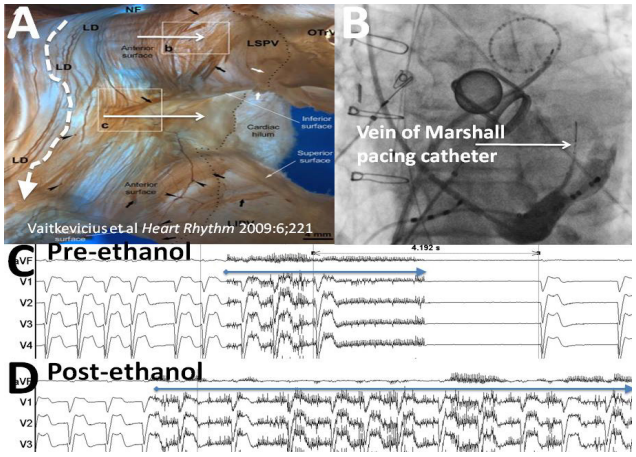


.**A**, Contrast injection in the CS showing the VOM take-off. **B**, VOM cannulation with angioplasty balloon. Contrast is injected prior to ethanol. A circular catheter is placed in the left inferior PV (LIPV). **C-D**, Voltage maps (scar in red, of 7 cm^2) of the left atrium pre-and post-ethanol. **E,** Signals from the LIPV during ethanol infusion, showing elimination of PV potentials (LASSO).

3.5 VOM ETHANOL INFUSION: A NOVEL TECHNIQUE FOR LOCAL VAGAL DENERVATION IN HUMANS

The location of the VOM coincides with that of the left dorsal pathway of vagal innervation to the intrinsic cardiac ganglia[102] (Figure 5). In our recent experience we have shown that high-frequency stimulation (30 Hz, 25 mA) in the VOM can induce vagal reflexes reaching the AV node (causing transient AV node conduction blockade) in 75% of patients (n=32) and inducing AF in 100%. Such responses are completely abolished in all patients after VOM ethanol infusion (Figure 5).Of note, because AF is consistently induced during high-frequency stimulation –due to direct left atrial capture-, vagal responses are only assessable by the presence of AV nodal block. Of the vagal plexi of the atria, it is the right inferior PV plexus that directly connects with the AV node.[103] The VOM is remote from the AV node, so inducing AV conduction slowing by VOM high-frequency stimulation supports VOM-to-right inferior PV plexus-to-AV node connection, and thus *supports that the VOM is a vascular route to the intrinsic cardiac ganglia* (see Figure 5A). Vagal responses were abolished in all patients in whom such responses were elicited at baseline, and AF induction by VOM high-frequency elimination was eliminated in all patients. Thus, VOM ethanol infusion is an effective strategy to achieve regional denervation of the human LA.[104]

Figure 5. Vagal denervation by VOM ethanol infusion

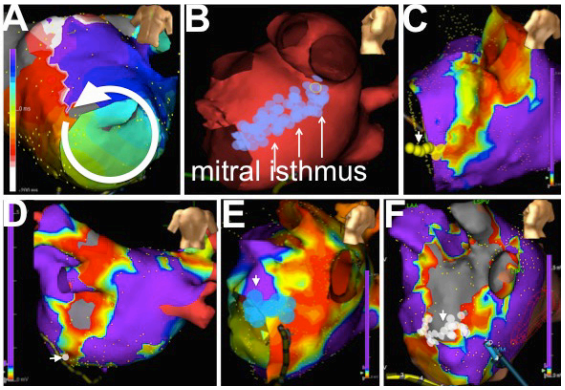


A, Vagal innervation (histochemical staining) of the LA. Dotted line is the location of the VOM, coinciding with the left dorsal (LD) tract of vagal nerves, connected with neural plexi (insets). From indicated reference. B, VOM cannulation with a quadripolar catheter to perform high-frequency stimulation, indicated by the blue arrow in C and D, Electrograms during high-frequency stimulation in the VOM during on-going AF. Pre-ethanol infusion (C), atrioventricular block with asystole of 4.1 s is induced. Such response is abolished after ethanol infusion (D).

3.6 VOM ETHANOL INFUSION AND PERIMITRAL FLUTTER (PMF)

Due to the frequent incidence of PMF, the difficulties in achieving perimitral bidirectional conduction block to treat it, and the potential risk of damaging the left circumflex coronary artery with RF ablation, there is a clinical need for new treatment strategies. We have evaluated the effect of VOM ethanol infusion on perimitral conduction in 43 patients (25 of which had PMF mapped prior to ethanol delivery). Although VOM ethanol infusion by itself only led to bidirectional perimitral block in 3 patients, this was easily achieved with minimal RF ablation in the most anterior aspect of the mitral isthmus (2.5±1.3 min), anterior to the scar created by ethanol, in 98% of patients.[105] Figure 6 shows examples. Considering the low success rate reported by RF ablation (32%[59], 64%,[60] or 71%[61]) –including epicardial ablation in the CS-, and the potential iatrogenic induction of recurrent flutters when bidirectional perimitral block is not achieved due to incomplete ablation, this novel technique promises to make a significant difference in the treatment of PMF.

Figure 6. PMF treated by VOM ethanol infusion



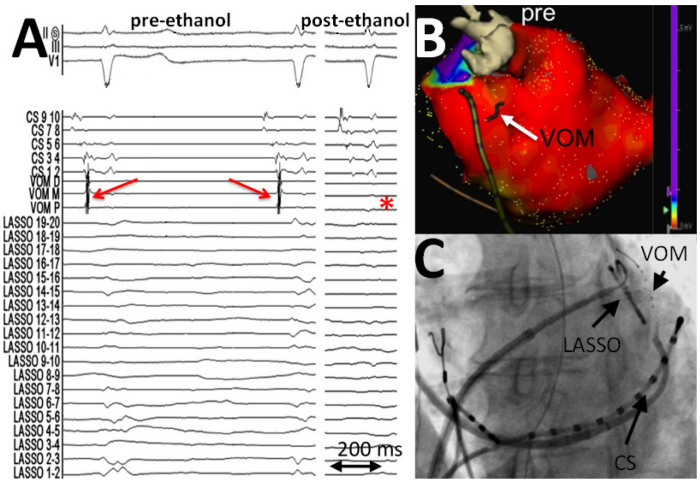
A, Example of PMF (counterclockwise, colors represent time). B, Conventional ablation sites (blue dots) in the mitral isthmus to treat PMF. C-F, Examples of ethanol-induced scar maps (voltage color scale) and locations of RF ablation lesions (arrowheads), required to achieve bidirectional mitral block.

3.7 ROLE OF VOM IN FAILED ABLATIONS

We have assessed the role of VOM activity in patients presenting for a repeat ablation procedure after a failed PVAI, as part of our R21 project. In 58 patients with recurrent AF, the VOM was cannulated in 51 and VOM signals were present in all of them, indicating that a *conventional PVAI procedure does not ablate VOM activity*. This was the case even in cases in which extensive LA ablation had been performed in the index procedure. Figure 7 shows an example that illustrates that, even with extensive LA ablation (that caused most of the LA endocardium to be scarred –without detectable electrograms) the VOM remains electrically active.

Thus, as a novel catheter ablation technique, our preliminary mechanistic data in humans supports that VOM ethanol infusion provides rapid tissue ablation of targeted areas, helps treat PMF and achieves regional LA vagal denervation. The VOM is not otherwise ablated by conventional PVAI.

Figure 7. Lack of VOM ablation by PVAI



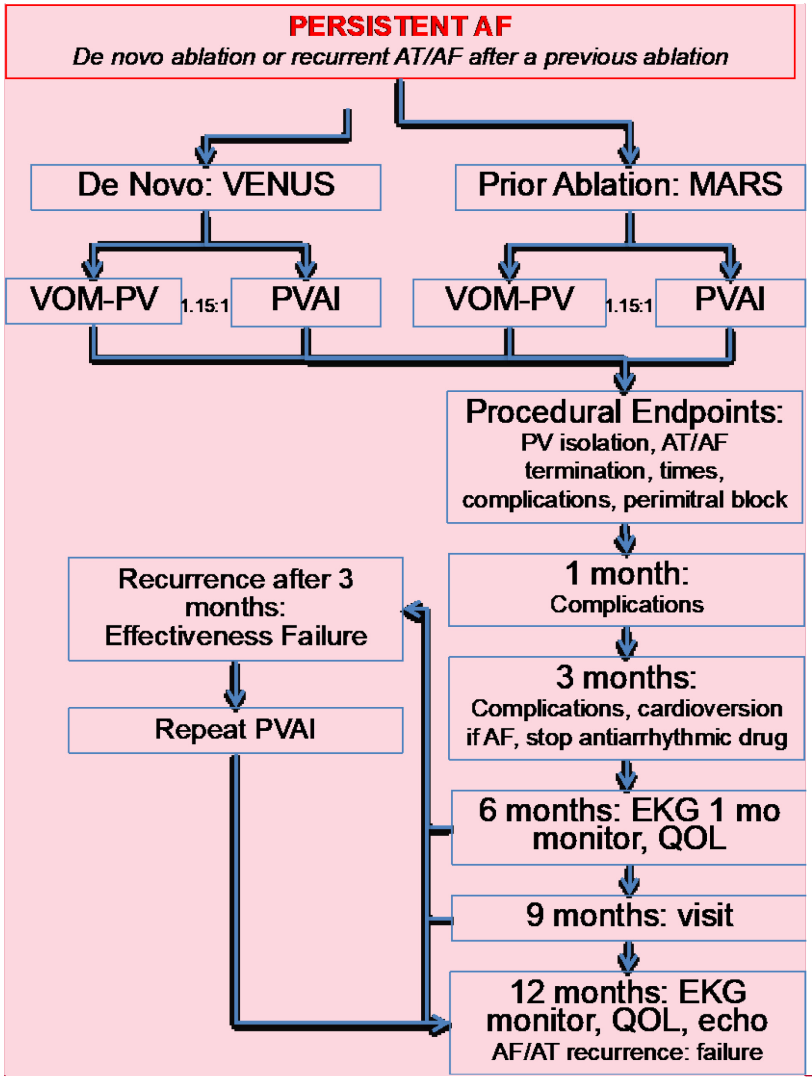
A patient with recurrent AF after an extensive PVAI underwent VOM ethanol infusion. A, intracardiac signals from the CS, VOM and the endocardium adjacent to the VOM (LASSO). Pre-ethanol infusion, large signals are recorded from the VOM (red arrows) that are abolished post-ethanol (*). B, 3D Voltage amplitude map of LA endocardium, showing scar (red) specifically on the endocardial side of the VOM. C, Fluoroscopic catheter position.

4.0 TRIAL OVERVIEW AND PRELIMINARY DATA

4.1 HYPOTHESIS

Our hypothesis is that a combined procedure of VOM ethanol infusion plus conventional PVAI (VOM-PV) is superior to PVAI alone in the catheter ablation treatment of persistent AF. We will compare the two treatments in a randomized fashion in 2 subsets of patients: *de novo* ablation, and repeat ablation in patients with persistent AF (Figure 8). Given the extent of tissue ablation required, we have chosen to use this technique in persistent AF, rather than in paroxysmal AF, in which less extensive tissue ablations may suffice. VOM ethanol infusion must be an add-on to the standard catheter ablation procedure, since it has no effect on other ablation targets such as the right PVs, septum, etc. Over our past experience we have established the safety of this procedure, uncovered novel mechanistic effects such as vagal denervation, and generated pilot data to support an improvement in outcomes.

Figure 8. Clinical Trial Design.



Commented [DE1]: Month 9 needs a description of what will happen at that time point

4.2 PRELIMINARY OUTCOMES DATA: RESULTS OF OUR PILOT EXPERIENCE

We have compared our ablation outcomes in persistent AF patients treated with VOM-PV with those treated with PVAI. In 174 patients undergoing conventional PVAI, our single-procedure success rate at one year has been 45% (consistent with literature reports of 27%,[45] 36%,[57] or 49%[58]). In contrast, in 66 patients with persistent AF subjected to VOM-PV, our success rate has been 61%. These data will be used for sample size statistical calculations for VENUS-AF, which enrolls a patient population undergoing their first AF ablation. Additionally, 62 patients with recurrent AF or flutter after a conventional ablation have undergone a repeat procedure including VOM-PV with a success rate of 76%. In our previous experience in such repeat procedures (n=169) our success rate was 42%, consistent with that of the literature. [45, 57, 58] These data will be used for ,MARS- AF which enrolls a patient population undergoing repeat ablations.

1. For all patients:
   a. Total procedure, RF ablation, and fluoroscopy times.
   b. RF time and success of bidirectional block across the mitral isthmus line tested by differential pacing.
   c. Pre-ablation 3D voltage maps.
   d. Ablation lesion sets: 3D maps (Carto or NavX), including ablated scar surface area, as measured by bipolar voltage less than 0.1 mV.
   e. Any procedural complications.
2. In patients randomized to VOM ethanol infusion:
   a. Successful vs. unsuccessful cannulation with angioplasty wire and balloon.
   b. Extent of tissue ablation achieved by ethanol infusion, defined as areas with local electrogram voltage <0.1 mV on 3D mapping. (Pre-PVAI voltage map).
   c. Added procedural and fluoroscopy time.
   d. Effect on AF: termination, conversion into flutter or no change.
   e. RF time to achieve block around the mitral annulus.
   f. Complications related to VOM instrumentation.
   g. Blood ethanol level measurement.
   h. LA instrumentation time.
   i. Ablation lesion sets: 3D maps (Carto or NavX) including total (RF plus ethanol) ablated scar surface area, as measured by bipolar voltage less than 0.1 mV
   j. Periprocedural data collection.

4.3 THREE MONTH BLANKING PERIOD

This protocol uses a three month “blanking period” as a period of time following an atrial fibrillation procedure in which atrial fibrillation episodes can occur as part of the body’s healing response. Any atrial fib/flutter activity during that blanking period is not counted in the study’s results and is not used in determining success or failure of the procedure as it is a common and expected outcome.

5.0 MATERIALS AND METHODS

5.1 SPECIFIC AIMS

Aim for VENUS is to assess the impact of VOM ethanol infusion in single-procedure success when added to <u>de novo *catheter ablation*</u> of persistent AF.

VOM triggers and innervation may play a role in persistent AF, and are not addressed by a standard PVAI. Our hypothesis is that VOM ethanol infusion will do so and lead to improved

outcomes. This is a prospective, multi-center, randomized study comparing a combined procedure including VOM ethanol infusion plus PVAI (VOM-PV) with PVAI alone in patients with persistent AF. The trial design incorporates a plan for possible repeat procedures if AF recurs after the 3-month blanking period, as this is common in clinical practice.

The aim of MARS is to assess the impact of VOM ethanol infusion <u>after a failed conventional ablation</u> of persistent AF.

Ablation failures requiring repeat procedures after PVAI are common, and can be due to recurrent AF, or new onset atrial flutters. We hypothesize that VOM ethanol infusion can be beneficial in these repeat procedures.

Our reported experience shows that VOM signals are uniformly intact in patients with recurrent AF after failed PVAI,[1] supporting the idea that VOM-dependent AF mechanisms are not addressed in conventional PVAI. Thus, we hypothesize that VOM ablation by ethanol infusion may have added value in this setting. Additionally, PVAI ablation failures may present as atrial flutters, including PMF and other circuits utilizing the mitral isthmus (e. g. reentry around the left PV antrum). We hypothesize, as supported by our recently reported data,[3] that VOM ethanol infusion can assist achieving bidirectional mitral isthmus block by ablating most of the mitral isthmus and requiring minimal RF applications in the most anterior aspect of the isthmus (Figure 6). Patients with AF or flutter occurring beyond 3 months after a previous standard PVAI performed outside this study will be randomized to either a repeat PVAI procedure alone or a VOM-PV. The goal is to assess the role of VOM as an added salvage procedure to a repeat ablation. Heterogeneities in the index PVAI procedure are likely, but will be characterized in detail in the pre-procedure scar map using voltage mapping. Additionally, patients with recurrences as flutter are expected to have a greater success, so randomization will be stratified per flutter vs. AF recurrence.

5.2 STUDY ENDPOINTS

Primary endpoints:

1. Primary Efficacy Endpoint Freedom from AT/AF clinical recurrence on follow-up visits AND less than 30 seconds of AT/AF on a 1 month continuous electrocardiographic monitor at 6 and 12 months.
2. Primary Safety Endpoint - Acute procedural complications and total mortality.

Secondary Endpoints

1. Freedom from AF/AT after >1 procedure.
2. Freedom from AF/AT on antiarrhythmic drugs.
3. AF burden (% time) on continuous monitoring at 6 and 12 months.
4. Procedural parameters: total procedure, fluoroscopy, total RF ablation time (first procedure), and total extent of ablated LA tissue.
5. Clinical/partial success: less than 25% AF burden on a continuous event monitor at 6 and 12 months from ablation procedure.
6. Sub-acute procedural complications (within 30 days).
7. Recurrence as persistent or paroxysmal AF, or flutter after 1 or 2 procedures.

8. LA function on Doppler echocardiography (LA strain[114ab]) at 12 months.
9. Incidence and mechanisms of atrial flutters.
10. Cardiovascular hospitalizations and
11. QOL as determined by AFEQT questionnaire.

5.3 INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

1. Patients between the ages of 21 and 85 years
2. Ablation History
   Patients for **VENUS** must meet the following:
   - Diagnosed with symptomatic not previously ablated persistent AF,
   - AF not spontaneously converting to sinus rhythm, persisting for ≥7 days

   Patients for **MARS** must meet the following
   - Recurrent AF or AT after a previous ablation of persistent AF at least 3 months prior to enrollment.
3. Resistant or intolerant to at least one class I, II, or III AAD
4. Patients deemed candidates for RF ablation of AF
5. Able and willing to comply with pre-, post-, and follow-up requirements.

Exclusion criteria

1. Left atrial thrombus.
   LAA thrombus can be determined by pre-procedural imaging: CT, TEE or MRI. Documentation by exception (ie. no LAA thrombus on imaging reports) is permitted for determination of eligibility.
2. LA diameter greater than 65 mm on long axis parasternal view, or left atrial volume more than 200 cc.
3. Left ventricular ejection fraction < 30%.
4. Cardiac surgery within the previous 90 days.
5. Expecting cardiac transplantation or other cardiac surgery within 180 days.
6. Coronary PTCA/stenting within the previous 90 days.
7. Documented history of a thrombo-embolic event within the previous 90 days.
8. Diagnosed atrial myxoma.
9. Significant restrictive, constrictive, or chronic obstructive pulmonary disease with chronic symptoms.
10. Significant congenital anomaly or medical problem that in the opinion of the investigator would preclude enrollment
11. Women who are pregnant or who plan to become pregnant during the study.
12. Acute illness or active infection at time of index procedure documented by either pain, fever, drainage, positive culture and/or leukocytosis (WBC > 11k/ mm^3) for which antibiotics have been or will be prescribed.
13. Creatinine > 2. 5 mg/dl (or > 221 µmol/L, except for patients in dialysis).
14. Unstable angina.

15. Myocardial infarction within the previous 60 days.
16. History of blood clotting or bleeding abnormalities.
17. Contraindication to anticoagulation.
18. Life expectancy less than 1 year.
19. Uncontrolled heart failure.
20. Presence of an intramural thrombus, tumor, or other abnormality that precludes catheter introduction or positioning.
21. Presence of a condition that precludes vascular access.
22. INR greater than 3.5 within 24 hours of procedure – for patients taking warfarin.
23. Cannot be removed from antiarrhythmic drugs for reasons other than AF.
24. Unwilling or unable to provide informed consent.

5.4 Informed Consent Process

The informed consent should be signed by the potential subject ***prior to any study-specific procedures taking place.***

An appropriately trained study team member will conduct the informed consent process with potential subjects in the approved manner for the institution, to include (at a minimum) the following procedures: ensuring the use of the most currently IRB approved document, allowing the potential subject sufficient time to read the consent and ask questions of the study staff, and ensure subjects have a clear understanding of the voluntary nature of their consent and the expectations for their commitment. The *process* above should be documented in the study record, apart from a copy being placed into the study file.

5.5 Study Procedures

SCREENING/BASELINE

1. Initial assessment.

After signing informed consent, the following data will be collected; a significant medical history and recent targeted physical exam, electrocardiogram (EKG), echocardiogram within one year prior to the procedure for evaluation of cardiac structure, and function, and laboratory tests. In addition, a review of medications that patient is taking (limited to AAD and anticoagulants), and a quality of life (QOL) questionnaire especially developed for AF (AFEQT)[113] will be filled out by patients.

Screening assessments performed pre-consent signature that are completed under standard of care can be included as viable source documents for patient study inclusion/exclusion and may be collected to screen potential study patients.

2. Pre-procedural imaging.

Imaging prior to enrollment is required to rule out structural heart disease and, as needed, to rule out the presence of LA appendage thrombus. *Ruling out LAA thrombus can be performed by the following: TEE, CT or MRI within 48 hours of the*

procedure; at least one month of oral anticoagulation prior to the procedure; or documented prior procedures of LAA occlusion or ligation. For ruling out structural heart disease, either a cardiac MRI, CT or transthoracic echocardiogram within 1 year prior to participation in the study is sufficient. Documentation by exception (ie. no LAA thrombus documented on imaging report) is permitted for determination of eligibility. Left atrial diameter and estimated left atrial volume will be obtained from any of these diagnostic modalities. There is no specific requirement for a pre-ablation CT or MR, since anatomical details of the LA can be obtained intra-procedurally with current mapping systems.

3 Randomization.

Randomization should take place after confirmation that all inclusion/exclusion criteria are met and measurements of LA volume are obtained. Patients will be randomized in a 1.15:1 fashion (to account for an 85% technical feasibility of the VOM procedure) to receive either VOM-PV or the conventional PVAI. Patients will be blinded to the randomization outcome.

PROCEDURE/DAY 0:

1 PVAI procedure

As part of a conventional catheter ablation of AF the following will be performed, all considered standard of care:

a) Electrophysiological catheters will be inserted, including a CS catheter, a duodecapolar circumferential catheter, and an ablation catheter. The last two will be inserted in the LA via trans-septal punctures.

b) Prior to ablation, geometry of the LA will be obtained using a 3-dimensional (3D) mapping system (any of the commercially available systems). This will generate a computerized geometry of the LA, including baseline voltage amplitudes in different regions. LA scar surface area (bipolar voltage less than 0.1 mV) will be collected.

c) Lesion sets delivered by RF application will include, in a step-wise fashion, the following ablations, starting with PVAI and added sequentially per the operator's judgment if AF persists after each step is completed:

    i) PVAI. RF should be applied 1 cm proximal to the PV ostia in a wide area circumferential pattern. Isolation will be verified by the absence of electrical activity from each PV and/or dissociated activity.

    ii) The greater PV antra, including posterior wall and roof.

    iii) Mitral isthmus: a line of RF ablation from the left inferior PV to the mitral annulus. Bidirectional block should be verified after completion by differential pacing.

iv) Areas of complex, fractionated potentials.

v) Sustained atrial flutters will be mapped and ablated as directed by the map and flutter location.

vi) Following step 4c, if AF persists after all the RF ablations, the patient will be cardioverted to restore sinus rhythm. Given the potential variability of the extent of ablations, maps of the lesion sets (see below) will be collected and maintained in an imaging core laboratory.

2 VOM procedure

In patients randomized to VOM-PV, ***prior to the conventional PVAI***, the following will be performed:

a) A 7F-9F sheath will be advanced in the CS via a right internal jugular vein access. Femoral vein access is also appropriate to cannulate the CS. Contrast injection in the CS will be performed via a sub-selector catheter (recommended 6F left internal mammary angiographic guide catheter) to identify the VOM. We will obtain a CS venogram and identify the location of the VOM. Cannulation of the VOM will be performed using the sub-selector catheter that can be torqued so that its tip is engaged in the ostium of the VOM. Contrast will be injected via the lumen of the sub-selector catheter to verify such engagement.

b) If large enough, the VOM will be cannulated with an angioplasty wire (0. 014") that will be advanced through the sub-selector catheter and into it. If the VOM is too small to accommodate the wire, venodilation with 200 µgm of nitroglycerine through the sub-selector catheter will be administered to facilitate VOM cannulation.

c) An angioplasty balloon (1.5 -2 mm diameter, 6-8 mm length) will be advanced over the wire and positioned in the ostium of the VOM. The balloon will be inflated to occlude the vein. Contrast venograms of the VOM will be recorded in left and right anterior oblique projections. The angioplasty balloon will be then advanced as distally as possible in the VOM and the first ethanol injection will be performed there after balloon inflation. The balloon will be then deflated and retracted 1-2 cm for a repeat inflation and ethanol injection. Up to four, 1 cc injections (depending on the VOM length) of 98% ethanol will be delivered in the VOM by sequentially retracting the balloon up to the VOM ostium.

d) The procedure will then continue with standard PVAI procedure as outlined in section 4.

3 Bipolar voltage amplitude maps to be performed:

Using an electro-anatomical mapping system, the extent of the scar –measured as bipolar voltage <0.1 mV- will be recorded:

a. At baseline after gaining trans-septal access to the LA in both randomization

groups.

b. After ethanol infusion, if randomized to VOM-PV.
c. After completion of the PVAI ablation lesions, in both randomization groups.

5.6 POST-PROCEDURAL DATA COLLECTION:

Patients may receive follow-up standard of care procedures (ECG, physical exam, review of medical history and concomitant medications) at the study site or at a provider of their choice. If an investigator at a study site does not perform the visit, the study staff will have the patient sign a Release of Medical Information and request the applicable medical records from the patient's provider. All ECG tracings must be reviewed and interpreted by a study investigator. The AFEQT questionnaire may be conducted by telephone call with the patient.

1. **Seven day telephone follow-up (+/- 3 days)** should be conducted by study coordinator to assess patient for symptoms of procedure related or disease related complications.
2. **Thirty-day (30D) follow-up (+/- 10 days).** Follow up evaluation will include an EKG, assessment for complications including a targeted physical exam and a review of adverse events and concomitant medications (limited to AAD and anticoagulants) will be documented. Routine medications, including AAD may be continued. Symptomatic AF or flutter should be treated with AAD or cardioversion as needed but will not be recorded for endpoint assessment.
3. **Three-month (90D) follow-up (+/- 30 days).** Evaluation will include an EKG, and assessment for complications, targeted physical exam and a review of adverse events and concomitant medications (limited to AAD and anticoagulants) will be documented. If the patient is in AF or flutter, a cardioversion will be performed electively within 2 weeks so that all patients are in sinus rhythm after the 3 month blanking period. AAD therapy will be discontinued in all patients at this time if they are clinically stable and in sinus rhythm.
4. **Six-month (180D) follow-up up (+/- 60 days)**. Follow up evaluation will include an EKG, and physical exam. Additionally, patients will fill out the AFEQT[113] QOL questionnaire and will undergo a 3-4 week continuous EKG monitor (4 weeks if tolerated by patient) (see *Core laboratories,* below). Subjects who have a miniaturized, implantable rhythm recording device (such as Medtronic LinQ and others) or an implanted pacemaker/defibrillator may have the continuous rhythm data obtained from that device, and may forego the portable recorder. The purpose of this EKG monitor (4 weeks if tolerated by patient) is to screen for recurrent AF that may prompt an early repeat procedure. Patients that have clinical or EKG recurrences will undergo a PVAI procedure (see below and Figure 8).
5. **Nine-month follow-up up (+/- 30 days).** Follow up evaluation will include an EKG and physical exam. Patients that have clinical or EKG recurrences will undergo a repeat PVAI procedure. The timing of a repeat procedure will be encouraged to be within 6 months of randomization.
6. **Twelve-month follow-up up (+/- 60 days)**. Follow up evaluation will include an EKG, and physical exam. Additionally, patients will fill out the AFEQT[113] QOL questionnaire

and undergo a continuous EKG monitor (4 weeks if tolerated by patient) to determine the primary efficacy endpoint.

Subjects who have a miniaturized, implantable rhythm recording device (such as Medtronic LinQ and others) or an implantable pacemaker/defibrillator may have the continuous rhythm data obtained from that device, and may forego the portable recorder.

Patients will fill out the AFEQT[113] QOL questionnaire. Additionally, echocardiographic assessment of LA function (LA ejection fraction, strain[114ab]) will be performed by a central reader at Houston Methodist Hospital.

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5.7 VENUS: Definitions of Procedural Success or Failure and Indications for Repeat Procedures

1. **Success:** Freedom from AT/AF clinical recurrence on follow-up visits AND less than 30 seconds of AT/AF on a 1 month continuous electrocardiographic monitor .

2. **Clinical Success.** Freedom from AT/AF clinical recurrence on follow-up visits but documented AF or flutter up to 25% of the time on a 1-month continuous electrocardiographic monitor. The rationale is to account for patients in whom a repeat procedure would not be clinically indicated, yet AF/AT would not be considered cured.

3. **Repeat procedures. A repeat procedure will constitute an effectiveness failure for the purpose of the primary efficacy endpoint.** However, repeat procedures and their outcomes will be recorded for secondary outcome analysis. First, repeat procedures are a clinical reality in persistent AF, and a single-procedure success endpoint –does not capture it. Second, it is possible that VOM-PV on a first procedure may increase success of a second procedure –e. g. if the recurrences in VOM-PV group are as AT instead of AF. Both represent a clinical failure of the procedure, but a repeat procedure for AT is more likely to succeed.[65] Repeat procedures will be encouraged to be timed within the first 6 months of the randomization procedure. Although this may seem short, it is our clinical experience that the bulk of AF recurrences tend to occur shortly after the blanking period.[115] Thus, we expect a minority of patients to recur late in this window.

   Indications for a repeat procedure include:

   a. Procedure failure: Symptomatic, recurrent persistent AF or flutter detected clinically during the scheduled follow-up visits.
   b. Less than partial/clinical success: AF or flutter burden on electrocardiographic monitoring exceeding 25% regardless of symptoms. Monitoring will be performed at 6 months post randomization procedure.
   c. Symptomatic AF or flutter detected on electrocardiographic monitoring regardless of AF or flutter burden. Monitoring will be performed at 6 months post randomization procedure.

Data to be collected during a repeat procedure will include

a. Documentation of PV isolation: number and location of reconnected PV at the baseline of the repeat procedure.
b. Perimitral conduction: presence or absence of perimitral block.
c. Mechanisms of atrial flutter, if present.
d. Documentation of other RF ablation sites
e. Documentation of RF time, time to perimitral block (if not already achieved), fluoroscopy time, LA instrumentation time, and procedure time.
f. Baseline and Final LA voltage map (using any commercially available electroanatomical mapping systems). Measurement of baseline and Final LA scar surface area.

4. Effectiveness (Treatment) Failures

Effectiveness failures towards the primary endpoint will include the following (see Figure 8):

a. Clinical recurrence of AF or AT after 3-months.
b. Documented AF or AT of 30 seconds or more on EKG monitor at obtained at 6 and 12 months.
c. Requirement of a repeat ablation procedure for recurrent AT-AF.
d. Death.

VENUS and MARS patients who have a recurrence after 3 months post randomization will have a repeat procedure (PVAI). Patients will be deemed to be effectiveness failure for the primary efficacy endpoint of the trial after repeat procedures. Still, subjects will:

a. Remain in the study for the purpose of all secondary endpoints: these include quality of life, AF burden on event monitoring, success after multiple procedures.
b. Undergo all clinically necessary procedures and treatments, including prescription of antiarrhythmic therapy and additional procedures needed to control atrial fibrillation or flutter. A crossover VOM procedure will be offered to those randomized to PVAI after 2 in-study procedures.
c. Data on such additional procedures or treatment will be collected in the Electronic Data Capture system (EDC).
d. An additional secondary endpoint will be created: total number of procedures performed and requirement of antiarrhythmic drugs.

5. VENUS: Cross-over of patients initially randomized to PVAI only.

If a VENUS patient is originally randomized to conventional PVAI and experiences a treatment failure after a repeat procedure he or she may undergo an additional conventional PVAI ablation procedure during the study. Crossover to VOM ethanol will only be allowed after 2 procedures are performed in the setting of study participation. This is allowed because certain recurrent flutters are particularly suited to respond to VOM ethanol. The primary and secondary endpoints will be computed following their original randomization group.

5.8 MARS: Definitions of Procedural Success or Failure and Indications for Repeat Procedures

1. **Success:** Freedom from AT/AF clinical recurrence on follow-up visits AND less than 30 seconds of AT/AF on a 1 month continuous electrocardiographic monitor .

2. **Clinical Success.** Freedom from AT/AF clinical recurrence on follow-up visits but documented AF or flutter up to 25% of the time on a 1-month continuous electrocardiographic monitor. The rationale is to account for patients in whom a repeat procedure would not be clinically indicated, yet AF/AT would not be considered cured.

3. **Repeat procedures. A repeat procedure will constitute an effectiveness failure for the purpose of the primary efficacy endpoint.** However, repeat procedures and their outcomes will be recorded for secondary outcome analysis. First, repeat procedures are a clinical reality in persistent AF, and a single-procedure success endpoint –does not capture it. Second, it is possible that VOM-PV on a first procedure may increase success of a second procedure –e. g. if the recurrences in VOM-PV group are as AT instead of AF. Both represent a clinical failure of the procedure, but a repeat procedure for AT is more likely to succeed.[65] Repeat procedures will be encouraged to be timed within the first 6 months of the randomization procedure. Although this may seem short, it is our clinical experience that the bulk of AF recurrences tend to occur shortly after the blanking period.[115] Thus, we expect a minority of patients to recur late in this window.

   Indications for a repeat procedure include:
   a. Procedure failure: Symptomatic, recurrent persistent AF or flutter detected clinically during the scheduled follow-up visits.
   b. Less than partial/clinical success: AF or flutter burden on electrocardiographic monitoring exceeding 25% regardless of symptoms. Monitoring will be performed at 6 months post randomization procedure.
   c. Symptomatic AF or flutter detected on electrocardiographic monitoring regardless of AF or flutter burden. Monitoring will be performed at 6 months post randomization procedure.

Data to be collected during a repeat procedure will include
   a. Documentation of PV isolation: number and location of reconnected PV at the baseline of the repeat procedure.
   b. Perimitral conduction: presence or absence of perimitral block.
   c. Mechanisms of atrial flutter, if present.
   d. Documentation of other RF ablation sites
   e. Documentation of RF time, time to perimitral block (if not already achieved), fluoroscopy time, LA instrumentation time, and procedure time.
   f. Baseline and Final LA voltage map (using any commercially available electroanatomical mapping systems). Measurement of baseline and Final LA scar surface area.

4. Effectiveness (Treatment) Failures

Effectiveness failures towards the primary endpoint will include the following (see Figure 8):

e. Clinical recurrence of AF or AT after 3-months.
f. Documented AF or AT of 30 seconds or more on EKG monitor at obtained at 6 and 12 months.
g. Requirement of a repeat ablation procedure for recurrent AT-AF.
h. Death.

Patients who have a recurrence after 3 months post randomization will have a repeat procedure (PVAI). Patients will be deemed to be effectiveness failure for the primary endpoint of the trial after repeat procedures. Still, subjects will:

e. Remain in the study for the purpose of all secondary endpoints: these include quality of life, AF burden on event monitoring, success after multiple procedures.
f. Undergo all clinically necessary procedures and treatments, including prescription of antiarrhythmic therapy and additional procedures needed to control atrial fibrillation or flutter. A crossover VOM procedure will be offered to those randomized to PVAI after 2 in-study procedures.
g. Data on such additional procedures or treatment will be collected in the Electronic Data Capture system (EDC).
h. An additional secondary endpoint will be created: total number of procedures performed and requirement of antiarrhythmic drugs.

5. Cross-over option for of patients initially randomized to PVAI only.
If a patient is originally randomized to conventional PVAI and experiences a treatment failure after a repeat procedure he or she may undergo an additional conventional PVAI ablation procedure during the study. Crossover to VOM ethanol will only be allowed after 2 procedures are performed in the setting of study participation. This is allowed because certain recurrent flutters are particularly suited to respond to VOM ethanol. The primary and secondary endpoints will be computed following their original randomization group.

6.0 STATISTICAL CONSIDERATIONS

A) Assumptions.

Single-procedure versus multiple-procedure success. Our initial preliminary data suggested an overall procedure success of 45% in patients undergoing PVAI and 61% for those undergoing VOM-PV (difference of 16%). This included patients with repeat procedures performed in some, but not all of the failed procedures (45% all patients in the PVAI group and 30% in the VOM-PV group). **The single-procedure success was 38% in patients undergoing PVAI and 56% in patients undergoing VOM-PV** (difference of 18%)**.** Thus, the endpoint of single-procedure success is likely to show greater differences amongst groups.

Mortality. The expected mortality is low in this study as it has been in AF ablation trials.

Mortality will be recorded as a safety endpoint.

One-year follow-up time. In previous versions of the protocol, additional follow-up time (up to 15 months) was included in the VENUS trial, in order to accommodate for appropriate follow-up of patients undergoing repeat procedures. Therefore, a trial duration of 12 months is sufficient if just single-procedure success is the primary efficacy endpoint. Procedural failures (events counted as the primary efficacy endpoint) occur mostly during the first year. Additionally, 12-month follow-up is consistent with the recommendations for clinical trials in AF by the HRS/EHRA/ECAS Catheter and Surgical Ablation consensus document.[2]

Unknown classification as success or failure. Patients who cannot be classified as successes or failures on the primary efficacy endpoint will be excluded from the primary analysis.

B) VENUS Group Sequential Clinical Trial Design

Power and sample size determination. Group sequential two proportions power analysis using simulation was performed using PASS V12 (Kaysville, UT). The following assumptions were made:

- Response rate in PV-VOM: p_1=0.56
- Response rate in PVAI: p_2= 0.38
- Hypotheses: H0: $p_1 = p_2$; H1: $p_1 \neq p_2$
- Test Statistic: Z-Test (Unpooled)
- Zero Adjustment Method: None
- Alpha-Spending Function: O'Brien-Fleming Analog
- Beta-Spending Function: None
- Futility Boundary Type: None
- Number of Looks: 3
- Simulations: 100000

Results. A group sequential trial with sample sizes of N1=180 and N2=156 at the final look achieves 91% power to detect a difference of 0.18 between a treatment group success proportion of 0.56 and a control group success proportion of 0.38 at the 0.05 significance level (alpha) using a two-sided Z-Test (Unpooled). The table below lists the sample sizes required for 91% power.

Table 5. Sample size requirements for a group sequential trial based on 100,000 iterations.

Value	95% LCL	95% UCL	Target	Actual	95% LCL	95% UCL	Beta
0.909	0.908	0.911	0.050	0.049	0.048	0.051	0.091

		----- Average Sample Size ----							
		-- Given H0 --		-- Given H1 --					
N1	N2	Grp1	Grp2	Grp1	Grp2	Diff0	Diff1	P1|H1	P2
180	156	179	155	144	125	0.00	0.18	0.56	0.38

Efficacy Monitoring. We propose to monitor efficacy at two interim time points and one final time period (i.e., three "looks") when primary outcome data (12 month follow-up) are available for 1/3, 2/3 and 3/3 of the total sample size of VENUS subjects. For the VENUS trial, these values are provide in the following table in terms of *information time*:

Table 6. Efficacy monitoring schedule for VENUS with cumulative sample size, significance boundaries and 95%CIs, and alpha- and beta-spending.

Accumulated primary outcomes for VENUS

	Accumulated Information	Accumulated Sample Size		
Look	Percent	VOM	PVAI	Total
1	33.33	60	52	112
2	66.67	120	104	224
3	100.00	180	156	336

Significance Boundaries with 95% Simulation Confidence Intervals

	Z-Value Boundary			P-Value Boundary		
Look	Value	95% LCL	95% UCL	Value	95% LCL	95% UCL
1	+/- 3.953	3.809	4.289	0.000	0.000	0.000
2	+/- 2.543	2.516	2.578	0.011	0.010	0.012
3	+/- 2.011	1.994	2.036	0.044	0.042	0.046

Alpha-Spending

	Signif. Boundary		Target		Actual		Proportion H1 Sims Outside Signif. Boundary	Cum. H1 Sims Outside Signif. Boundary
Look	Z-Value Scale	P-Value Scale	Spending Function Alpha	Cum. Spending Function Alpha	Alpha Spent	Cum. Alpha Spent		
1	+/- 3.953	0.000	0.000	0.000	0.000	0.000	0.033	0.033
2	+/- 2.543	0.011	0.012	0.012	0.011	0.012	0.547	0.579
3	+/- 2.011	0.044	0.038	0.050	0.038	0.049	0.329	0.909

The hypothesis test applied at the *k*th look is a two-tailed test of equality of two independent proportions, functionally composed as

$$Z_k = \frac{\hat{p}_{1k} - \hat{p}_{2k}}{\sqrt{\frac{\hat{p}_{1k}(1-\hat{p}_{1k})}{n_{1k}} + \frac{\hat{p}_{2k}(1-\hat{p}_{2k})}{n_{2k}}}}$$

where $\hat{p}_{1k}$ is the proportion of successful primary outcomes in the PVAI-VOM arm of VENUS at the *k*th look, and $\hat{p}_{2k}$ is the proportion of successful primary outcomes within the PVAI arm of VENUS at the *k*th look. Z_k follows a standard normal distribution, N(0,1). If during the first look when at least N=60 VOM and N=52 PVAI primary outcomes have been observed (N=112 total), if Z_1 exceeds 3.953, then the trial will be evaluated for early termination due to beneficial efficacy, whereas if the power is 30% or less, the trial will be evaluated for early termination for futility. However, if the power of the test falls in the "promising zone" (30-70%), we will continue the trial. The same rule applies for the 2nd look when at least N=120 VOM and 104 PVAI primary outcomes (224 total) have been observed in both arms, for which the tabled critical value of Z_2 is ±2.543. The overall efficacy of the trial will be determined when at least N=180 VOM and N=156 (336 total) primary outcomes have been observed, for which the critical value of Z_3 is ±2.011.

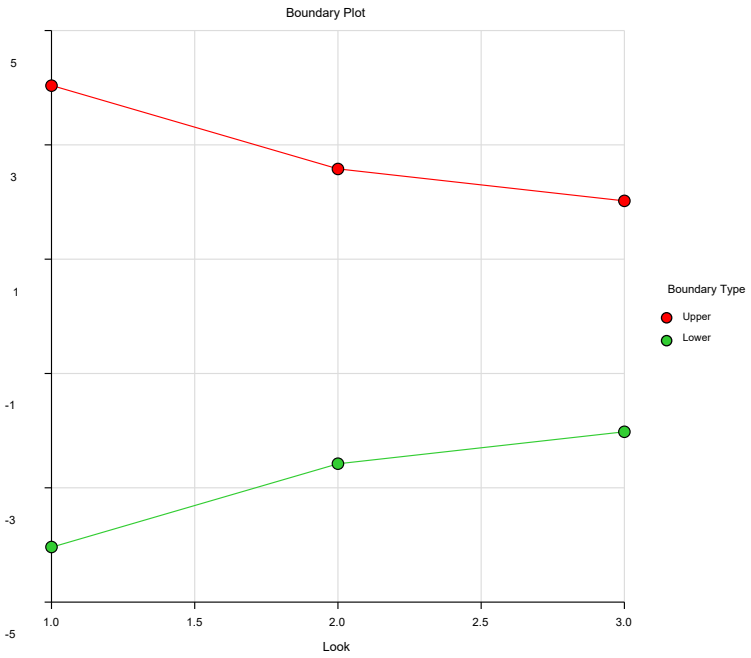

Figure S1. Efficacy boundaries at 33%, 66%, and 100% accrual of VENUS primary outcomes.

VENUS Interim Analysis - Conditional Power and Futility For Various Test Results. During the interim analysis, estimations of conditional power and futility will be performed, to provide information for clinical trial continuation decisions. The sample size will not be subject to any changes.

Conditional power runs were made using PASS 12 (Kaysville, UT). During the first look at 33% information time, there will be 60 VOM and 52 PVAI primary outcomes available. Using a one-sided (α=0.025) test of two proportions, $\theta=p_2-p_1$, where p_2 is the PVAI success rate and p_1 is the VOM success rate, the expectation is that the test statistic Z_k is less than zero, since H_a: $p_2<p_1$. The table below list the conditional power and futility at the first look for a range of Z_k values:

Table 7. VENUS Conditional power and futility at the first look (33% information, 60 VOM, 52 PVAI) for a range of Z_k values from a one-sided test of two independent proportions.

Cond. Power	Pred. Power	Total Sample Size VOM/PVAI	Current Sample Size n1k|n2k	Prop. Group 1 P1	Prop. Group 2 P2	Test Statistic Z_k	Alpha	Futility
0.99994	1	180|156	60|52	0.56	0.38	-5	0.025	0.00006
0.99974	0.99998	180|156	60|52	0.56	0.38	-4.5	0.025	0.00026
0.9991	0.99978	180|156	60|52	0.56	0.38	-4	0.025	0.0009
0.99717	0.99814	180|156	60|52	0.56	0.38	-3.5	0.025	0.00283
0.99209	0.98894	180|156	60|52	0.56	0.38	-3	0.025	0.00791
0.98027	0.95313	180|156	60|52	0.56	0.38	-2.5	0.025	0.01973
0.95597	0.85624	180|156	60|52	0.56	0.38	-2	0.025	0.04403
0.91184	0.67408	180|156	60|52	0.56	0.38	-1.5	0.025	0.08816
0.84101	0.43598	180|156	60|52	0.56	0.38	-1	0.025	0.15899
0.74056	0.2196	180|156	60|52	0.56	0.38	-0.5	0.025	0.25944
0.61467	0.08289	180|156	60|52	0.56	0.38	0	0.025	0.38533

Table 8. VENUS Conditional power and futility at the second look (66% information, 120 VOM, 104 PVAI) for a range of Z_k values from a one-sided test of two independent proportions.

Cond. Power	Pred. Power	Total Sample Size VOM/PVAI	Current Sample Size n1k|n2k	Prop. Group 1 P1	Prop. Group 2 P2	Test Statistic Z_k	Alpha	Futility
1.00000	1.00000	180|156	120|104	0.56	0.38	-5.0	0.025	0
1.00000	1.00000	180|156	120|104	0.56	0.38	-4.5	0.025	0
0.99998	0.99998	180|156	120|104	0.56	0.38	-4.0	0.025	0.00002
0.99973	0.99950	180|156	120|104	0.56	0.38	-3.5	0.025	0.00027
0.99703	0.99233	180|156	120|104	0.56	0.38	-3.0	0.025	0.00297
0.97954	0.94042	180|156	120|104	0.56	0.38	-2.5	0.025	0.02046
0.90942	0.75562	180|156	120|104	0.56	0.38	-2.0	0.025	0.09058
0.73567	0.43104	180|156	120|104	0.56	0.38	-1.5	0.025	0.26433
0.46930	0.14923	180|156	120|104	0.56	0.38	-1.0	0.025	0.5307
0.21648	0.02834	180|156	120|104	0.56	0.38	-0.5	0.025	0.78352
0.06795	0.00279	180|156	120|104	0.56	0.38	0.0	0.025	0.93205

C) MARS Group Sequential Clinical Trial Design

MARS Preliminary Data. For patients that had a history of previous ablation (original MARS trial sample size calculations) the pilot data showed that among 169 patients we observed a response rate of p_1=42% for repeat PVAI and p_2=76% for 32 patients undergoing VOM-PV.

Power and sample size determination. Group sequential two proportions power analysis using simulation was performed using PASS V12 (Kaysville, UT). The following assumptions were made:

- Response rate in PV-VOM: p_1=0.76

- Response rate in PVAI: p_2= 0.42
- Hypotheses: H0: $p_1 = p_2$; H1: $p_1 \neq p_2$
- Test Statistic: Z-Test (Unpooled)
- Zero Adjustment Method: None
- Alpha-Spending Function: O'Brien-Fleming Analog
- Beta-Spending Function: None
- Futility Boundary Type: None
- Number of Looks: 3
- Simulations: 100000

Table 9. Sample size requirements for a group sequential trial based on 100,000 iterations.

Power			Alpha				
Value	95% LCL	95% UCL	Target	Actual	95% LCL	95% UCL	Beta
0.810	0.807	0.812	0.050	0.049	0.047	0.050	0.190

		Average Sample Size							
		Given H0		Given H1					
N1	N2	Grp1	Grp2	Grp1	Grp2	Diff0	Diff1	P1|H1	P2
33	33	33	33	28	28	0.00	0.34	0.76	0.42

Results. Group sequential trials with sample sizes of 33 and 33 at the final look achieve 81% power to detect a difference of 0.34 between a treatment group proportion of 0.76 and a control group proportion of 0.42 at the 0.05 significance level (alpha) using a two-sided Z-Test (Unpooled).

Efficacy Monitoring. We propose to monitor efficacy at two interim time points and one final time period (i.e., three "looks") when primary outcome data (12-15 month follow-up) are available for 1/3, 2/3 and 3/3 of the total sample size of MARS subjects. For the MARS trial, these values are provide in the following table in terms of *information time*:

Table 10. Efficacy monitoring schedule for MARS with cumulative sample size, significance boundaries and 95%CIs, and alpha- and beta-spending.

Accumulated primary outcomes for MARS

Look	Accumulated Information Percent	Accumulated Sample Size		
		Group 1	Group 2	Total
1	33.33	11	11	22
2	66.67	22	22	44
3	100.00	33	33	66

Significance Boundaries with 95% Simulation Confidence Intervals for Scenario 1

	Z-Value Boundary			P-Value Boundary		
Look	Value	95% LCL	95% UCL	Value	95% LCL	95% UCL
1	+/- 5.014	5.014	6.675	0.000	0.000	0.000
2	+/- 2.708	2.640	2.708	0.007	0.007	0.008
3	+/- 2.055	2.055	2.080	0.040	0.037	0.040

Alpha-Spending

Look	--- Signif. Z-Value Scale	Boundary--- P-Value Scale	--------- Target --------- Spending Function Alpha	--------- Actual ---------- Cum. Spending Function Alpha	Proportion Alpha Spent	Cum. Cum. Alpha Spent	H1 Sims Outside Signif. Boundary	H1 Sims Outside Signif. Boundary
1	+/- 5.014	0.000	0.000	0.000	0.000	0.000	0.017	0.017
2	+/- 2.708	0.007	0.012	0.012	0.010	0.011	0.404	0.422
3	+/- 2.055	0.040	0.038	0.050	0.038	0.049	0.388	0.810

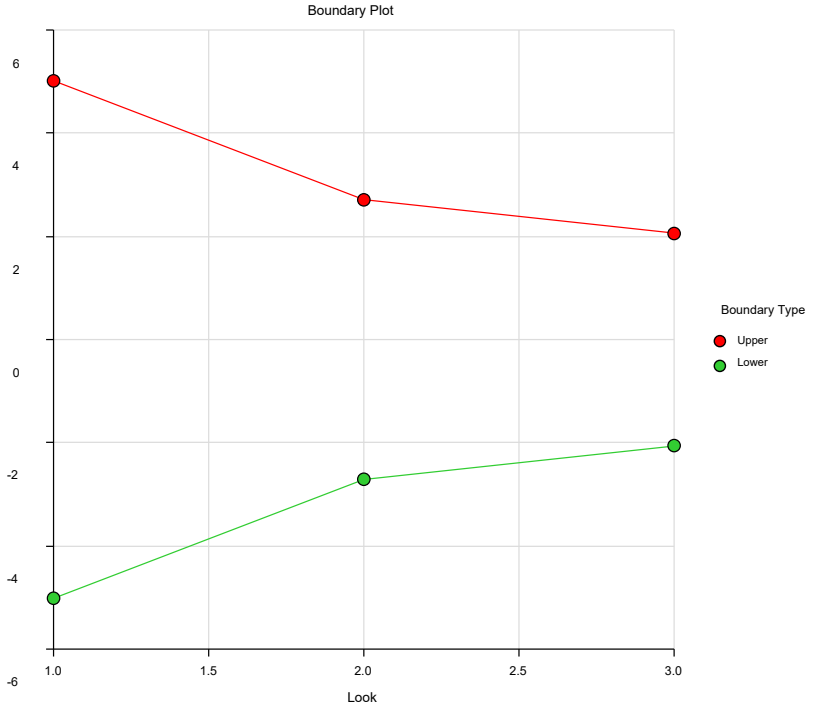

Figure S2. Efficacy boundaries at 33%, 66%, and 100% accrual of MARS primary outcomes.

During the interim analysis, estimations of conditional power and futility will be performed, to provide information for clinical trial continuation decisions. The sample size will not be subject to any changes.

Table 11. MARS Conditional power and futility at the first look (33% information, 11 VOM, 11 PVAI) for a range of Z_k values from a one-sided test of two independent proportions.

Cond. Power	Pred. Power	Total Sample Size VOM/PVAI	Current Sample Size n1k|n2k	Prop. Group 1 P1	Prop. Group 2 P2	Test Statistic Z_k	Alpha	Futility
0.9997	1	33|33	11|11	0.76	0.42	-5	0.025	0.0003
0.99894	0.99998	33|33	11|11	0.76	0.42	-4.5	0.025	0.00106
0.99674	0.99978	33|33	11|11	0.76	0.42	-4	0.025	0.00326
0.99104	0.99814	33|33	11|11	0.76	0.42	-3.5	0.025	0.00896
0.97798	0.98894	33|33	11|11	0.76	0.42	-3	0.025	0.02202
0.95155	0.95313	33|33	11|11	0.76	0.42	-2.5	0.025	0.04845
0.90431	0.85624	33|33	11|11	0.76	0.42	-2	0.025	0.09569
0.82969	0.67408	33|33	11|11	0.76	0.42	-1.5	0.025	0.17031
0.72555	0.43598	33|33	11|11	0.76	0.42	-1	0.025	0.27445
0.5971	0.2196	33|33	11|11	0.76	0.42	-0.5	0.025	0.4029
0.45712	0.08289	33|33	11|11	0.76	0.42	0	0.025	0.54288

Table 12. MARS Conditional power and futility at the second look (66% information, 22 VOM, 22 PVAI) for a range of Z_k values from a one-sided test of two independent proportions.

Cond. Power	Pred. Power	Total Sample Size VOM/PVAI	Current Sample Size n1k|n2k	Prop. Group 1 P1	Prop. Group 2 P2	Test Statistic Z_k	Alpha	Futility
1.00000	1.00000	33|33	22|22	0.76	0.42	-5	0.025	0
1.00000	1.00000	33|33	22|22	0.76	0.42	-4.5	0.025	0
0.99995	0.99998	33|33	22|22	0.76	0.42	-4	0.025	0.00005
0.99925	0.9995	33|33	22|22	0.76	0.42	-3.5	0.025	0.00075
0.99323	0.99233	33|33	22|22	0.76	0.42	-3	0.025	0.00677
0.96097	0.94042	33|33	22|22	0.76	0.42	-2.5	0.025	0.03903
0.85426	0.75562	33|33	22|22	0.76	0.42	-2	0.025	0.14574
0.636	0.43104	33|33	22|22	0.76	0.42	-1.5	0.025	0.364
0.35968	0.14923	33|33	22|22	0.76	0.42	-1	0.025	0.64032
0.14311	0.02834	33|33	22|22	0.76	0.42	-0.5	0.025	0.85689
0.03807	0.00279	33|33	22|22	0.76	0.42	0	0.025	0.96193

C) Statistical Analyses.

Pre-specified subgroup analyses: The following subgroups are defined to assess potential impact on outcomes:

- Male vs female.

- Longstanding persistent AF (duration of more than 1 year) vs persistent AF of less than one year
- Left atrial volume strata: defined as mild or no left atrial enlargement (LA volume - up to 75 ml/m^2), moderate enlargement (76-89 ml/m^2), or severe enlargement -90+ ml/m^2)
- Enrollment as AF or AT –for MARS-AF trial only.
- Pre-existing low voltage scar and extent of low-voltage scar after ablation procedure (divided in tertiles).

Primary Outcome. Hypothesis tests for the equality of two proportions (unpooled standard errors) will be employed for determining whether or not the VOM success rate is significantly greater than the success rate for PVAI. The test statistic is a z-score which is standard normal distributed and the relevant lookup critical values (percentage points) are listed in the interim analysis section for group sequential designs. From a post-hoc perspective, we may use the stratified Mantel-Haenszel odds ratio test of proportions if we learn that success rates track with a particular covariate, such as LA volume or AF duration, and the strata weights are not highly imbalanced.

Secondary Outcomes. The secondary outcomes are listed below along with their corresponding storage location (various tables or Excel .csv files on output after report generation). Model building strategies (MBS) will be employed using univariate and multivariable regression models for post-hoc analyses of secondary outcomes. During MBS, univariate predictors whose $p<0.25$ will be selected as multiple variable model candidates. MBS regression methods may include linear, logistic, Poisson, and Cox proportional hazards (PH) along with regression diagnostics using the relevant goodness-of-fit criteria, residuals, variance inflation factors (VIF), ROC-AUC, and assumption-checking techniques (e.g. normally-distributed standardized residuals for linear regression). Regression diagnostics for linear regression will include estimation and filtering of overly influential records based on residuals, standardized, residuals, deletion residuals, Cook's distance, leverage, DFFITS, DFBETAS, and VIFs. Regression diagnostics for logistic and Poisson regression will include filtering on Pearson, deviance, and leverage residuals and the Hosmer-Lemeshow test for logistic regression GOF. Cox PH regression diagnostics will include Schoenfeld and Nelson-Aelen residuals, and possible employment of stratified models when the PH assumption fails.

The table below lists the primary outcomes which are to be analyzed during each interim analysis, as well as the secondary outcomes which will be analyzed and reported prior to all DSMB review meetings.

Primary Endpoints	EDC Pages	Data Fields
1. Freedom from symptomatic AF or flutter AND reduction of AF/flutter to less than 30 seconds in a continuous 4 week monitor at 6 and 12 months	• 12 month continuous ECG page- MARS • 12 month continuous ECG page- Venus	• 6-12 month AF/AT Burden less than 30 seconds on continuous ECG monitoring
2. Safety: Acute procedural complications	• AE pages Day 7 Telephone FU	• Acute AE's related to Day 0 procedure • Day 7 reported complications
Secondary Endpoints:		
1. Single vs. 2-procedure success.	• Status change page	• Single: reached primary endpoint #1 after first procedure with no repeats • Two procedure: reached primary endpoint #1 after second procedure with no 3rd procedure
2. AF burden (% time) on continuous monitoring at 12 months.	• 6-12 month continuous ECG page- MARS • 12 month continuous ECG page- Venus	• 6- 12 month AF/AT Burden (%) on continuous ECG monitoring
3. Procedural parameters: total procedure, fluoroscopy, total RF ablation time (first procedure), and total extent of ablated LA tissue.	• PVAI Page • VOM page	• Total procedure time, PVAI only • Total procedure time, VOM procedure • Total fluoro time • Scar measurements pre and post PVAI and VOM
4. Clinical success: freedom from symptomatic AF/flutter but AF/flutter > 1 min/day < than 1% at 12 months.	• 12 month continuous ECG page- MARS • 12 month continuous ECG page- Venus	• 12 month AF/AT Burden less than 25% continuous event monitor at 6 and 12 months from ablation procedure
5 Sub-acute procedural complications (within 30 days).	• Symptoms page • AE page	Day 30 reported, procedure related complications via symptoms and/or AEs
6 Recurrence as persistent or paroxysmal AF, or flutter after 1 or 2 procedures.	• 12 lead Ecg page • Evaluation for repeat procedure page • AE page	• Type of recurrence (rhythm) • Characterization of recurrence e.g. persistent or paroxysmal for a fib; typical or atypical for a flutter.
7. LA function on Doppler echocardiography (LA strain114ab) at 12 months.	Central echocardiogram page	LA Strain
8. Incidence and mechanisms of atrial flutters.	• 12 lead ECG page • AE page • Evaluation for repeat procedure page	• Date of occurrence • Type of flutter (typical vs atypical)
9. Cardiovascular hospitalizations and QOL.	• Hospitalizations • SAEs • QOL	• Total # of CV related hospitalizations • QOL score

Use of propensity scores in multivariate models. An ideal goal for observational etiological

studies is to allocate randomly subjects into different treatment groups in order to guarantee on average that there are no systematic differences in covariates between groups.[120] After randomization, there is nevertheless a possibility for observing large differences in confounders which may lead to bias in results. The propensity score provides a scalar summary of covariate information and is defined as the propensity (probability) that a subject's covariate profile represents subjects truly assigned to a given treatment group. Propensity scores based on significantly different confounder variables can be used to create a quasi-randomized experiment with adjustment to the treatment effect. We will assess the role of propensity scores in prediction models in order to reduce the effects of baseline factors that may be significantly different among subjects in different treatment groups. Firstly, we will identify baseline covariates which are significantly different across treatment groups (using t-tests with skew-zero transformed covariates or Mann-Whitney tests). Significant covariates will be incorporated into a logistic regression model (y=0 PVAI, 1-VOM-PV) to generate subject-specific logits, which are normally-distributed.[121, 122] Treatment-subject-specific logits will then be used for matching subjects across the treatment groups in order to construct a sample of subjects with balanced covariates. We suspect that propensity matching will not be required to tackle the problem of extreme confounder differences, but will nevertheless evaluate the effect of propensity matching prior to logistic regression to determine treatment effect possibly adjusted for age.

Missing data. The critical piece of data required for endpoint analysis is the electrocardiographic event monitor. Failure to comply with wearing the monitor will lead to missing data. We request patients to wear monitors for 1–month. However, only a minimum of 1-week of monitored time is required for Endpoint assessment. Patients with less than 1 –week of monitoring will be considered as missing data. Patients who die before then study end will be considered not to have a response to treatment. For patients with missing primary outcomes, we will perform multiple imputation (MI) based on Monte Carlo Markov chain (MCMC) methods (Refs 1-3). MI will only be used for dealing with missing data as a secondary analysis tool of the primary endpoint. In Stata, MI is available for many procedures, especially the regression modules (linear, logistic, Poisson, Cox PH). MI can be performed to iteratively impute central estimates of missing outcome measures based on subjects' covariate patterns. The most straightforward example can be envisioned in this study, where logistic regression with MI is employed to train a model based on primary outcome (0-failure,1-success) as the dependent variable and age, gender, baseline AF duration, and baseline LA volume as independent predictors to impute $P(y=1|\mathbf{x})$ for subjects with missing primary outcome.

Sensitivity analysis. Following methods introduced in Proschan et al. (Ref 4), we simulated success rates for patients with missing 12-month outcomes in VOM and PVAI arms for VENUS and MARS at 33%, 66%, and 100% information time (looks 1-3). B=100,000 iterations were used with proportions of P_m=0, 0.05, 0.10, 0.15, and 0.2 representing the amount of missing data in both VOM and PVAI arms. At look k, let the success rate in the VOM arm be p_{1k} and the success rate in the PVAI arm be p_{2k}, n_{1k} and n_{2k} the number of patients accrued in the VOM and PVAI arms, $n_{1k}^m = n_{1k}P_m$ and $n_{2k}^m = n_{2k}P_m$ the number of patients in VOM and PVAI arms with missing outcome data, and $n_{1k}^0 = n_{1k} - n_{1k}^m$ and $n_{2k}^0 = n_{2k} - n_{2k}^m$ the number of patients in VOM and PVAI arms without missing outcomes. Next, for VOM patients with missing

outcomes, simulate the number of successes by taking random draws of a binomial variate with parameters (n_{1k}^{m}, p_2) , and the number of successes among PVAI patients with missing outcomes as $B(n_{2k}^{m}, p_1)$. Note that the random draws of binomial variates are based on the success rate in the opposing arm, which enforces a high level of conservatism. A test statistic (unpooled variance) at the *b*th iteration is

$$Z_k^{(b)} = \frac{\hat{p}_{1k} - \hat{p}_{2k}}{\sqrt{\frac{\hat{p}_{1k}(1-\hat{p}_{1k})}{n_{1k}} + \frac{\hat{p}_{2k}(1-\hat{p}_{2k})}{n_{2k}}}},$$

where $\hat{p}_{1k} = [n_{1k}^{0} p_{1k} + B(n_{1k}^{m}, p_2)]/n_{1k}$ is the unobserved success rate among VOM patients with and without missing data, and $\hat{p}_{2k} = [n_{2k}^{0} p_{2k} + B(n_{2k}^{m}, p_1)]/n_{2k}$. The power of the test is equal to the proportion of rejections among the *B* iterations, given in the form

$$Power = \frac{\#\{b: Z_k^{(b)} > 1.96\}}{B}.$$

The tables below present power as a function of VOM and PVAI success rates, and the proportion of patients with missing data for the VENUS and MARS trials.

VENUS 33% (n1=60,n2=52)		PVAI Success				
VOM Success	Missing	0.28	0.33	0.38	0.43	0.48
0.46	0	1.000	0.000	0.000	0.000	0.000
	0.05	0.778	0.055	0.000	0.000	0.000
	0.1	0.462	0.054	0.002	0.000	0.000
	0.15	0.247	0.051	0.003	0.000	0.000
	0.2	0.197	0.052	0.007	0.001	0.000
0.51	0	1.000	1.000	0.000	0.000	0.000
	0.05	1.000	0.584	0.059	0.000	0.000
	0.1	0.855	0.461	0.045	0.001	0.000
	0.15	0.601	0.213	0.043	0.002	0.000
	0.2	0.445	0.159	0.042	0.008	0.001
0.56	0	1.000	1.000	1.000	0.000	0.000
	0.05	1.000	1.000	0.476	0.060	0.000
	0.1	1.000	0.854	0.459	0.049	0.001
	0.15	0.907	0.514	0.218	0.040	0.003
	0.2	0.709	0.397	0.154	0.045	0.011
0.61	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	1.000	0.477	0.058
	0.1	1.000	1.000	0.860	0.452	0.053
	0.15	0.990	0.879	0.510	0.227	0.034
	0.2	0.901	0.696	0.383	0.146	0.049
0.66	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	0.478
	0.1	1.000	1.000	1.000	0.868	0.434

	0.15	1.000	0.991	0.866	0.513	0.233
	0.2	0.982	0.905	0.682	0.367	0.153

VENUS 66% (n1=120,n2=104)

VOM Success	Missing	PVAI Success 0.28	0.33	0.38	0.43	0.48
0.46	0	1.000	1.000	0.000	0.000	0.000
	0.05	1.000	0.766	0.008	0.000	0.000
	0.1	0.976	0.473	0.024	0.000	0.000
	0.15	0.777	0.243	0.018	0.000	0.000
	0.2	0.532	0.159	0.020	0.001	0.000
0.51	0	1.000	1.000	1.000	0.000	0.000
	0.05	1.000	1.000	0.770	0.007	0.000
	0.1	1.000	0.976	0.476	0.025	0.000
	0.15	0.981	0.745	0.227	0.019	0.000
	0.2	0.860	0.487	0.148	0.020	0.001
0.56	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	1.000	0.770	0.003
	0.1	1.000	1.000	0.971	0.429	0.022
	0.15	1.000	0.978	0.700	0.214	0.018
	0.2	0.983	0.834	0.468	0.131	0.022
0.61	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	0.768
	0.1	1.000	1.000	1.000	0.969	0.466
	0.15	1.000	1.000	0.978	0.698	0.198
	0.2	1.000	0.981	0.812	0.483	0.129
0.66	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	1.000	0.979
	0.15	1.000	1.000	1.000	0.981	0.710
	0.2	1.000	0.999	0.980	0.821	0.479

VENUS 100% (n1=180,n2=156)

VOM Success	Missing	PVAI Success 0.28	0.33	0.38	0.43	0.48
0.46	0	1.000	1.000	0.000	0.000	0.000
	0.05	1.000	0.994	0.086	0.000	0.000
	0.1	1.000	0.811	0.072	0.000	0.000
	0.15	0.979	0.594	0.069	0.001	0.000
	0.2	0.827	0.363	0.048	0.002	0.000
0.51	0	1.000	1.000	1.000	0.000	0.000
	0.05	1.000	1.000	0.993	0.083	0.000
	0.1	1.000	1.000	0.781	0.059	0.000
	0.15	1.000	0.973	0.572	0.061	0.001
	0.2	0.987	0.808	0.335	0.047	0.002

0.56	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	1.000	0.992	0.081
	0.1	1.000	1.000	0.999	0.781	0.057
	0.15	1.000	1.000	0.966	0.540	0.063
	0.2	1.000	0.984	0.787	0.321	0.048
0.61	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	0.991
	0.1	1.000	1.000	1.000	0.999	0.795
	0.15	1.000	1.000	1.000	0.966	0.552
	0.2	1.000	1.000	0.983	0.784	0.329
0.66	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	1.000	1.000
	0.15	1.000	1.000	1.000	1.000	0.970
	0.2	1.000	1.000	1.000	0.985	0.800

MARS 33% (n1=11,n2=11)		PVAI Success				
VOM Success	Missing	0.32	0.37	0.42	0.47	0.52
0.66	0	1.000	0.000	0.000	0.000	0.000
	0.05	0.109	0.127	0.000	0.000	0.000
	0.1	0.108	0.126	0.000	0.000	0.000
	0.15	0.107	0.132	0.020	0.026	0.000
	0.2	0.108	0.015	0.021	0.026	0.000
0.71	0	1.000	1.000	0.000	0.000	0.000
	0.05	0.518	0.108	0.124	0.000	0.000
	0.1	0.517	0.108	0.121	0.000	0.000
	0.15	0.357	0.108	0.129	0.018	0.023
	0.2	0.087	0.107	0.015	0.019	0.024
0.76	0	1.000	1.000	1.000	0.000	0.000
	0.05	0.483	0.521	0.420	0.113	0.124
	0.1	0.482	0.522	0.102	0.113	0.000
	0.15	0.314	0.356	0.102	0.121	0.015
	0.2	0.312	0.084	0.103	0.093	0.015
0.81	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	0.530	0.472	0.097
	0.1	1.000	0.489	0.529	0.089	0.098
	0.15	0.554	0.311	0.357	0.094	0.111
	0.2	0.270	0.313	0.194	0.095	0.092
0.86	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	0.545	0.588
	0.1	1.000	1.000	0.499	0.544	0.517
	0.15	0.659	0.613	0.309	0.358	0.277
	0.2	0.660	0.265	0.310	0.232	0.080

MARS 66% (n1=22,n2=22)

VOM Success	Missing	PVAI Success 0.32	0.37	0.42	0.47	0.52
0.66	0	1.000	1.000	1.000	0.000	0.000
	0.05	1.000	0.584	0.141	0.000	0.000
	0.1	0.799	0.445	0.154	0.026	0.000
	0.15	0.643	0.363	0.152	0.042	0.006
	0.2	0.252	0.112	0.050	0.052	0.012
0.71	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	0.588	0.134	0.000
	0.1	1.000	0.799	0.448	0.151	0.023
	0.15	0.887	0.642	0.362	0.147	0.038
	0.2	0.461	0.251	0.119	0.114	0.046
0.76	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	0.603	0.128
	0.1	1.000	1.000	0.805	0.447	0.141
	0.15	0.859	0.890	0.647	0.359	0.140
	0.2	0.702	0.459	0.248	0.120	0.116
0.81	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	0.815	0.449
	0.15	1.000	0.868	0.897	0.655	0.359
	0.2	0.908	0.710	0.464	0.336	0.235
0.86	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	1.000	0.830
	0.15	1.000	1.000	1.000	0.906	0.667
	0.2	1.000	0.914	0.718	0.465	0.380

MARS 100% (n1=33,n2=33)

VOM Success	Missing	PVAI Success 0.32	0.37	0.42	0.47	0.52
0.66	0	1.000	1.000	1.000	0.000	0.000
	0.05	1.000	1.000	0.703	0.181	0.000
	0.1	1.000	0.928	0.414	0.183	0.005
	0.15	0.742	0.501	0.319	0.056	0.019
	0.2	0.515	0.371	0.115	0.061	0.028
0.71	0	1.000	1.000	1.000	1.000	0.000
	0.05	1.000	1.000	1.000	0.859	0.177
	0.1	1.000	1.000	0.931	0.414	0.179
	0.15	0.973	0.863	0.499	0.316	0.118
	0.2	0.661	0.513	0.372	0.126	0.056
0.76	0	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	0.867

	0.1	1.000	1.000	1.000	0.934	0.414
	0.15	1.000	0.975	0.896	0.502	0.315
	0.2	0.934	0.660	0.517	0.372	0.231
0.81	**0**	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	1.000	0.941
	0.15	1.000	1.000	0.977	0.903	0.619
	0.2	0.984	0.939	0.734	0.516	0.369
0.86	**0**	1.000	1.000	1.000	1.000	1.000
	0.05	1.000	1.000	1.000	1.000	1.000
	0.1	1.000	1.000	1.000	1.000	1.000
	0.15	1.000	1.000	1.000	0.981	0.913
	0.2	1.000	0.986	0.944	0.852	0.619

D) Stopping rules. The trials will be stopped if one of the following occurs:

a. Futility/efficacy boundaries reached. As illustrated in Figures S1 and S2, if the upper or lower boundary is reached at the 1/3 or 2/3 data assessment for beneficial efficacy or futility respectively, the trial will be evaluated for early termination.

b. Safety. Events will be reported to FDA, NHLBI, and DSMB according to FDA/OHRP requirements and NHLBI adverse event and unanticipated problem reporting policy. Expedited reporting will occur within 7 days of initial receipt of information for fatal or life-threatening unexpected serious reactions and within 15 calendar days for non-fatal, non-life threatening unexpected events. The DSMB will otherwise evaluate overall safety events on a bi-annual basis. An excess of ***procedural*** adverse events attributable to study procedure will be evaluated for early termination. Procedural adverse events include those that occur within 24 hours of the procedure or those that may be delayed but procedure-related (atrio-esophageal fistula or delayed pericardial effusion). The following are expected to be rare. One event may occur by chance in either treatment groups. Two of the same events in either arm will trigger consideration for study termination after detailed case review:

- Mortality.
- Stroke-Transient ischemic attack or systemic embolus.
- Pericardial effusion requiring drainage

1. Raghunathan, T. E., J. M. Lepkowski, J. Van Hoewyk, and P. Solenberger. 2001. A multivariate technique for multiply imputing missing values using a sequence of regression models. Survey Methodology 27: 85–95.
2. Rubin, D. B. 1987. Multiple Imputation for Nonresponse in Surveys. New York: Wiley.
3. van Buuren, S. 2007. Multiple imputation of discrete and continuous data by fully conditional specification. Statistical Methods in Medical Research 16: 219–242.
4. Proschan, MA, McMahon, RP, Shih, JH, Hunsberger, SA, Geller, NL, Knatterud, G, Wittes, J. 2001. Sensitivity analysis using an imputation method for missing binary data in clinical trials. J. Statistical Planning and Inference. 96: 155-165.

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7.0 STUDY ORGANIZATION

7.1 SCHEDULE OF EVENTS

Visit ID	1	2	3	4	5	6	7	8	9
Procedure	screen/ baseline	procedure day 0	prior to discharge	7 days (± 3)	30 day FU (±10) 1mo	90 day FU (±30d) 3mo	180 day FU (±60d) 6mo	9 mo FU (±30d)	360 day FU (±60d) 12 mos.
Informed Consent (prior to study specific procedures	X								
Eligibility Checklist (PMH, medications, verification of Inclusion and exclusion criteria)	X								
General **Medical History** & **Cardiovascular History** with CHADS2-VASC Score)	X								
LAA thrombus exclusion Transesophageal Echocardiogram (within 48 hours) or anticoagulation/LAA exclusion)	X[(l)]								
Cardiac MRI or CT or Echocardiogram (showing structure & function within 1 year)	X[(m)]								
Randomization	X								
Targeted **Physical Exam**	X				X	X	X	X	X
12 lead **EKG**	X	X pre-procedure	X		X	X	X	X	X
Laboratory: CBC, Serum creatinine, and INR – (INR only if patient is taking warfarin[(g)])	X								
AF **Quality of Life** Questionnaire (AFEQT)	X						X	X	X
Pre-procedure 3D **Mapping**[(b)(j)]		X							
VOM Procedure w/ ETOH Injection and Post ethanol map		X[(i)]							
PVAI Procedure		X							
Post Procedure 3 D **Mapping**		X[(c,j)]							
Follow-Up Phone Call[(f)]				X					
3-4 Week EKG (**Event Monitor**)[(d,n)]							X		X
Echocardiogram , Central Reader (Dr. Nagueh)									X[(k)]
Repeat Procedure allowed [(e)]						X	X	X	
AAD Therapy	If appl.	If appl.	If appl.	If appl.	If appl.	Stop AAD[(a)]			
Anticoagulation	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X

Footnote Key

(a) Must be in sinus rhythm prior to stopping AAD, may stop if clinically indicated
(b) Required for both VENUS-AF and MARS-AF groups. For subjects in VENUS-AF, there should be little to no scarring for first procedure. For subjects in MARS-AF group and study patients requiring repeat procedures, maps will assess pre-existing extent of previous ablation lesions and presence or absence of PV reconnection.
(c) To delineate extent of scar in both PVAI alone and PVAI/VOM groups.
(d) Continuous 4 week EKG monitoring, will take place at month 6, and 12
(e) Repeat procedures will be allowed if certain criteria met
(f) Seven day follow-up phone call to assess post procedure symptoms.
(g) Baseline INR must be within 24 hours prior to procedure for patients taking warfarin;
(h) Not applicable.
(i) For patients randomized to receive VOM ethanol infusion, and who have a vein of Marshall that can be cannulated.
(j) For subjects who undergo repeat on-study procedures, mapping will be done a second time
(k) Echocardiogram for LA Strain will be performed at study end at 12 months, and read centrally by core lab.
(l) Imaging prior to enrollment is required to rule out the presence of LA appendage thrombus. Ruling out LAA thrombus can be performed by the following: TEE within 48 hours of the procedure; at least one month of oral anticoagulation prior to the procedure; or documented prior procedures of LAA occlusion or ligation.
(m) Imaging prior to enrollment is required to rule out structural heart disease. For ruling out structural heart disease, either a cardiac MRI or CT or transthoracic echocardiogram within 1 year prior to participation in the study is sufficient.
(n) Options for continuous EKG monitoring include: event monitoring include: external event monitor, implanted miniaturized rhythm monitor, or implanted pacemaker.

7.2 Patient Recruitment, Procedures And Follow-Up

Patients will be recruited from Cardiac Electrophysiology consultation services at all sites. Qualified, trained investigators will perform the procedures at the centers. These investigators will be unblinded. Patients will be followed up by qualified and trained at each study center.

The clinicians will follow the patients and evaluate adverse events and clinical primary endpoints.

7.3 Clinical Research Nurses/Coordinators

Each site will have designated research nurses and/or study support staff. Coordinating center may provide research nurse and/or study support staff as needed to conduct the trial efficiently. All study staff will keep in close communication with the Project Manager at the coordinating center, in order to ensure the study process runs smoothly. The coordinating center will train all study coordinators in the same manner.

7.4 Data Coordinating Center (DCC)

A DCC has been set up at the Dan L. Duncan Institute for Clinical and Translational Research at Baylor College of Medicine. Coordinators at each site will use a web-based data entry and collection system, which is capable of image collection (including maps) and FISMA-compliant. Methodist coordinating center will oversee data collection, integrity and quality. A statistician with extensive experience handling large data sets has been recruited to independently lead data analysis. He will meet periodically with DCC and lead blinded data analysis of the proposed endpoints and SAEs. Data will be reported to the DSMB with pre-specified criteria for stopping the trial if safety and futility boundaries are reached. See below in “Protection of Human Subjects”.

The DCC will design, develop and maintain the secure, web-based electronic database systems for this trial. The electronic data management system (EDC) is a secure, web-based system which will require the participants to have an internet-accessible computer/tablet with an Internet browser. This electronic data management system will have logical checks and audit logs built into the system to ensure data correctness and data integrity. All automated alerts and notifications requested by the project team will be implemented in this electronic data management system. This system will also have reports and queries that are requested by the project team and the DSMB to monitor and manage the study. Also provided will be backend access to statistical software with data connectivity to facilitate data analyses. At various time points in the study, as requested by the study team, snapshots and locking of the database will occur, and clean data sets will be provided to the study team for review and data analyses.

7.5 STUDY DRUG

Investigational Product: The VOM injections will be performed using Dehydrated Alcohol Injection, USP, (multiple manufacturers). The product is commercially available and is indicated for therapeutic neurolysis in a number of medical situations, mainly for chronic pain.

Supply: The alcohol will be obtained commercially by the each site, and stored in a locked, limited access area under the appropriate temperature conditions. The number of ml used in each procedure will be documented in the surgical record by the surgeon. Site specific handling and accountability procedures, if different than above, shall be approved by the sponsor and outlined in the Clinical Trial Management Plan.

Labeling and accountability: The supply obtained for this study will be clearly marked for Investigational use per the FDA requirements, regardless of its approval status. Records will be kept of the date, patient use, and lot # of study drug used from this supply. No unused study drug will be retained. After opening, used and unused product will be destroyed on site per institutional policy. No drug supplies shall be returned to the sponsor.

Storage and Maintenance: Study drug will be stored in a cool place away from a heat source, as indicated on package insert.

Administration and Dosing: Study drug is administered intravenously. Dosing is dependent upon the surgeon achievement of sufficient neurolysis for successful ablation (up to 4 injections of 1cc ethanol.

7.6 BLINDING

Patients and personnel involved in data analysis, will be blinded to the treatment provided. Upon enrollment, the operators will be informed of the randomization outcome. After the procedure is performed, data will be collected and analyzed with treatment groups as the only identifier. The DSMB will receive the data identification for their assessment.

The primary endpoint of freedom from AF as determined by electrocardiographic monitoring by either external monitors or implanted devices will be adjudicated *in an independent and blinded manner* by the external EKG monitoring laboratory, respectively.

7.7 CORE LABORATORIES: ECHOCARDIOGRAPHY, AND EKG MONITORING

Electrocardiographic monitoring will be performed by continuous 3-4 week monitors as described. We have secured a commitment from a qualified vendor to provide with storage of *continuous data* (i.e. e. all the heart beats) for the 3-4 week monitoring time that will allow precise determination of the AF burden (percentage of time in AF). *Data will be reviewed by technicians unaware of the treatment mode, thus AF occurrence and AF burden quantification will be blinded*. Additionally, analysis such as heart rate variability may be performed: If VOM ethanol causes effective vagal denervation, and vagal innervation modulates dynamics of heart rate variability[123] then we expect differences between the two treatment groups. The core echocardiographic laboratory is a national leader in echocardiography with particular expertise in LA function.[114b] LA volumes, ejection fraction and strain will be collected as described and reviewed and analyzed in the echocardiography core laboratory at Houston Methodist Hospital.[114ab]

Alternate rhythm monitoring

Certain patients may be eligible for implantation of a miniaturized subcutaneous recording device (Medtronic LinQ device) or other equivalent implanted permanent monitoring devices. These implanted devices provide continuous electrocardiographic monitoring for up to 3 years including data on atrial fibrillation or flutter burden, episode duration per day and other quantified data. For patients that choose to have this kind of monitoring, AF data will be quantified for the primary and secondary endpoints using this device as opposed to 3-4 week electrocardiographic monitoring.

Certain patients may already have an implanted pacemaker previously inserted prior to study enrollment. These devices yield interrogation reports that provide sufficient data on atrial fibrillation or flutter burden, episode duration per day and other quantified data. For patients that already undergo this kind of monitoring, AF data will be quantified for the primary and secondary endpoints using these devices as opposed to 3-4 week electrocardiographic monitoring.

7.8 SAFETY CONSIDERATIONS

Ethanol infusion for the treatment of hypertrophic cardiomyopathy has been used for more than a decade.[124] Complications derive from collateral damage (i.e. e. AV block) or spillage of ethanol in unintended arterial branches.[124] VOM infusion is retrograde, and spilled ethanol drains via the CS into the right atrium to be diluted to non-damaging concentrations. Ethanol passage into the systemic circulation via the LA, albeit seemingly dangerous, is necessary for its ablative effects in the atrial myocardium. In order to achieve rapid dilution and avoid systemic effects, a slow infusion rate is critical. Mixed blood ethanols have been undetectable. VOM venograms performed after VOM ethanol infusion can show varying degrees of myocardial staining, but macroscopic extravasation into the epicardial space has not occurred. Adverse events of the VOM procedure included one CS dissection, which had no clinical consequences. Two patients developed sub-acute pericardial effusion 4 and 6 weeks after the procedure, respectively. The role of VOM ethanol is unclear, since this complication is also well described in conventional ablation.[56] No systemic effects were

detected at the doses tested (total 4 ml). This is an FDA Investigational New Drug (IND # 105083) project, which will continue.

Added procedure and fluoroscopy times in our previous experience average 45 and 8 minutes, respectively. Reported fluoroscopy times of conventional ablation can be up to 100-120 minutes,[125, 126] so 8 minutes do not represent a major fluoroscopy time increase. Given that VOM ethanol may lead to ablation of otherwise targeted tissue (including LIPV isolation),[1] and facilitate perimitral block,[3] it may reduce the need of RF ablation in these areas. Thus VOM ethanol may potentially save procedure and fluoroscopy times downstream.

7.8.1 Adverse Event Reporting

The adverse event reporting period for this trial begins at the time the subjects sign the informed consent form, and will continue through the final month follow-up visit or withdrawal from the study. Reportable events will be reported per institution specific IRB policy.

Only AE's related to the catheter ablation procedure, ethanol ablation, and disease process will be captured.

Anticipated (Expected) Adverse Events (AE's)

Patients may experience certain clinical events that are attributable to the ablation procedure or the disease process of the patient. ***The following list of AE's are expected based on previous clinical and research experience.***

- Atrial Arrhythmias
- Chest pain or Angina
- Standard of care cardioversions for arrhythmias
- Headache
- Minor bleeding
- Hypertension or hypotension
- Vasovagal reactions
- self-limiting pericarditis attributable to the ablation procedure defined as pleuritic chest discomfort with or without pericardial rub
- pacemaker implantation for nodal dysfunction rhythms (sick sinus syndrome, sinus bradycardia, sinus arrest or AV blocks) that resulted in symptomatic bradycardia (unrelated to the ablation procedure or related to pre-existing disease state)
- Incision site pain/soreness
- Incision site infection
- Inadvertent AV block: Second or third degree heart block
- Palpitations
- Pulmonary edema
- ECG changes that did not require additional hospitalization
- Pericarditis
- Anxiety
- Hematoma

7.8.2 Serious Adverse Events (SAE) Reporting

An adverse event that meets one or more of the following criteria/outcomes will be classified as serious: These events will be treated accordingly and reported per local & federal regulations and institutional policies & requirements.

- Results in a life-threatening illness or injury.
- Results in permanent impairment of a body structure or a body function.
- Requires inpatient hospitalization ≥ 24 hours (other than the ablation procedure) or prolongation of existing hospitalization.
- Requires a medical or surgical intervention to prevent permanent impairment to body structure.
- Death

SAE's will be reported in accordance with current institutional policies.

NOTE: Unexpected serious adverse events deemed related, probably or possibly related to the VOM study procedure will warrant a hold on the study until further review and approval by the IRB and DSMB.

Screen Failure AEs will be documented as follows: Adverse events that occur for subjects prior to the intervention, will be documented in the study record and will not be reported to the IRB or sponsor (HMRI) unless unexpected or the PI determines the event should be reported to the IRB as non-study intervention related event. Subjects who are deemed screen failures and experience an event that meets the general SAE criteria will be followed until resolution of the event and those events will be reported to HMRI as the sponsor of the IND, and to the IRB per institutional policies for reporting SAE's.

7.8.3 Data Safety and Monitoring Plan

Data Safety Monitoring Board (DSMB)

A study-specific DSMB has been created by the NHLBI (National Heart, Lung, and Blood Institute) which is funding this clinical trial (R01 HL115003). None of the members of the DSMB are listed on the protocol as sub-investigators or have conflicting interests in the trial results. The DSMB is made up of electrophysiology consultants familiar with ablation procedures that will have insights into the specific clinical scenarios that can occur in AF ablation. Additionally, the DSMB will have a dedicated statistician. The NHLBI will administer the DSMB with the assistance of the project manager and data center.

Data Reports to DSMB

Specific data reports will be supplied to the DSMB Executive Secretary at the NHLBI for reporting to the DSMB for review on a semi-annual basis or as requested. The reports

will contain un-blinded data in order to properly ascertain adverse events attributable to the VOM procedure. The DSMB reports and voting results will subsequently be provided to the IRB and the FDA as part of the IND oversight process.

7.8.4 *Minimization of Other Risks*

Procedural Risks: There are known risks to the conventional pulmonary vein ablation procedure, and they remain present for every patient undergoing ablation of AF. Additional risks specific to the Vein of Marshall procedure are listed in the consent and expected outcomes are fully explained to each consented subject. Standard safety precautions will be taken to minimize risk. The Principal Investigator of the study is very familiar with the risks of catheter ablation procedures and is experienced in its resolution and treatment.

Risks to PHI: All data will be de-identified and only the research personnel will have access to subjects protected health information; all source documents will be kept onsite and stored with the principal investigator. The CRFs for this Study will be created by the PI as hard copy (paper) and as electronic CRFs. If electronic CRFs are used, the source document will be the electronic CRF, with appropriate password controls. The forms are designed to record observations and other data pertinent to the Study on each participant enrolled in the Study. The CRFs will be completed by the Investigator and/or designated staff. All data will be entered into a computer and stored in a secure database, accessible to approved personnel only. All hardcopies will be stored in a secure location and will be only accessible to approved personnel.

8.0 STUDY ADMINISTRATION & OVERSIGHT

8.1 PI Oversight

Principal Investigator, Dr. Miguel Valderrábano, will have general and scientific oversight of the project. Dr. Valderrábano will oversee the quality of clinical measurements obtained in the study and ensuring adherence to the protocol. Additionally, he will be responsible for patient recruitment, which includes site start-up activities and training for all site personnel.

8.2 Coordinating Center

Houston Methodist Research Institute d/b/a The Methodist Hospital Research Institute will serve as the coordinating center for this study, led by the project PI, Dr. Miguel Valderrábano. A trial administrator-manager at HMRI will oversee day-to-day operations of the clinical study as it relates to participant enrollment, clinical site administration, and data administration.

8.3 Scientific Advisory Board

Constituted by outside experts in clinical research, autonomic nervous system and evidence-based medicine research, or the use of ethanol ablation, this board will have the following functions:

1. Reviewing the operational conduct of the study, including adherence to the study protocol. The board will assist in facilitating resolution of problems that may arise concerning these issues.
2. Reviewing and rendering advice concerning potential changes to the protocol. Such changes would require approval by the DSMB.
3. Recommending publication policies, as well as overseeing the publications and presentations review process. This includes reviewing scientific reports, analysis, ancillary study proposals, and publications resulting from data that are obtained during the study; review and approval of any revisions to the publication guidelines for the study; and determination of data analyses, not currently included in the protocol, for the purpose of furthering scientific understanding in the field.
4. Reviewing recommendations from the DSMB and providing advice and guidance regarding potential study issues.

8.4 DATA SAFETY MONITORING BOARD (DSMB)

An NIH-based, study-specific DSMB will oversee safety issues for the study as described in section 7.7.3 Data Safety Monitoring Plan.

9.0 TRIAL MANAGEMENT

9.1 STATISTICAL MANAGEMENT

The primary functions of the individuals in the statistical core laboratory from Houston Methodist Hospital will be to contribute to data analysis and to create systems for randomization. Data from the Data Coordinating Center (see below) will be available for blinded statistical analysis for interim analysis, applicable DSMB or FDA reports, and prior to publications or presentations.

9.2 DATA MANAGEMENT

Data Coordinating Center

Data Coordinating Center (DCC) will be responsible for the integrity of data collection – blinded to the specific treatment provided. Statistical analysis will be provided by HMRI statistics team, who will have access to the DCC data. Clinical analysis will be handled by an expert EP researcher from Methodist.

Data flow From Remote Sites

The Investigator at each investigative site is responsible for the completion and timely web-based submission of case report forms (CRFs) for each patient according to visit

requirements as detailed in the Schedule of Events. All electronic data will be stored as a HIPAA-compliant limited data set in a password-protected database. Research nurses at each site will be responsible for entering the data in the system.

Data collection and record-keeping

An electronic Case Report Form (EDC) will be completed for each subject enrolled into the clinical study. The investigator will review, approve and sign/date each completed patient case report record; the investigator's signature serving as attestation of the investigator's responsibility for ensuring that all clinical and laboratory data entered on the EDC are complete, accurate and authentic.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

Study Records Access

The investigator will maintain all records in accordance with Good Clinical Practice guidelines. Regulatory documents are maintained in a locked file cabinet in the AOCT office, with limited access. Sponsor personnel viewing any site-related PHI will follow all rules of the institution and regulations regarding protection of PHI.

Case report forms will not contain any subject identifiers and will be labeled with only subject ID numbers. Study data is recorded on a secure, limited-access electronic database constructed by the DCC, and compliant with all electronic data regulations.

Any paper records, such as consent forms, that contain direct subject identifiers (e.g., name, social security number) will be stored in a separate locked filing cabinet in the study coordinator's office. Only the study coordinator and the Investigator will have access to this information.

Missing data processing plan

Critical data fields are those variables necessary for final study analysis. They will be agreed upon by the PI and the Clinical Data Manager, and detailed within the Data Management Plan. For those critical fields that are discrepant or not completed on the case report form (CRF), a query will be issued to the investigative site. Missing or overdue patient CRFs will also be queried.

9.3 STUDY MONITORING

The study sponsor will provide or contract a clinical study monitor to monitor the clinical trial. Monitoring visits will begin as soon as subjects are consented and enrolled and will

continue until all subjects have been taken off of the clinical trial and the trial has been terminated. Monitoring visits will include review of informed consent process, eligibility, adherence to the clinical protocol, and adverse events. Safety issues and/or trends in data errors or deviations will be managed by the administrative study team (principal investigator, project manager, IND sponsor representative, et. al). The monitoring process is outlined in the clinical monitoring plan which will be maintained by the coordinating center.

9.4 PROJECT MANAGEMENT

HMRI will have a project manager who will coordinate the sites with regard to regulatory set-up and maintenance, IRB, DSMB and other committee approvals and submissions, case report form completion, problem solving, and timeline enforcement as appropriate. Management of the trial and oversight is delineated in the Clinical Trial Management Plan.

Non Local Clinical Trial Sites

The organization of this trial is centralized at HMRI, which will act as a coordinating center for other clinical sites. Additional eligible, experienced AF treatment sites will be contracted to enroll patients and receive reimbursement on a per-patient basis. Sites will be trained on the protocol prior to initiation to minimize protocol deviations, avoid breaches of blinding procedures and other violations. Sites should be structured with 3 levels of personnel: operators, blinded clinicians that would follow the primary endpoints; and research nurses. This process and training is explained in the Clinical Trial Management Plan.

10.0 GUIDE TO ACRONYMS / DEFNITIONS

AAD Antiarrhythmic Drug

CS Coronary Sinus

DCC Data Coordinating Center. This is Dan L. Duncan Institute for Clinical and Translational Research (ICTR). Will be referred to as DCC

DSMB Data Safety Monitoring Board

ECG/EKG Electrocardiogram

EDC Electronic Data Capture (also, electronic case report form)

HMRI Houston Methodist Hospital doing business as Houston Methodist Research Institute or The Methodist Hospital Research Institute (IND Sponsor)

LA Left Atrium

MARS Vein of **M**arshall **A**lcohol in **R**epeat ablation of per**S**istent **A**trial **F**ibrillation.

NIH National Institutes of Health (sponsor of this study)

Persistent AF: continuous AF that is sustained beyond seven days. Episodes of AF in which a decision is made to electrically or pharmacologically cardiovert the patient after 48 hours of AF, but prior to 7 days, should also be classified as persistent AF episodes.

PMF Perimitral Flutter

PVAI Pulmonary Vein Antrum Isolation (traditional A. Fib. Procedure)

RF Radiofrequency

VENUS **VENUS-AF**. **V**ein of Marshall **E**thanol i**N**fusion in **U**ntreated per**S**istent **A**trial **F**ibrillation.

VOM Vein of Marshall

VOM-PV Vein of Marshall infusion plus conventional PVAI. Also VOM+PVAI

11.0 CLASSES OF ANTI-ARRHYTHMIC DRUGS (VAUGHN-WILLIAMS CLASSIFICATION)[127]

Class	Known as	Examples	Mechanism	Clinical uses in cardiology [2]
Ia	fast-channel blockers- affect QRS complex	• Quinidine • Procainamide • Disopyramide	(Na^+) channel block (intermediate association/dissociation)	• Ventricular arrhythmias • prevention of paroxysmal recurrent atrial fibrillation (triggered by vagal over activity) • procainamide in Wolff-Parkinson-White syndrome
Ib	Do not affect QRS complex	• Lidocaine • Phenytoin • Mexiletine • Tocainide	(Na^+) channel block (fast association/dissociation)	• treatment and prevention during and immediately after myocardial infarction, though this practice is now discouraged given the increased risk of systole • ventricular tachycardia
Ic		• Encainide • Flecainide • Propafenone • Moricizine	(Na^+) channel block (slow association/dissociation)	• prevents paroxysmal atrial fibrillation • treats recurrent tachyarrhythmias of abnormal conduction system. • contraindicated immediately post-myocardial infarction.
II	Beta-blockers	• Propranolol • Esmolol • Timolol • Metoprolol • Atenolol • Bisoprolol	beta blocking Propranolol also shows some class I action	• decrease myocardial infarction mortality • prevent recurrence of tachyarrhythmias
III		• Amiodarone • Sotalol • Ibutilide • Dofetilide • Dronedarone • E-4031	K^+ channel blocker Sotalol is also a beta blocker[3] Amiodarone has Class I, II, III & IV activity	• In Wolff-Parkinson-White syndrome • (sotalol:) ventricular tachycardias and atrial fibrillation • (Ibutilide:) atrial flutter and atrial fibrillation
IV	slow-channel blockers	• Verapamil • Diltiazem	Ca^{2+} channel blocker	• prevent recurrence of paroxysmal supraventricular tachycardia • reduce ventricular rate in patients with atrial fibrillation
V		• Adenosine • Digoxin • Magnesium Sulfate	Work by other or unknown mechanisms (Direct nodal inhibition).	Used in supraventricular arrhythmias, especially in Heart Failure with Atrial Fibrillation, contraindicated in ventricular arrhythmias. Or in the case of Magnesium Sulfate, used in Torsades de Pointes.

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