Vein of Marshall ethanol infusion for persistent atrial fibrillation: VENUS and MARS clinical trial design

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Background Although pulmonary vein isolation (PVI) is effective in the treatment of paroxysmal atrial fibrillation (AF), its success rates in persistent AF are suboptimal. Ablation strategies to improve outcomes including additional lesions beyond PVI have not consistently shown benefit. Recurrence as perimitral flutter (PMF) is a common form of ablation failure. The vein of Marshall (VOM) contains myocardial connections and abundant sympathetic and parasympathetic innervation implicated in the genesis and maintenance of AF, and is anatomically co-localized with the mitral isthmus, the ablation target of PMF. VOM ethanol infusion is effective in targeting these arrhythmia substrates.

Objective To test the safety and efficacy of VOM ethanol infusion when added to PVI in patients undergoing either de novo ablation of persistent AF or after a previous ablation failure.

Study design VENUS-AF and MARS-AF are prospective, multicenter, randomized, controlled trials. VENUS-AF will enroll patients undergoing their first catheter ablation of persistent AF. MARS-AF will enroll patients undergoing ablation after previous ablation failure(s). Patients (n = 405) will be randomized to PVI alone or in combination with VOM ethanol infusion. The primary endpoints include procedural safety and freedom from AF or atrial tachycardia (AT) of more than 30 seconds on 30-day continuous event monitors at 6 and 12 months after randomization procedure (single-procedure success), off antiarrhythmic drugs. Key secondary endpoints include AF burden, freedom from AF/AT after repeat procedures and quality of life.

Conclusions The VENUS-AF and MARS-AF will determine the safety and potential rhythm control benefit of VOM ethanol infusion when added to PVI in patients with persistent AF undergoing de novo or repeat ablation, respectively. (Am Heart J 2019;215:52-61.)

Catheter ablation has become a key component of the atrial fibrillation (AF) management. Isolation of the pulmonary veins (PVs)[1] and adjacent LA (PVI)[2,3] is the accepted procedural strategy, based on the mechanistic concept that atrial extrasystoles arising from the PVs initiate paroxysmal AF.[4] The link between PV extrasystoles and AF is clear in paroxysmal AF, but not in persistent AF, in which the mechanisms of AF may be more related to a chronic atrial substrate than to acute triggers.[5] In persistent AF, the PVI procedure is less successful than paroxysmal AF,[6] with single procedure success reported as low as 27%,[7] 36%,[8] or 49%,[9] but up to 61%[10] or 67%,[11] In order to achieve overall acceptable success rates (which can reach up to 79%-94%),[7,10,12] there is a need for repeat procedures and concomitant use of antiarrhythmic drugs. The rate of repeat procedures for persistent AF can reach up to 70% to 80%.[13-16] Ablation strategies to add additional lesions -beyond isolation of the PVs, including ablation of complex, fractionated potentials, linear lesions, or areas of low voltage have failed to produce improved outcomes.[17-20]

Clinical failures of a first ablation procedure are caused in a significant portion of patients by atrial flutters[21-23] rather than recurrent AF. Such atrial flutters may be caused by perimitral reentry (PMF) in up to 33–60% of the patients.[21,23,24] Catheter ablation of PMF involves the creation of a linear lesion from the mitral annulus to the left inferior PV (i.e., mitral isthmus).[25,26] Achieving a complete ablation (defined by bidirectional conduction block across the ablation line) can be very difficult,[14,26,27] with success rates reported as 32%,[28] 64%,[29] or 71%.[30] It sometimes requires ablation inside the coronary sinus (CS),[31] in close proximity to the circumflex coronary artery, which could be damaged.[29] Incomplete ablation of the mitral isthmus is arrhythmogenic,[32,33] increasing the risk of recurrent flutter by up to 4 times.[34]

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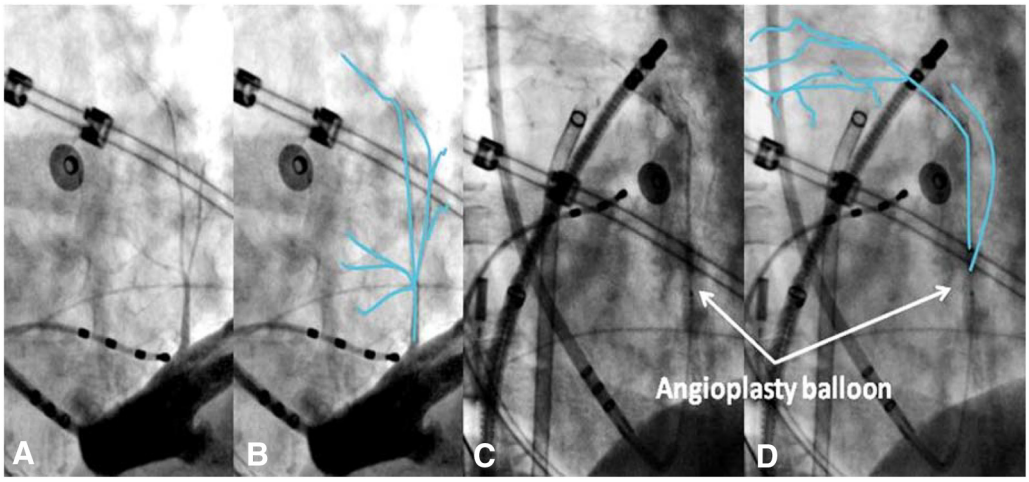
Figure 1



VOM ethanol infusion procedure. A, Contrast injection in the CS lumen through the sub-selector catheter with its tip close to the VOM, showing the VOM takeoff 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).

The ligament and vein of Marshall (VOM) can be a source of ectopic beats leading to AF[35] and of dual sympathetic[36] and parasympathetic innervation[37] that have been implicated in the genesis and maintenance of AF. Additionally, the VOM anatomical location—connecting the coronary sinus with the PVs—coincides with the location of the posterior mitral isthmus, commonly ablated to treat perimitral flutter. We developed the technique of retrograde balloon cannulation and ethanol infusion in animals,[38] and demonstrated its safety and feasibility in humans.[39] VOM ethanol infusion leads to ablation of the VOM and its intrinsic electrical activity,[38,39] as well as the neighboring myocardium, its associated PV connections,[40] the mitral isthmus,[41] and the associated parasympathetic innervation.[42] The goal of the VENUS and MARS trials is to ascertain whether VOM ethanol infusion leads to improved freedom from AF in patients undergoing de novo or repeat catheter ablation of persistent AF, respectively.

Methods

VOM ethanol infusion: technique

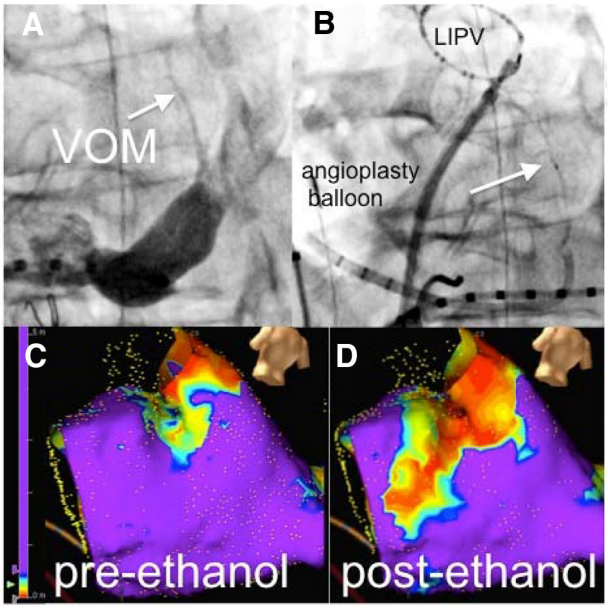
The technique has been previously described by our group[38-43] and reproduced by others. We enter the CS with a sheath advanced from the right internal jugular vein (a femoral vein access can also be used). A sub-selector catheter with a ~90° angle at the tip (typically, a left internal mammary artery 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 1 shows an example.

In our pilot experience, we had 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%, $P < .05$), suggesting that success is not only determined by anatomical factors (e.g., size and tortuosity of the VOM), but also by operator experience. We estimate a 15% incidence of failure to cannulate the VOM.

VOM ethanol ablative effects

Anatomically, the VOM is an epicardial vein and ethanol exerts its ablative effects via transmyocardial circulation into the LA cavity,[43] with an epicardial-to-endocardial gradient. 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 extending variably to areas around the left PVs, and towards the mitral annulus, including a large portion of the mitral isthmus (Figure 2). This is uniquely suited to treat PMF. In our initial experience, we tested perimitral conduction in 77 patients (25 of which had PMF mapped prior to

Figure 2



Tissue ablation by VOM ethanol. 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 cm2) of the left atrium pre- and post-ethanol.

ethanol delivery). Although VOM ethanol infusion by itself only led to bidirectional perimitral block in 3 patients, block 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.[41] Considering the low success rate reported by RF ablation (32%,[28] 64%,[29] or 71%[30]) –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.

VOM ethanol and denervation

The location of the VOM coincides with that of the left dorsal pathway of vagal innervation to the intrinsic cardiac ganglia[44] (Figure 3). In our recent experience we have shown that high-frequency stimulation (30 Hz, 25 mA) in the VOM can induce parasympathetic reflexes reaching the AV node (causing transient AV node conduction blockade). Such responses are completely abolished in all patients after VOM ethanol infusion.[42] Of the atrial ganglionated plexi, it is the right inferior PV plexus that directly connects with the AV node.[45] The VOM is remote from the AV node. 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*. Parasympathetic responses were abolished by ethanol in all patients in whom such responses were elicited at baseline, and AF induction by VOM high-frequency elimination was eliminated.[42] Thus, VOM ethanol infusion is an effective strategy to achieve regional denervation of the LA.

Overview of the clinical trials

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 4 shows the clinical trial design.

VENUS-AF. Vein of Marshall Ethanol iNfusion in Untreated perSistent Atrial Fibrillation: To assess the role of VOM ethanol infusion in de novo catheter ablation of persistent AF.
MARS-AF. Vein of Marshall Alcohol in Repeat ablation of perSistent Atrial Fibrillation: To assess the impact of VOM ethanol infusion after a failed conventional ablation of persistent AF.

Study endpoints are listed in Table I.

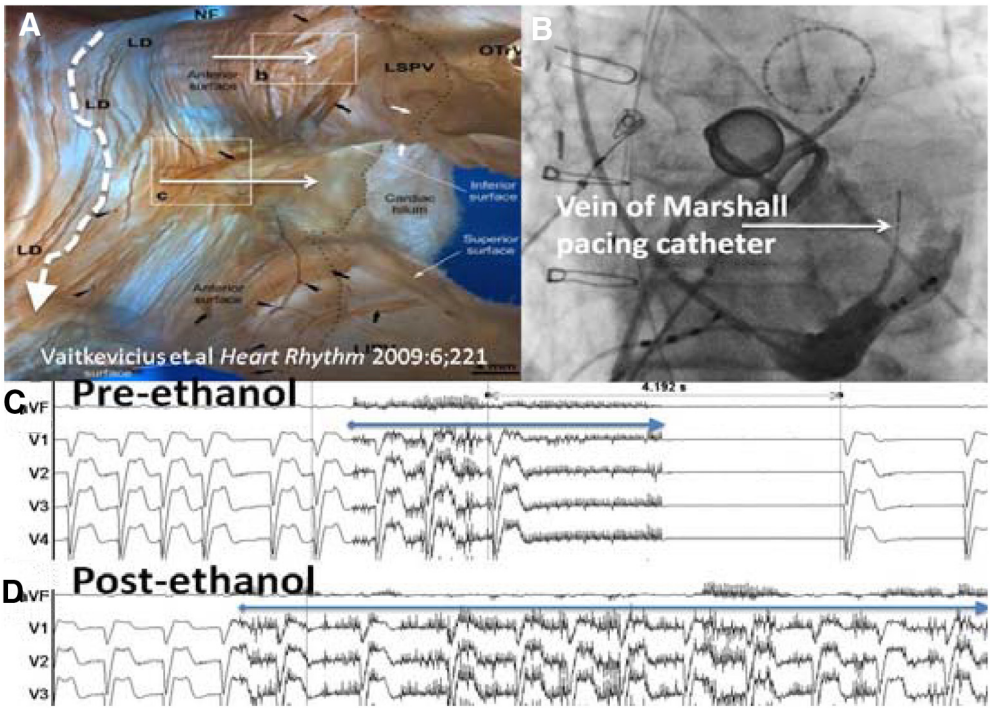
Preliminary pilot experience

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 1 year has been 45% (consistent with literature reports of 27%,[7] 36%,[8] or 49%[9]). 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.[7-9] These data will be used for MARS-AF.

Patient population and number of planned subjects

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. Table II lists inclusion and exclusion criteria. Total number of planned subjects for inclusion: 405. Randomization is 1.15:1, with 15% more patients enrolled in VOM groups to compensate for technical failures of VOM cannulation. The

Figure 3



Atrial denervation by VOM ethanol. Parasympathetic 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).

purpose of the uneven enrollment numbers is to analyze outcomes in the two groups *both as intention-to-treat as well as as-treated*, in order to establish the feasibility, safety and outcome differences of successfully VOM-ethanol-treated patients.

VENUS: 180 (VOM + PVAI) + 156 (PVAI) = 336
MARS: 37 (VOM + PVAI) + 32 (PVAI) = 69

Procedural considerations

Pre-procedural imaging. Imaging prior to enrollment with a 2D echocardiogram 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 1 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.

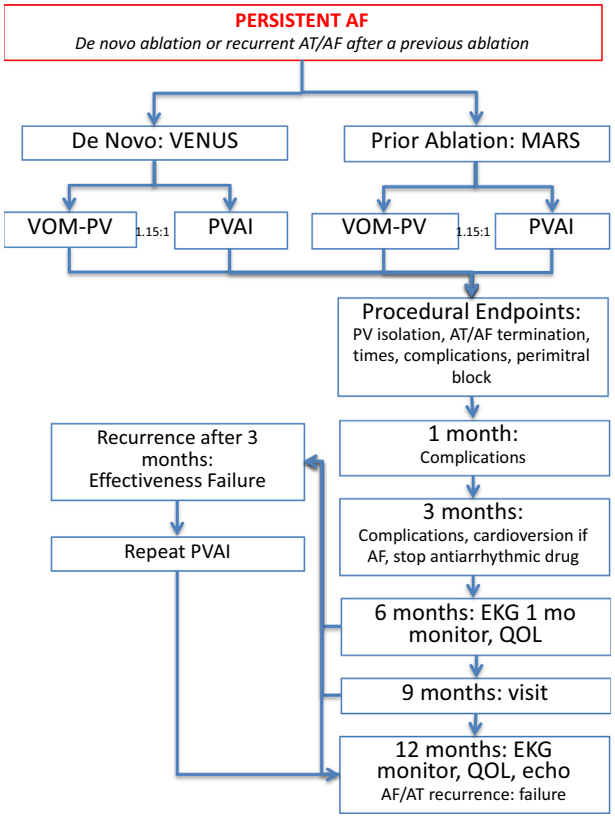
PVAI and VOM ablation strategies. See Table III.

Effectiveness (treatment) failures

a. Clinical recurrence of AF or AT after 3-months (blanking period).
b. Documented AF or AT of 30 seconds or more on a 1-month EKG monitor at obtained at 6 and 12 months. Accepted substitutes include pacemaker, defibrillator, or implanted recorder interrogations.
c. Requirement of a repeat ablation procedure for recurrent AT-AF.
d. Death.

Randomization. In VENUS, randomization will be stratified by AF history duration in 3 groups (less than 6 months, 6-24 months, and greater than 24 months). Additionally, VENUS randomization will be stratified by

Figure 4



Clinical trial design. Patients with drug-refractory persistent AF undergoing their first (VENUS) or repeat (MARS) ablation will be randomized to either PVAI alone or in combination with VOM ethanol infusion (VOM-PV).

Table I. 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. Success at obtaining bidirectional perimitral block, and ablation time required.
6. Clinical/partial success: less than 25% AF burden on a continuous event monitor at 6 and 12 months from ablation procedure.
7. Sub-acute procedural complications (within 30 days)
8. Recurrence as persistent or paroxysmal AF, or flutter after 1 or 2 procedures.
9. LA function on Doppler echocardiography (LA strain at 12 months.
10. Incidence and mechanisms of atrial flutters.
11. Cardiovascular hospitalizations and
12. Quality of life as determined by AFEQT questionnaire.

left atrial volume: normal LA volume - up to 75 ml/m2), moderate enlargement (76-89 mL/m^2), or severe enlargement– 90+ mL/m^2). In MARS, randomization will be stratified by recurrence type (AF or atrial tachycardia).

Patient follow-up post procedure. Patients will be followed at 1, 3, 6, 9, and 12 months post-procedure. Anytime during the blanking period (0–3 months post-procedure), patients may be treated with antiarrhythmic drugs or cardioversion as needed.

Cross-over of patients initially randomized to PVAI only. The VOM procedure can only be performed once. If a patient is originally randomized to conventional PVAI and experiences a treatment failure, 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.

Sources of funding

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Statistical considerations for VENUS

Assumptions. Our initial pilot 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 among groups.

The expected mortality is low in this study as it has been in AF ablation trials. Mortality will be recorded as a safety endpoint. During the initial planning phase, a follow-up time of 15 months was considered for the VENUS trial, in order to accommodate for appropriate follow-up of patients undergoing repeat procedures. However, because of time and funding constraints, a total follow-up duration of 12 months was chosen, which is appropriate for single-

Table II. 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 thromboembolic 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 >11 k/ 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), or GFR <30 ml/min/1.73 m^2.
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.

Table III. Procedural strategies

PVAI procedure.

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:

1) 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.
2) The greater PV antra, including posterior wall and roof.
3) 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.
4) Areas of complex, fractionated potentials.
5) Sustained atrial flutters will be mapped and ablated as directed by the map and flutter location.
6) If AF persists after all the RF ablations, the patient will be cardioverted to restore sinus rhythm.

VOM procedure

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

1) A 9-10F 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. Selective on selective cannulation of the VOM will be performed using the sub-selector catheter. Contrast will be injected via the lumen of the sub-selector catheter to verify such engagement.
2) The VOM will be cannulated with an angioplasty wire (0. 014″) that will be advanced through the sub-selector catheter and into it.
3) An angioplasty balloon (1.5-2 mm diameter, 6-8 mm length) will be advanced over the wire 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.
4) The procedure will then continue with standard PVAI procedure as above.
5) VOM treated patients will undergo mitral isthmus ablation, with the goal to achieve perimitral block.

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:

1. At baseline after gaining trans-septal access to the LA in both randomization groups.
2. After ethanol infusion, if randomized to VOM-PV.
3. After completion of the PVAI ablation lesions, in both randomization groups.

procedure success as the primary efficacy endpoint. Procedural failures (events counted as the primary efficacy endpoint) occur mostly during the first year. Additionally, a 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.[1]

Group sequential clinical trial design. A group sequential randomized trial design was employed, for which power and sample size determination was determined for a inequality test of two success proportions using PASS V12 (Kaysville, UT). The following assumptions were made for the VENUS study of de novo AF subjects:

- 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

Computational results indicated that 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).

Primary outcome efficacy will be monitored 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 subjects. This equates to information times (sample sizes) of n1 = 60 and n2 = 52 (total n = 112), n1 = 120 and n2 = 104 (total n = 224), and n1 = 180 and n2 = 156 (total n = 336) at the three looks, respectively. The significance boundaries are +/− 3.953 (3.809, 4.289), +/− 2.543 (2.516, 2.578), and +/− 2.011 (1.994, 2.036) for the 3 looks. At each look, the hypothesis test applied 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 kth look, and $\hat{p}_{2k}$ is the proportion of successful primary outcomes within the PVAI arm of VENUS at the kth look. Z_k follows a standard normal distribution, N(0,1). If during the first look when at least n1 = 60 (VOM) and n2 = 52 (PVAI) primary outcomes have been observed (n = 112 total), Z_1 exceeds 3.953, then the efficacy of VOM would normally be considered to indicate early stopping due to a beneficial outcome, whereas if Z_1 falls anywhere between these limits, then continuation of the trial would be necessary. The same rule applies to the 2nd look when at least n1 = 120 and n2 = 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 n1 = 180 and n2 = 156 (336 total) primary outcomes have been observed, for which the critical value of Z_3 is ±2.011. Figure 5 illustrates the significance limits for primary efficacy over the information time.

Statistical analysis. For intent-to-treat (ITT) subjects, the primary analysis will include two-tailed hypothesis tests for inequality of two proportions (unpooled standard errors) to determine whether or not the VOM success rate is significantly different than the success rate for PVAI. Resampling methods with Z-scores will be employed to evaluate varying levels of missingness of efficacy caused by withdrawals, subjects lost to follow-up, or subjects with missing primary outcomes. 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. Post-hoc analysis for ITT subjects and per-protocol subjects will include evaluations of loss of rhythm (LOR) and secondary outcomes using model building strategies (MBS) based on univariate and multivariable regression. During MBS, univariate predictors whose $P < .25$ 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.

Statistical considerations for MARS

Assumptions. 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.

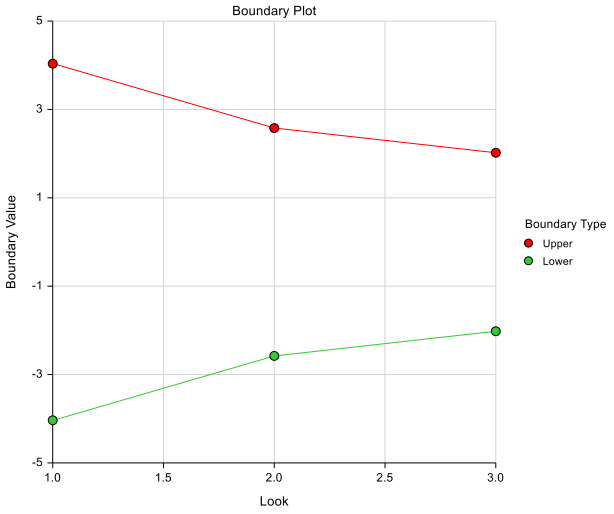
Power and sample size determination. Group sequential two proportions power analysis using simulation was performed using PASS V12 (Kaysville, UT). The following assumptions were made:

- 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

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

Figure 5



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

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 month follow-up) are available for 1/3, 2/3 and 3/3 of the total sample size of MARS subjects. 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.

Safety

A study-specific data safety monitoring board (DSMB) has been established by the NHLBI (National Heart, Lung, and Blood Institute) which is funding this clinical trial (R01 HL115003). The DSMB will review safety events, efficacy interim analyses, monitored study conduct and will review and approve protocol modifications. 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 and bioethicist, an expert in clinical trials, and an NIH official. A steering committee is responsible for study design and conduct. A data coordinating center has been constituted at the Dan L. Duncan Institute for Clinical & Translational Research of Baylor College of Medicine. Independent study monitoring of data integrity will be conducted at all sites. The study is also overseen by the FDA (IND# 115,060). and registered as NCT 01898221 at www.clinicaltrials.gov. Adverse events will be monitored by an investigator non involved in the procedure or patient follow-up, and blinded to the randomization outcome (NSK).

Rhythm core laboratory

The primary endpoint will be determined by clinical follow-ups and by 1-month continuous monitoring performed at 6- and 12-months post-randomization. External event monitors will be provided by MediLynx (Plano, TX). Data from implanted devices (pacemaker, defibrillators, or implantable loop recorders) able to provide 1-month AF burden will be acceptable substitutes. Data will be verified by a core laboratory led by an investigator not involved in the procedure or patient follow-up, and blinded to the randomization outcome (ASD).

Echocardiographic core laboratory

Indices of left atrial function will be assessed at 12 months post-randomization by the core echocardiography laboratory led by an investigator blinded to the randomization outcome (SN).

Data monitoring

An independent monitor will assess data accuracy and integrity in all sites after site activation and continue periodically based on enrollment until all subjects have completed trial visits or 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, trial manager).

Each site's first 3 enrolled patients will be monitored at 100% of data items and thereafter, every 10th patient will be monitored. Exclusion/inclusion criteria and adverse events will be monitored in every patient.

Trial sites and investigator training

The initial sites include Houston Methodist DeBakey Heart Center, Houston DeBakey VA Medical Center, both in Houston, and Texas Cardiac Arrhythmia Institute in St David's Medical Center in Austin, Texas. The trial will open at additional sites, including Arizona Heart Rhythm Center, University of Southern California, Virginia Commonwealth University, and others. Given the VOM ethanol procedural complexity, operators will undergo dedicated training prior to site qualification, including formal review of procedural steps and pre-recorded video, as well as on-site proctoring during site-initiation visit.

Conclusions

The VENUS and MARS trials will define the role of VOM ethanol infusion in the treatment of persistent AF.

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