2-Hydroxybenzylamine (2-HOBA) to Prevent Early Recurrence of Atrial Fibrillation after Catheter-based Ablation

Principal Investigator:

Greg Michaud, MD
Professor of Medicine
Chief, Arrhythmia Section
Division of Cardiovascular Medicine
Vanderbilt University Medical Center

Co-Principal Investigator:

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

Table of Contents:

1.0 BACKGROUND

1.1 New therapeutic strategies for atrial fibrillation (AF) are needed. Current rhythm control therapies for AF include the antiarrhythmic drugs (sodium and potassium channel blockers) and AF ablation (catheter or surgical).[1] The recurrence rate for antiarrhythmic drugs is ~50%, while the recurrence rate for catheter ablation is up to 50% following a single procedure with 20-40% of patients requiring a second procedure.[2] The antiarrhythmic drugs not only have limited and unpredictable efficacy but also a narrow therapeutic window with potentially fatal ventricular arrhythmias as a major concern for all agents,[1] and clinical trials of catheter-based ablation have reported a rate of major complications of at least 1% including stroke, tamponade, and death.[1] The limited efficacy and inherent risk of the antiarrhythmic drug class and AF ablation drive the need to develop alternative therapeutic strategies for AF.

1.2 Inflammation and oxidative stress promote AF and its clinical risk factors. Numerous clinical risk factors such as obesity, hypertension, diabetes mellitus/metabolic syndrome, and aging are known to increase the risk of AF, but the molecular pathways linking these comorbidities to AF are incompletely understood.[3-5] It is well recognized that many of these comorbidities are associated with inflammation, which is believed to be a major shared mechanism.[6] Inflammation is the immune response to cellular injury generated by oxidative stress resulting in overabundance of reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$) and hydroxyl radicals. Inflammation and oxidative stress are closely related, and each promotes the generation of the other. Oxidative stress is known to result in damage to lipids, proteins, and DNA, and is implicated in the development of a wide range of diseases including cancer, atherosclerotic cardiovascular disease, autoimmune disease, neurological disorders, as well as the pathogenesis and progression of AF.

1.3 An optimal antioxidant or anti-inflammatory drug to treat AF has not been identified. As oxidative stress became increasingly recognized for its role in AF pathogenesis[7-9], numerous studies were conducted investigating the use of dietary antioxidants such as vitamins C, E, and omega-3 polyunsaturated fatty acid (n-3 PUFAs or "fish oil") for the treatment of AF. The major limitation of treatment with vitamins C and E are that they fail to significantly suppress oxidative stress, which is their therapeutic target.[10] This is also a problem for n-3 PUFA.[11] In a clinical trial conducted by our research group, 190 patients with AF were randomized to high dose n-3 PUFA or matching placebo and followed for 6-months for AF recurrence. The rate of AF recurrence was 59% in the n-3 PUFA group compared to 47% in the placebo group with no difference in the primary endpoint of time-to-AF recurrence (HR=1.2 [95% CI: 0.8-1.8] P=0.39). Furthermore, there was no difference in the concentration of inflammatory cytokines (Interleukins 6, 8, 10; tumor necrosis factor alpha) or measures of oxidative stress (urinary F2-isoprostane) between n-3 PUFA and placebo groups.[11] Given a large amount of ROS can be generated by a variety of different cell types (neutrophils, monocytes, myofibroblasts), a major problem with the antioxidant drug class appears to be that they cannot achieve sufficient in-vivo concentrations to reduce ROS levels.[12] Anti-inflammatory drugs, steroids and colchicine, can both reduce AF after ablation or cardiac surgery, but the side-effects preclude widespread long-term use. Steroids are associated with immunosuppression, glucose intolerance, and agitation; and a meta-analysis of studies using colchicine for AF found a 21% rate of GI intolerance (diarrhea, nausea, abdominal pain) in the colchicine group compared to 8% in placebo (P<0.001).[13]

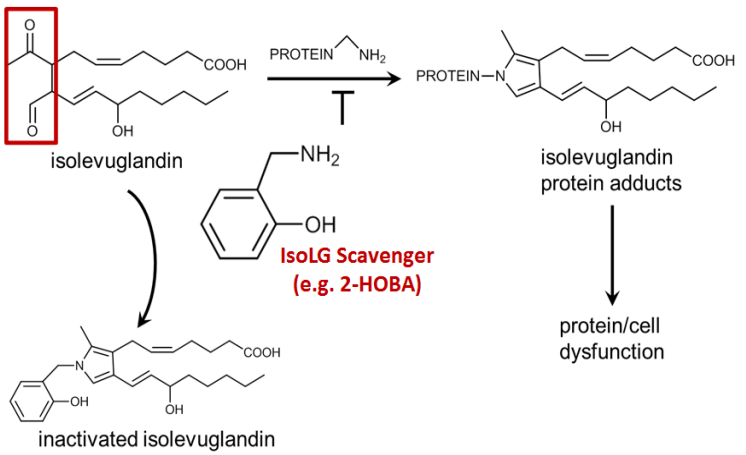

Figure 1: The dicarbonyl motif of isolevuglandin (IsoLG) binds rapidly to lysine side chains to form a dysfunctional IsoLG-protein adduct. However, in the presence of an IsoLG scavenger such as 2-HOBA, IsoLG preferentially binds to 2-HOBA resulting in an inactive byproduct.

1.4 Isolevuglandin scavengers are compounds that target oxidative stress but are NOT anti-inflammatories or antioxidants. Given the failure of the anti-inflammatory and antioxidant drug classes to effectively treat AF in humans, the discovery of a new group of

compounds that serve as downstream mediators of oxidative stress damage, the isolevuglandins (IsoLGs), represents an important conceptual advance in attacking this pathway to AF. IsoLGs are the product of lipid peroxidation, which occurs when ROS react with polyunsaturated fatty acids present in cell membranes or freely circulating.[14] IsoLGs are highly reactive and following their formation bind within seconds to macromolecules with lysine residues forming IsoLG adducts (**Figure 1**).[15] IsoLG adducts are irreversible modifications that cause dysfunction of protein targets, including structures relevant to cardiomyocyte homeostasis (e.g. ion channel proteins, proteasomes, chromatin, mitochondria). IsoLGs are so highly reactive that they cannot be measured in their unreacted form and must instead be measured as IsoLG-adducts.[15] The discovery of the IsoLGs has identified a critical component in the oxidative stress pathway that may be targeted therapeutically.[16] IsoLG scavenger compounds, such as 2-HOBA, can bind IsoLG orders of magnitude more rapidly than IsoLG can bind with the lysine on other macromolecules, thereby generating permanently inactivated IsoLG compounds rather than IsoLG-adducts.[16] This study aims to test the efficacy of a therapeutic strategy targeting IsoLG for treatment of AF using the IsoLG scavenger 2-HOBA.

1.5 Early recurrence of AF after catheter-based ablation can be used to study AF due to inflammation and oxidative stress. Over the past decade, post-operative AF has been studied extensively as a model of AF due to inflammation and oxidative stress.[17-20] However, AF research in the post-cardiac surgery setting is complicated because the patients often have severe comorbid coronary and valvular heart disease, may be on a highly variable amount of vasopressors, may remain intubated and sedated for days, often have large volume shifts from intravenous fluids and blood products, and may have an unknown history of AF prior to surgery. We propose here to study early recurrence of AF in patients who have undergone catheter ablation for AF as an alternative to the post-cardiac surgery setting.

Reference	N	Study Drugs	Risk
Koyama	128	Prednisone	-22%
Kim	138	Methylprednisolone	-25%
Deftereos	161	Colchicine	-18%
Deftereos	223	Colchicine	-18%

Table 1: Studies of early AF recurrence with anti-inflammatories.

Early recurrence of AF is conventionally defined as any recurrence of AF, atrial flutter, or atrial tachycardia lasting >30 seconds within 90-days following ablation.[2] Approximately 50% of patients experience early arrhythmia recurrence and the vast majority of those recurrences are within the first 30-days.[2,21] Compelling evidence supports oxidative stress and inflammation caused by ablation-related atrial tissue injury as the main contributor to early recurrence.[22-26] Up to 75% of patients experience symptoms from pericarditis following AF ablation, and numerous studies have demonstrated that the use of anti-inflammatory medications (corticosteroids, colchicine) reduces early AF recurrence (**Table 1**).[22-26] Despite these promising results, common side-effects from steroids (immunosuppression, agitation, glucose intolerance) and colchicine (gastrointestinal intolerance) preclude widespread long-term use of these agents for AF.

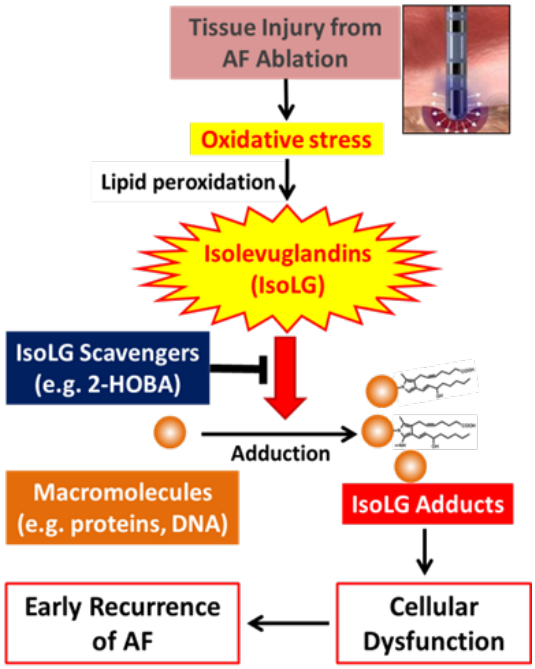

Figure 2: Tissue injury from AF ablation generates oxidative stress resulting in IsoLG-adduct formation that contributes to early recurrence of AF.

2.0 RATIONALE AND SPECIFIC AIMS

We hypothesize that oxidative stress causes AF through the production of isolevuglandins and that treatment with IsoLG scavengers will significantly reduce AF. The proposed studies will test this hypothesis by randomizing patients with AF to 2-HOBA or placebo 3 days prior to AF ablation to allow 2-HOBA to reach steady-state levels. We hypothesize that tissue injury from AF ablation causes a large release of ROS that react with lipids to generate IsoLGs (**Figure 2**). In the absence of 2-HOBA, IsoLGs will react within seconds to form IsoLG-macromolecule adducts in atrial tissue, promoting early recurrence of AF. In the presence of 2-HOBA, IsoLGs will rapidly react to form IsoLG-macromolecule adducts in atrial tissue, promoting early

recurrence of AF. In the presence of 2-HOBA, IsoLG will preferentially bind to and therefore be inactivated by 2-HOBA thereby sparing injury to the atrial tissue caused by oxidative stress and its contribution to early recurrence of AF. Early recurrence of AF will be measured by ECGs that are recorded once per day by a smartwatch (Apple Watch, Apple Inc., Cupertino, CA) with additional ECGs recorded by the participant if they experience symptoms of AF, or if the smartwatch alerts the participant of a possible AF episode via its auto-detection AF monitoring algorithm. The Apple Watch's AF algorithm is based on sampling of heart rate and variability and will give an audible alarm if those parameters indicate a possible episode of AF. The smartwatch records a single-lead ECG if the participant touches the watch with their contralateral hand. The day and time of the episode is also stored by the smartwatch. At the end of the 28-day follow-up period, study personnel will review the stored ECGs. Blood will be drawn prior to ablation and on post-procedure Day 1 for measurement of IsoLG-adduct levels. DNA will be extracted to explore a pharmacogenomic interaction with haplotypes at the chromosome 4q25 AF risk locus, which: 1) is strongly associated with the development of AF and the early recurrence of AF after ablation[27]; and 2) has been reported to be a regulator of an anti-oxidant gene program in response to cardiac injury.[28]

The proposed double-blind, randomized, placebo-controlled trial of 2-HOBA in patients undergoing AF ablation is designed to address the following Specific Aims:

Specific Aim 1: **To test the hypothesis that treatment with 2-HOBA reduces early recurrence of AF (clinical endpoint)**

Specific Aim 2: **To test the hypothesis that treatment with 2-HOBA reduces circulating levels of IsoLG-adducts (biochemical endpoint)**

Specific Aim 3: **To explore the idea that genetic variation at the 4q25 (*PITX2*) AF susceptibility locus modulates the clinical and biochemical response to 2-HOBA**

3.0 ANIMAL STUDIES AND PREVIOUS HUMAN STUDIES

3.1. The IsoLG scavenger, 2-HOBA, is remarkably effective in experimental models of AF

Oxidative stress contributes to the development of numerous diseases through oxidative protein modification. Early results in mouse models of Alzheimer's disease and hypertension demonstrated that treatment using 2-HOBA effectively reduced cognitive impairment in the Alzheimer's model and lowered blood pressure in the hypertension model.[29,30] Our research group has been investigating the use of IsoLG scavengers to treat AF in experimental mouse models.[31] Obesity and hypertension are two of the strongest clinical risk factors for the development of AF and are well-established states of high inflammation and oxidative stress.[32] In a diet-induced obesity mouse model, treatment with 2-HOBA significantly reduced AF inducibility compared to controls. The control group included mice treated with an inactive structural analog, 4-HOBA, which does not scavenge IsoLG (**Figure 3**). In an angiotensin-II hypertension mouse model, AF was readily induced and treatment with 2-HOBA successfully reduced inducibility compared to 4-HOBA while a group treated with antihypertensive medications (hydralazine and hydrochlorothiazide) also had less AF (**Figure 3**).[31] AF is also inducible in a new mouse model of inflammation without hypertension. Further in the models of inducible AF, IsoLG adducts elevated at baseline in

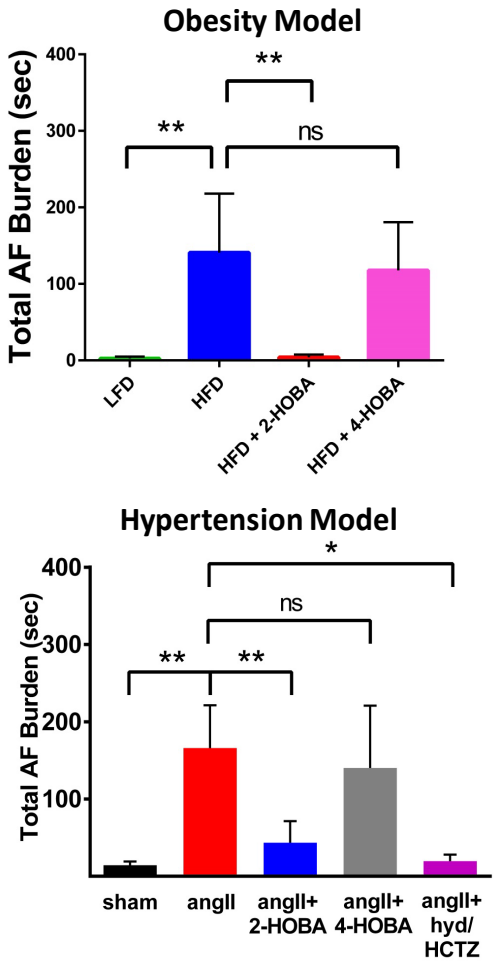

Figure 3: Murine models of obesity and hypertension have inducible AF with rapid atrial pacing. Treatment with an IsoLG scavenger, 2-HOBA, reduces AF inducibility, but treatment with an inactive structural analog, 4-HOBA, does not. (LFD=low fat diet, HFD=high fat diet, angII=angiotensin II, hyd=hydralazine, HCTZ=hydrochlorothiazide).

the atria are lowered by 2-HOBA. Taken together, a therapeutic strategy aimed at reducing reactive IsoLG-adduct levels through the use of scavenger compounds such as 2-HOBA has yielded positive results in multiple mouse models of oxidative-stress induced AF and provides the basis for advancing this line of research to humans.

3.2. A Phase I clinical trial of the IsoLG scavenger 2-HOBA

A first-in-humans Phase I clinical trial using oral 2-HOBA was recently completed to assess its safety and pharmacologic profile. A single dose study (NCT-3176940) and a multidose study (NCT-03555682) were conducted. In the single dose escalation study, 6 ascending doses of 2-HOBA were studied. Three participants were enrolled at each dose and 18 participants successfully completed the study.[33] The highest planned dose (825 mg) was reached. There were no serious adverse events. There were 5 total adverse events (AEs). They were all determined to be mild in intensity and not dose-dependent. Reported AEs included: frequent urination (N=2), headache (N=1), itchy throat (N=1), rash (N=1), sleepiness (N=1) and abdominal bloating (N=1). No clinically significant changes in ECG recordings, vital signs, or laboratory parameters were observed.

In the multi-dose randomized, double-blind clinical trial, 18 healthy volunteers were given 2-HOBA or placebo for two weeks. Two doses of 2-HOBA were tested, 500 mg or 750 mg. The study assessed the safety, tolerability, and pharmacokinetics of dosing over a two week period, and demonstrated a safe and well-tolerated profile with three-times daily dosing. 9 subjects were studied at each dose level, with 6 receiving the ADI and 3 receiving placebo. These doses were chosen to achieve similar plasma levels at steady state compared with the single dose study. No serious AEs were observed. 14 patients reported at least 1 adverse event (**Table 2**). Reported AEs included headache (N=6), GI distress (N=3), rash/itching (N=3), urine odor (N=2), dry mouth (N=2), nasal congestion (N=2), lethargy/sleepiness (N=2), hypertension (N=1), and eye irritation (N=1). One volunteer experienced a rash of moderate intensity, and was withdrawn from the study, though this AE was not determined to be study-related or dose-dependent.

	2-Hydroxybenzylamine acetate dose			
	Placebo (*n*=6)	500 mg (*n*=6)	750 mg (*n*=6)	Total (*n*=18)
Any event, n (%)	4 (67)	6 (100)	4 (67)	14 (78)
Headache	2 (33)	2 (33)	2 (33)	6 (33)
GI distress (nausea, bloating, constipation)	2 (33)	1 (17)	0 (0)	3 (17)
Rash/itching	1 (17)	1 (17)	1 (17)	3 (17)
Urine odor	0 (0)	2 (33)	0 (0)	2 (11)
Dry mouth	1 (17)	1 (17)	0 (0)	2 (11)
Nasal congestion	0 (0)	2 (33)	0 (0)	2 (11)
Lethargy/sleepiness	0 (0)	1 (17)	1 (17)	2 (11)
Hypertension	0 (0)	1 (17)	0 (0)	1 (6)
Eye irritation	0 (0)	1 (17)	0 (0)	1 (6)

Table 2. Summary of reported adverse events by dose in multi-dose Phase 1 study of 2-HOBA

The pharmacokinetics observed in the multi-dose trial largely resembled those obtained in the single dose study. 2-HOBA had a T_{max} of 1-2 h and achieved steady-state C_{max} in the range of those observed with the highest dose in the single dose study - 3177 ± 1993 ng/mL after 15 days of dosing at 500 mg and 2292 ± 913 ng/mL after 15 days of dosing at 750 mg. Both groups (500 and 750 mg doses) experienced progressive AUC increases over the 15 days, yielding accumulation ratios of 1.25-1.50. The half-life in this dosing interval was slightly longer than that observed in the single dose study, contributing to the increased accumulation of 2-HOBA. Average systemic exposure (AUC and $C_{max)}$ was similar for the 500 and 750 mg doses. Response to 2-HOBA revealed considerable variability among subjects, as those dosed at the 500 mg regimen showed greater exposure on average than volunteers dosed at 750 mg. Oral bioavailability is not currently established in humans, and limits the interpretation of these data. Notably, the major metabolite of 2-HOBA, salicylic acid, showed a peak plasma

concentration of 12.8 ± 3.7 mg/L, considerably lower than the accepted therapeutic range of 150-300 mg/L required for anti-inflammatory activity. Consequently, inhibition of cyclooxygenases was not observed, as determined by urinary metabolites of 3 major prostaglandins, pGE-M, TxB_2-M, and PGI-M.]

No evaluation of the compound in pediatric settings or in patients with reported history of disease has been undertaken. A greater range of age and ethnicity should be explored in additional clinical studies to gain a better understanding of its clinical profile.

3.3. The peak plasma concentration of 2-HOBA in humans will reach the level demonstrated to reduce IsoLG-adduct formation in experimental models

Prior studies in mice demonstrated that the plasma concentration of 2-HOBA needed for efficacy was ~2.7 μM.[16] To date, the peak plasma concentration of 2-HOBA in humans has been analyzed from subjects given the 50mg and 100mg doses in our Phase I clinical trial. The peak plasma concentration of 2-HOBA from subjects given a single 100mg dose was 1.7 μM. The single dose escalation study has administered the 550mg dose without any adverse events, which based on estimates using first-order kinetics, is expected to achieve a peak plasma concentration (8.5 μM) that significantly exceeds the level found to be efficacious in experimental models. Therefore, 2-HOBA is safely tolerated at doses that will produce plasma concentrations known to reduce formation of IsoLG-adducts.

3.4. A pilot study demonstrates that AF ablation increases IsoLG adduct levels

In preparation for this trial, a pilot study (IRB #171722) was conducted to demonstrate the feasibility of the study protocol, demonstrate that IsoLG-adduct levels increase following AF ablation, and generate preliminary data for the power/sample size analysis. These measurements are made using a cellular system (since IsoLG adducts are not free in the circulation), and has used IsoLG measurements in monocytes and dendritic cells (DC).[30] Over an 8-week period, 11 subjects who met the eligibility criteria for our proposed clinical trial were prospectively enrolled and underwent AF ablation. The data from our pilot study support the following conclusions:

1. The rate of participant enrollment was sufficient to meet the enrollment target of this proposal. We have proposed to enroll 1 participant per week to accrue 162 participants over 36 months. We enrolled 1.375 participants per week in the pilot study.

2. AF ablation resulted in a statistically significant increase in circulating IsoLG-adduct levels. IsoLG-adduct levels were measured in blood samples collected pre-ablation and on post-procedure day #1. IsoLG-adduct levels increased in 82% (9/11) of subjects, and the mean increase was 23% in DCs (Pre: 41±16% vs. Post: 63±17%; P=0.02; **Figure 4, top panel**) and 21% (Pre: 38±19% vs. Post: 59±18%; P=0.04) in monocytes following ablation.

3. There was an early recurrence of AF in 45% of participants (5/11). This demonstrates that, similar to other published reports, early recurrence of AF is common in our study population and is sufficient to power the primary endpoint of Aim 1, which is AF recurrence within 28-days following ablation (**Figure 4, bottom panel**). This is comparable to the early recurrence rate of AF from numerous other published reports, which is ~50%.[21,23]

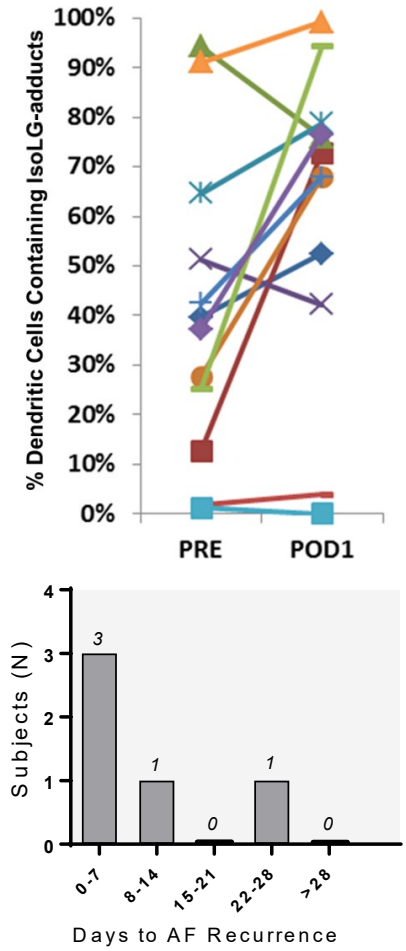

Figure 4: (**Top**) There is a significant increase in the proportion of dendritic cells containing IsoLG-adducts from pre-ablation to post-operative day #1 (POD1; P=0.02). (**Bottom**) 45% of participants had an early recurrence of AF within our proposed 28-day monitoring period.

4.0 INCLUSION/EXCLUSION CRITERIA

INCLUSION	EXCLUSION
First time AF ablation with radiofrequency or cryo ablation	Planned surgical or hybrid (surgical + catheter) ablation
Repeat AF ablation if the patient has persistent AF and ablation of non-pulmonary vein substrate is planned (e.g. posterior wall ablation, mitral or roof line, etc)	Current use of amiodarone or within past 3 months
Able to provide written, informed consent	Use of oral steroids or colchicine
22 years of age or older	Pro-inflammatory, rheumatologic disorder (e.g. RA, SLE, IBD, psoriasis, ankylosing spondylitis)
	NYHA Class III/IV Heart Failure
	Active ischemia
	Hypertrophic Cardiomyopathy
	Cardiac or thoracic surgery within 6 months
	Expected life span < 1 year
	Creatinine clearance <30 ml/min
	Prior or planned heart transplantation
	Pregnant women
	Aspirin allergy
	Current use of MAO-I

RA=rheumatoid arthritis; SLE=systemic lupus erythematosus; IBD=inflammatory bowel disease (Crohn's, Ulcerative Colitis); NYHA=New York Heart Association; LVEF=left ventricular ejection fraction

Table 3: Eligibility Criteria.

5.0 ENROLLMENT AND RANDOMIZATION

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

Randomization: This will be a double-blind, randomized study. Eligible subjects will be randomized according to a stratified permuted block scheme with a block size of 8. This ensures that the cumulative number of assignments to each treatment (2-HOBA or placebo) will be in balance after each block of assignments had been made. Stratification will be made based on: 1) the planned ablation technique, cyroballoon vs. radiofrequency, 2) paroxysmal vs. persistent atrial fibrillation, 3) *de novo* vs. repeat ablation. A statistician will design the randomization table and enable the randomization tool within REDCap. After a patient enrolls for the study, the study nurse will determine the treatment assignment using the randomization tool in REDCap.

The study drug will be started 3 days prior to ablation. The dose of 2-HOBA will be 750mg three times per day (TID). At least 24 hours in advance, the study nurse will notify IDS to randomize the participant and prepare the study drug. MTI will provide 2-HOBA to the IDS as a 250 mg capsule. MTI will also provide matching

placebo capsules. The study nurse will arrange for the study drug to be given to the participant in-person or mailed. The total duration of the study drug is 31 days (3 days before ablation + 28 days after ablation).

6.0 STUDY PROCEDURES

Encounter 1: *Enrollment and Informed Consent*

- Performed by a member of the study team either in person in the Vanderbilt Arrhythmia Clinic or by phone with an electronic signature.
- In person meetings will occur in either: 1) the Vanderbilt Cardiac MRI Suite (if a pre-ablation cardiac MRI is scheduled), 2) the Vanderbilt Cardiac CT Suite (if a pre-ablation cardiac CT is scheduled), 3) the VHVI Research Room (5th Floor Medical Center East, South Tower), or at their cardiology or imaging appointments at the One Hundred Oaks location (if applicable).
- A supply of the study drug will be given to the participant along with dosing instructions either in-person or via the mail.

Encounter 2: *Day of ablation*

- The participant will arrive the morning of ablation as instructed by the clinical team
- Prior to ablation, the participant will be taken to the EP pre-op holding room. A dose of the study drug will be taken at this time (~30 minutes prior to the procedure).
- The study team will provide the participant with the smartwatch as well as an accompanying smart phone (if the patient does not own their own iPhone). The study team will initialize the smartwatch and phone with configurations to collect data, and instruct patients on how to record ECGs from the smartwatch.
- After the patient arrives in the operating room, they will undergo standard of care preparation for surgery including sterile drape and administration of general anesthesia using endotracheal intubation and mechanical ventilation. Central venous catheters are placed in the femoral veins as part of the procedure.
- A 30 mL blood sample will be collected from one of the central venous catheters prior to ablation. 10 mL of blood (divided between purple and blue top tubes) will be delivered to the Core Laboratory for Cardiovascular Translational and Clinical Research, and 20 mL will be put in green top tubes, placed on ice, and delivered to the research laboratory for culturing of monoctyes and dendritic cells for measurement of isolevuglandin-adduct levels.
- AF ablation will then be performed as per standard of care by the primary operator and clinical team.
- A post ablation blood draw will be collected at the conclusion of the AF ablation prior to the central venous catheter removal. We will collect an additional 30 mL blood sample for research, with 10 mL of blood delivered to the Core Laboratory for Cardiovascular Translational and Clinical Research and 20 mL put in green top tubes and delivered to the research laboratory for culturing of monocytes and dendritic cells for measurement of isolevuglandin-adduct levels
- Following ablation, the patient will be extubated while in the operating room and returned to the EP holding room awake and alert.
- After removal of central venous catheters and when the patient is ready to begin eating and drinking, another dose of the study drug will be given.

Encounter 3: *Post-ablation day #28*

- The participant will be met in either the: 1) VHVI Arrhythmia Outpatient Clinic at the time of their 1-month Post-op Clinical Visit (Medical Center East or One Hundred Oaks locations), or 2) the VHVI Research Room.
- A 10 mL blood sample (divided between purple and blue top tubes) will be delivered to the Core Laboratory for Cardiovascular Clinical and Translational Research.
- The study personnel will record any ECGs recorded from the smartwatch from the day of the ablation until the end of the 28-day of follow-up period
- The study personnel will record remaining pills of either placebo or 2-HOBA at the conclusion of the study
- Participants will be allowed to keep the smartwatch
- Should the participant have required an iPhone as well as the smartwatch, the phone will be returned to the study personnel

Encounter 4: *6 and/or 12 month follow up visits*

- Participants undergoing ablation are routinely seen at 6 and 12 months during follow up as part of clinical care
- During these visits, study team personnel may review medical records and meet with participants regarding clinical outcomes of their ablation procedure
- If 6 or 12 month follow up visits are not planned, study personnel may call patients during the 12 month time frame to see if any clinical events have occurred

7.0 RISKS

Patients undergoing AF ablation are at risk for complications by nature of the procedure. These risks are inherent to the patient population studied here. The risks of obtaining blood samples from phlebotomy of peripheral veins and existing venous lines are minimal.

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

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

Data and Safety Monitoring Plan

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

Data Safety Monitoring Board (DSMB) will be formed to review safety data, study progress, and data quality every 6 months or after enrollment of every 30 subjects, whichever occurs earlier. The DSMB will be chaired by Dr. Julia Wattacheril, MD, MPH Associate Professor of Medicine, Director, Nonalcoholic Fatty Liver Disease Program, Columbia University – NY Presbyterian Hospital; an external member will be Dr. Christine Albert, MD, MPH, Professor of Medicine, Brigham and Women's Hospital, Director of the Center for Arrhythmia Prevention, who has expertise in clinical trials of AF; and a biostatistical member, Gregory Dan Ayers, who has expertise in biostatistical review for DSMBs. The DSMB will review all serious adverse events (AEs). Any serious AE will be reported to the DSMB, IRB, and FDA as soon as possible, but not more than three days from the investigators' awareness of the event. Any untoward medical event will be classified as an AE, regardless of its causal relationship with the study.

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

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

Adverse events include:

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

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

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

1. results in death
2. is life-threatening
3. requires inpatient hospitalization or prolongation of existing hospitalization
4. results in persistent or significant disability/ incapacity (as per reporter’s opinion)
5. is a congenital anomaly/birth defect
6. is another medically important condition
7. The term "life-threatening" in the definition of "serious" refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

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

Definition of severity of adverse events:

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

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

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

Definition of adverse event causality:

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

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

Likely: The AE occurred during or after administration of the study treatment or a pre-existing event worsened within an appropriate period of time, and at least one of the following criteria is applicable:

- the event could not be explained by the clinical condition or history of the subject, environmental or toxic factors, or other diagnostic or therapeutic measure;
- was an expected ADR associated with study treatment or a class-labeled drug effect;
- AE subsided or disappeared after withdrawal or dose reduction of study treatment; or
- AE recurred after re-exposure to study treatment.

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

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

Adverse event reporting

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

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

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

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

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

9.0 STUDY WITHDRAWAL/DISCONTINUATION

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

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

i. The participant, for any reason, does not undergo AF ablation.

ii. The participant does not comply with the study protocol, such as failure to attend research encounters, or inability to wear the smartwatch.

iii. The participant experienced an adverse event related to the study drug/protocol necessitating withdrawal.

10.0 STATISTICAL CONSIDERATIONS

Dr. Fei Ye (Associate Professor of Biostatistics, Vanderbilt University Medical Center) formulated the plan and will direct the statistical analysis for this trial.
Analysis Sets: 1) Full analysis set (FAS)**:** All randomized participants. The FAS will be used in the analysis of all efficacy endpoints. Participants will be included in FAS according to the treatment to which they are randomized. 2) Safety analysis set (SAS)**:** All randomized participants who have received 2-HOBA. The SAS will be used in the analyses of all safety endpoints. Participants will be included in the analyses according to the treatment they actually received.

General Statistical Strategies: Patient demographics/other baseline characteristics will be listed by patient and/or summarized descriptively by treatment group and by stratification factors. Categorical data will be presented as frequencies and percentages. Binary and categorical data will be analyzed with Fisher's exact test and chi-squared test. For continuous data, summary statistics will be presented. For single time-point data, between-group differences will be assessed with t-test (2 groups) or analysis of variance (3 or more groups) based on a continuous variable. Nonparametric counterparts, Wilcoxon rank sum test and Kruskal-Wallis test, will be used when assumptions for parametric methods are not met. For correlated data (e.g., repeated measurements) will be analyzed with generalized estimating equation (GEE) or linear mixed models (LMM). Multiple comparison issues will be corrected using the Bonferroni's approach. All statistical analysis will be performed using R 3.4.2 or a newer version.

10.1. Aim 1 (Clinical Endpoint)

The primary hypothesis will test whether 2-HOBA reduces the rate of early recurrence of AF, atrial tachycardia, or atrial flutter following AF ablation within 28 days follow-up. For simplicity we will refer to this as "AF recurrence".
Measurement of Outcome (Aim 1): Participants enrolled in the study will wear a smartwatch linked to an iPhone to continually record heart rate, variability and detection of arrhythmias. For detection of the primary endpoint, participants will record a daily ECG each morning upon waking via the watch. In addition, participants will be notified by the smartwatch of 1) detection of atrial fibrillation or atrial flutter, 2) persistent high HR (> 110 bpm) outside of exercise. Upon prompting or upon patient self-report of symptoms, participants will utilize the ECG capture function of the watch and record the ECG for study personnel. A continuous tracing of 30 seconds (the full duration of a Watch EKG tracing) will be considered as having met the primary endpoint.

Sample size and power (Aim 1): The sample size of this study is based on a logistic regression of the binary primary endpoint of early AF recurrence by the end of follow-up. A total sample size of 162 with 81 in each arm will provide 81% power to detect a reduction in the rate of early recurrence of AF if the event rate is 29% in the treatment arm. This change corresponds to an odds ratio of 0.4 (treatment vs control), which is nearly equivalent to the odds ratio observed in similar trials using colchicine (colchicine OR 0.38)[26] at the significance level of 0.046 using a 1-sided Farrington and Manning likelihood score test, and a 48% rate of early recurrence in the placebo (control) group. The estimate 48% rate of early recurrence was extrapolated from our pilot data, which found a 45% rate of recurrence, but did not utilize continuous monitoring as will be performed in our trial. An interim analysis will be performed for superiority after half of the patients (n=40 per group) have completed their 28-day assessment of the primary endpoint at the significance level of 0.006. The final analysis will be performed after all patients have completed their 28-day assessment of the primary point at two-sided significance level of 0.041. Efficacy will be declared early at the interim analysis if the efficacy boundary is crossed ($p<0.007$). All on-study patients will be followed for efficacy and safety until the planned end-of-follow-up. If the interim analysis indicates that the treatment is not better than the control by 13% (non-binding futility boundary $p>0.4$), we may close the recruitment into study with regard to any recommendations of DSMB. The O'Brien-Fleming boundaries was used for the alpha- and beta-spending functions to preserve the overall Type I and Type II error.

Primary Analysis (Aim 1): All FAS will be included in the primary analysis of AF recurrence, the primary efficacy endpoint. Binary recurrence of AF will be compared between treatment and control using a chi-square test. The primary analysis will be performed using a multivariable logistic regression model, where the dependent/outcome variable is the early AF recurrence (yes/no) by the end of the 28-day follow-up and the primary independent/predictor variable is the treatment arm (2-HOBA or placebo). Adjustment will be made for age, gender, AF type (paroxysmal versus persistent), method for pulmonary vein isolation (radiofrequency versus cryoablation), additional ablation lesions (yes/no), BMI (continuous), and hours of monitoring (0-672 hours). To avoid over-fitting our multivariable logistic regression model, a 10:1 ratio for degrees of freedom per the less frequent outcome event will be used in our modeling. Nonlinear form of the continuous predictors (age, BMI, and hours of monitoring) will be considered when building the model with restricted cubic splines. Models will be evaluated for goodness-of-fit, and internally validated for the calibration and discrimination performance using the bootstrap method.
Secondary Analysis (Aim 1): A secondary analysis will analyze a surrogate of AF burden as the endpoint and designed to account for the impact of cardioversion on AF burden assessment. AF burden is the percentage of time in AF, atrial tachycardia, or atrial flutter over total time monitored. The secondary analysis will use the data

provided by the continuous HR monitor provided by the smartwatch. The analysis will be performed using a linear mixed models (LMM) in which the AF burden is assessed weekly during the 4-week (28-day) follow-up period. Same covariates mentioned in the primary analysis will be considered. Additionally, AF burden will also be analyzed in ordinal scale using generalized estimating equation (GEE), ranked based on severity according to increasing AF burden and whether or not a cardioversion was performed. For any missing data in the covariates, we will use a multiple imputation method that incorporates predictive mean matching and flexible additive imputation models as implemented in the aregImpute function in the *Hmisc* R package. The impact of missing data on the validity of results will be examined with a pattern-mixture approach using the R package *SensMice*. This will be done for Aims 1 and 2. Additional secondary analyses will include: 1) multivariable modeling of participants who were excluded from the primary analysis due to the use of colchicine or steroids during the follow-up period (will examine clinical and biochemical endpoints); 2) multivariable modeling with adjustment for 2-HOBA levels for the clinical and biochemical endpoints; 3) multivariable modeling with adjustment for duration of smartwatch use.

Interim Analyses (Aim 1)**:** An interim analysis will be performed by the study team and reviewed by the DSMB. When 50% of the target enrollment is reached testing for an association between 2-HOBA or placebo and significant adverse events (see Ethical Aspects of the Proposed Research for details), superiority and futility will be performed (see above: Sample Size and Power Aim 1). The interim analysis will include early stopping rules for superiority, futility, and harm.

- Interim Analysis for Harm: although none of our Phase I pre-clinical or clinical data have raised safety concerns, and no adverse events have been observed, we planned the assessment of safety which will be based mainly on the frequency of adverse events and on the number of laboratory values that fall outside of pre-determined ranges. Other safety data such as vital signs will be considered as appropriate. All safety data will be listed. Patients will be summarized by treatment and other covariates. All adverse events recorded during the study will be summarized. The incidence of treatment-emergent adverse events (new or worsening from baseline) will be summarized by body system, severity, type of adverse event, and relation to the investigational regimen. Severe adverse events and non-fatal serious adverse events are not expected.
- Interim Analysis for Efficacy and Futility: early stopping rules for efficacy and futility will be based on the primary endpoint in Aim 1, which is arrhythmia recurrence during the 28-day follow-up period. There will be no early stopping rules for the primary endpoints in Aim 2 (biochemical endpoint) or Aim 3 (pharmacogenomic analysis). Both of those aims will be considered exploratory.

10.2. Aim 2 (Biochemical Endpoint)

Primary hypothesis: The primary endpoint for Aim 2 will be the IsoLG-adduct content of dendritic cells expressed as a percentage (IsoLG-adducted dendritic cells/total dendritic cells x 100). We will test the hypothesis that 2-HOBA reduces the change in IsoLG-adduct levels that occurs with AF ablation (as measured on post-procedure day #1 minus pre-ablation blood samples).

Sample size and power (Aim 2)**:** Our pilot data shows that the change in dendritic cell IsoLG-adduct level is 23% following ablation in our placebo (control) group and we expect the range of possible observed reduction to be 20-40%. With n=81 participants in each arm, the study provides at least 80% power to detect a minimal 22% reduction in IsoLG-adduct level at two-sided significance level of 5%.

Primary Analysis (Aim 2): Our primary analysis will use multivariable linear regression to test whether our primary determinant (2-HOBA versus placebo), significantly reduces the change in IsoLG-adduct levels (continuous), adjusted for baseline pre-ablation IsoLG-adduct level and other potential confounding factors: age, sex, method for pulmonary vein isolation (radiofrequency versus cryo), additional ablation lesions (yes/no), and BMI (continuous). The IsoLG content of dendritic cells adjusted for baseline level will be presented for both treatment arms, together with corresponding 95% confidence intervals (CIs) and p-values will be graphically interpreted using boxplots, partial effect plots, residual plots, etc.

Secondary Analysis (Aim 2): A secondary endpoint will be the IsoLG-adduct level in monocytes. Due to the prohibitively labor-intensive process required to measure IsoLG content in dendritic cells and monocytes, only the pre-ablation and post-procedure day#1 samples will be analyzed for the analysis. However, blood samples from 2 additional time points (baseline and post-procedure day #28) will be available for pharmacokinetic

analysis and secondary analysis of other serological markers. These will include malondialdehyde and other biomarkers of oxidative stress.

10.3. Aim 3 (Exploratory Genomics): This aim will use the 162 subjects enrolled in Aims 1 and 2. DNA will be extracted and genotyping will be performed on a GWAS chip that includes the 3 SNPs located at chromosome 4q25 that are independently associated with AF risk (rs2200733, rs17570669, and rs3853445).[34] The 4q25 GRS will be a weighted score calculated by using the OR for AF multiplied by the number of risk alleles present from each of the 3 independent 4q25 SNPs. We hypothesize that a statistically significant interaction will exist between the 4q25 GRS and the treatment group (2-HOBA or placebo) for an association with our clinical and biochemical endpoints. In Aim 3, we will test for an interaction between the 4q25 GRS and the treatment group for an association with the primary clinical endpoint (early recurrence of AF). Adjustment will be made for the covariates used in Aim 1. We will then add the 4q25 GRS to the multivariable linear regression model used in Aim 2 and test whether there is a statistical interaction between the GRS and the treatment group for an association with the primary biochemical endpoint (change in dendritic cell IsoLG-adduct level with ablation) by comparing models with and without the interaction term using likelihood ratio test. The added predictive value of 4q25 GRS and the interaction of GRS and treatment will be evaluated by comparing nested models with and without the predictor with regard to c statistics and the integrated discrimination improvement. Models involving 4q25 GRS will be internally validated using the aforementioned bootstrap method for the evaluation and correction of potential optimism in the performance of the model by balancing the bias and variance in the prediction error. All statistical tests will be considered significant at two-sided 5% level.

11.0 PRIVACY/CONFIDENTIALITY ISSUES

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

12.0 FOLLOW-UP AND RECORD RETENTION

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

13.0 REFERENCES

1. January CT, Wann LS, Alpert JS, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the Heart Rhythm Society. Circulation 2014;130:2071-104.
2. Calkins H, Kuck KH, Cappato R, et al. 2012 HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design: a report of the Heart Rhythm Society (HRS) Task Force on Catheter and Surgical Ablation of Atrial Fibrillation. Developed in partnership with the European Heart Rhythm Association (EHRA), a registered branch of the European Society of Cardiology (ESC) and the European Cardiac Arrhythmia Society (ECAS); and in collaboration with the American College of Cardiology (ACC), American Heart Association (AHA), the Asia Pacific Heart Rhythm Society (APHRS), and the Society of Thoracic Surgeons (STS). Endorsed by the governing bodies of the American College of Cardiology

Foundation, the American Heart Association, the European Cardiac Arrhythmia Society, the European Heart Rhythm Association, the Society of Thoracic Surgeons, the Asia Pacific Heart Rhythm Society, and the Heart Rhythm Society. Heart rhythm : the official journal of the Heart Rhythm Society 2012;9:632-96 e21.
3. Wang TJ, Parise H, Levy D, et al. Obesity and the risk of new-onset atrial fibrillation. Jama 2004;292:2471-7.
4. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. Jama 1994;271:840-4.
5. Darbar D, Roden DM. Genetic mechanisms of atrial fibrillation: impact on response to treatment. Nat Rev Cardiol 2013;10:317-29.
6. Van Wagoner DR, Piccini JP, Albert CM, et al. Progress toward the prevention and treatment of atrial fibrillation: A summary of the Heart Rhythm Society Research Forum on the Treatment and Prevention of Atrial Fibrillation, Washington, DC, December 9-10, 2013. Heart rhythm : the official journal of the Heart Rhythm Society 2015;12:e5-e29.
7. Sakabe M, Shiroshita-Takeshita A, Maguy A, et al. Omega-3 polyunsaturated fatty acids prevent atrial fibrillation associated with heart failure but not atrial tachycardia remodeling. Circulation 2007;116:2101-9.
8. Jahangiri A, Leifert WR, Patten GS, McMurchie EJ. Termination of asynchronous contractile activity in rat atrial myocytes by n-3 polyunsaturated fatty acids. Mol Cell Biochem 2000;206:33-41.
9. Carnes CA, Chung MK, Nakayama T, et al. Ascorbate attenuates atrial pacing-induced peroxynitrite formation and electrical remodeling and decreases the incidence of postoperative atrial fibrillation. Circulation research 2001;89:E32-8.
10. Roberts LJ, 2nd, Oates JA, Linton MF, et al. The relationship between dose of vitamin E and suppression of oxidative stress in humans. Free Radic Biol Med 2007;43:1388-93.
11. Darghosian L, Free M, Li J, et al. Effect of omega-three polyunsaturated fatty acids on inflammation, oxidative stress, and recurrence of atrial fibrillation. The American journal of cardiology 2015;115:196-201.
12. Gutierrez A, Van Wagoner DR. Oxidant and Inflammatory Mechanisms and Targeted Therapy in Atrial Fibrillation: An Update. Journal of cardiovascular pharmacology 2015;66:523-9.
13. Lennerz C, Barman M, Tantawy M, Sopher M, Whittaker P. Colchicine for primary prevention of atrial fibrillation after open-heart surgery: Systematic review and meta-analysis. Int J Cardiol 2017;249:127-37.
14. Roberts LJ, 2nd, Salomon RG, Morrow JD, Brame CJ. New developments in the isoprostane pathway: identification of novel highly reactive gamma-ketoaldehydes (isolevuglandins) and characterization of their protein adducts. FASEB J 1999;13:1157-68.
15. Brame CJ, Salomon RG, Morrow JD, Roberts LJ, 2nd. Identification of extremely reactive gamma-ketoaldehydes (isolevuglandins) as products of the isoprostane pathway and characterization of their lysyl protein adducts. J Biol Chem 1999;274:13139-46.
16. Zagol-Ikapitte IA, Matafonova E, Amarnath V, et al. Determination of the Pharmacokinetics and Oral Bioavailability of Salicylamine, a Potent gamma-Ketoaldehyde Scavenger, by LC/MS/MS. Pharmaceutics 2010;2:18-29.
17. Marin F, Pascual DA, Roldan V, et al. Statins and postoperative risk of atrial fibrillation following coronary artery bypass grafting. The American journal of cardiology 2006;97:55-60.
18. Calo L, Bianconi L, Colivicchi F, et al. N-3 Fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial. Journal of the American College of Cardiology 2005;45:1723-8.
19. Cheruku KK, Ghani A, Ahmad F, et al. Efficacy of nonsteroidal anti-inflammatory medications for prevention of atrial fibrillation following coronary artery bypass graft surgery. Prev Cardiol 2004;7:13-8.
20. Halonen J, Halonen P, Jarvinen O, et al. Corticosteroids for the prevention of atrial fibrillation after cardiac surgery: a randomized controlled trial. Jama 2007;297:1562-7.
21. Andrade JG, Khairy P, Macle L, et al. Incidence and significance of early recurrences of atrial fibrillation after cryoballoon ablation: insights from the multicenter Sustained Treatment of Paroxysmal Atrial Fibrillation (STOP AF) Trial. Circulation Arrhythmia and electrophysiology 2014;7:69-75.
22. Deftereos S, Giannopoulos G, Kossyvakis C, et al. Colchicine for prevention of early atrial fibrillation recurrence after pulmonary vein isolation: a randomized controlled study. Journal of the American College of Cardiology 2012;60:1790-6.

23. Kim YR, Nam GB, Han S, et al. Effect of Short-Term Steroid Therapy on Early Recurrence During the Blanking Period After Catheter Ablation of Atrial Fibrillation. Circulation Arrhythmia and electrophysiology 2015;8:1366-72.
24. Kim DR, Won H, Uhm JS, et al. Comparison of two different doses of single bolus steroid injection to prevent atrial fibrillation recurrence after radiofrequency catheter ablation. Yonsei Med J 2015;56:324-31.
25. Koyama T, Tada H, Sekiguchi Y, et al. Prevention of atrial fibrillation recurrence with corticosteroids after radiofrequency catheter ablation: a randomized controlled trial. Journal of the American College of Cardiology 2010;56:1463-72.
26. Deftereos S, Giannopoulos G, Efremidis M, et al. Colchicine for prevention of atrial fibrillation recurrence after pulmonary vein isolation: mid-term efficacy and effect on quality of life. Heart rhythm : the official journal of the Heart Rhythm Society 2014;11:620-8.
27. Shoemaker MB, Bollmann A, Lubitz SA, et al. Common genetic variants and response to atrial fibrillation ablation. Circulation Arrhythmia and electrophysiology 2015;8:296-302.
28. Tao G, Kahr PC, Morikawa Y, et al. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. Nature 2016;534:119-23.
29. Davies SS, Bodine C, Matafonova E, et al. Treatment with a gamma-ketoaldehyde scavenger prevents working memory deficits in hApoE4 mice. J Alzheimers Dis 2011;27:49-59.
30. Kirabo A, Fontana V, de Faria AP, et al. DC isoketal-modified proteins activate T cells and promote hypertension. J Clin Invest 2014;124:4642-56.
31. Prinsen JK ST, Yermaliskaya LV, Norlander AE, Kirabo, Madhur MS, Barnett JV, Boutaud O, Kannankeril PJ, Harrison DG, Murray KT. Reactive gamma-ketoaldehydes promote protein misfolding and atrial arrhythmia susceptibility in experimental hypertension. Heart rhythm : the official journal of the Heart Rhythm Society 2016;13.
32. Magnussen C, Niiranen TJ, Ojeda FM, et al. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarCaRE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). Circulation 2017;136:1588-97.
33. Fuller JC, Jr., Pitchford LM, Morrison RD, et al. In vitro safety pharmacology evaluation of 2-hydroxybenzylamine acetate. Food Chem Toxicol 2018;121:541-8.
34. Lubitz SA, Sinner MF, Lunetta KL, et al. Independent susceptibility markers for atrial fibrillation on chromosome 4q25. Circulation 2010;122:976-84.