A PHASE II RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLINDED STUDY EVALUATING THE EFFECTS OF ATACIGUAT (HMR1766) ON AORTIC VALVE CALCIFICATION IN PATIENTS WITH MODERATE CALCIFIC AORTIC VALVE STENOSIS

NCT02481258

1/8/2014

A PHASE II RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLINDED STUDY EVALUATING THE EFFECTS OF ATACIGUAT (HMR1766) ON AORTIC VALVE CALCIFICATION IN PATIENTS WITH MODERATE CALCIFIC AORTIC VALVE STENOSIS

Regulatory Sponsor:	Jordan D. Miller, PhD Department of Surgery Mayo Clinic Rochester, Minnesota
Funding Sponsor: (Optional)	National Center for Accelerating Translational Sciences National Institutes of Health 301-435-0824 (Dr. Christine Colvis, Program Officer)
Study Product:	Ataciguat
Protocol Number: (IRBe)	IRBe # 14-006469
IND Number:	119,829

Initial version:1/8/2014 Version **5**

Table of Contents

List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CAVS	Calcific Aortic Valve Stenosis
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	A Phase II randomized, placebo-controlled, double-blinded study evaluating the safety and efficacy of ataciguat (HMR1766) in patients with moderate calcific aortic valve stenosis
Running Title	Long term efficacy of Ataciguat in patients with moderate CAVS
Protocol Number	14-006469
Phase	Phase II
Methodology	Double Blinded, randomized, placebo-controlled study
Overall Study Duration	12 months
Subject Participation Duration	12 months (Screen Visit + 2 Follow Up Visits)
Single or Multi-Site	Single site
Objectives	The primary objective of the current study is to determine whether Ataciguat (HMR1766) slows progression of valve calcification in patients with moderate calcific aortic valve stenosis. A secondary objective is to determine whether Ataciguat slows progression of left ventricular dysfunction in patients with moderate calcific aortic valve stenosis.
Number of Subjects	100
Diagnosis and Main Inclusion Criteria	Mild to moderate calcific aortic valve stenosis. Aortic valve area greater than 1.0 cm^2 but less than 2.0 cm^2 by echocardiographic evaluation and valuvlar calcium levels >150 AU by chest CT scanning.

Study Product, Dose, Route, Regimen	• Ataciguat/HMR1766 and matching placebo
	• Oral administration of 200 mg gelatin capsules packaged in alu/alu blister packs
	• Dosing
	  • Anticipated dose of 200 mg ataciguat or matching placebo (= 4 capsules of 50 mg or matching placebo) given once daily in the morning, with food. This dosing will be continued for the duration of the study period.
	  • If the drug is not tolerated, the patient's involvement in the study will be terminated (i.e., there is no de-escalation option)

Duration of Administration	12 months
Reference therapy	Placebo capsules will be provided as a reference.
Statistical Methodology	A repeated measures ANOVA with Bonferonni-corrected T-tests will be used to detect the impact of Ataciguat/HMR1766 on aortic valve calcification (assessed using CT scanning), aortic valve function (assessed using echocardiography), or left ventricular function (using echocardiography).

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Hemodynamically significant calcific aortic valve stenosis (CAVS) affects 3% of the population over age 65, and patients with even moderate aortic valve stenosis (peak velocity of 3-4 m/sec) have a 5 year event-free survival of less than 40%. Presently, there are no effective treatments to slow progression of aortic valve calcification, and aortic valve replacement is the only available treatment for advanced CAVS. Thus, major aims of our research program include: 1) the use of integrative approaches to identify mechanisms contributing to initiation and progression of CAVS, and 2) the use of integrative approaches to identify therapeutic interventions that slow progression of CAVS without negatively impacting other organ systems/tissues in vivo (e.g., skeletal ossification). In the present UH2/UH3 application, we propose that activators of oxidized soluble guanylate cyclase (sGC) are a novel therapeutic intervention that can slow progression of CAVS. During the UH3 phase of the grant, we aim to provide key proof-of-concept data that Ataciguat/HMR1766: 1) is well tolerated by patients with mild to moderate CAVS, 2) slows progression of CAVS in a robust mouse model of valvular calcification and stenosis, 3) reduces osteogenic signaling in human aortic valve interstitial cells in vitro, and 4) effectively activates oxidized sGC and attenuates osteogenic signaling in valves from patients with severe CAVS. Upon meeting appropriate milestones during the UH2 phase of the grant, we will rapidly move towards the UH3 phase of the grant, where we will examine the effects of chronic ataciguat treatment on accumulation of aortic valve calcium, progression of aortic valve and ventricular dysfunction, and inflammatory cytokine levels in patients with mild to moderate CAVS. Collectively, we believe the proposed studies have a high likelihood of not only providing new insight into fundamental mechanisms regulating gene expression in CAVS, but are also likely to identify Ataciguat as a novel therapeutic agent to slow progression of CAVS in humans.

Hemodynamically significant aortic valve stenosis affects 3% of the population over age 65, and patients with even moderate aortic valve stenosis (peak velocity of 3-4 m/sec) have a 5 year event-free survival of less than 40%. Presently, there are no effective treatments to slow progression of aortic valve calcification, and aortic valve replacement is the only available treatment for advanced aortic valve stenosis. This proposal tests the novel hypothesis that activation of oxidized soluble guanylate cyclase with Ataciguat/HMR1766 will slow progression of valve calcification and dysfunction. Our UH2/UH3 application is uniquely designed to not only demonstrate efficacy of treatment in humans, but also to lend key insights into the biological processes underlying changes in aortic valve function using robust animal models, in vitro cell assays, and in vivo measurements of sGC activation and suppression of osteogenic signaling in human valves. Collectively, we believe Ataciguat/HMR1766 provides an innovative therapeutic approach to reduce morbidity and mortality associated with aortic valve calcification in humans.

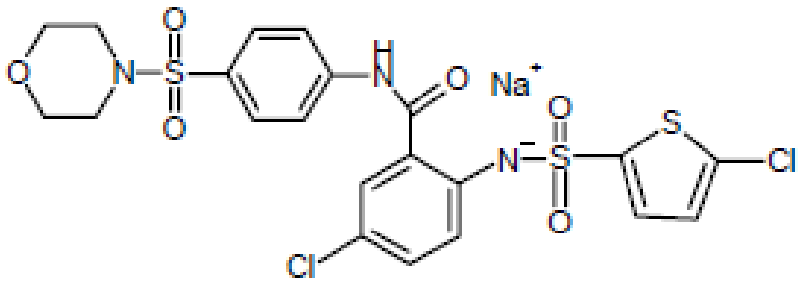
Brief overview and Clinical Importance: Hemodynamically significant aortic valve stenosis (CAVS) is a tremendously important clinical problem affecting ~3% of the population over age 65[9], and patients with even moderate aortic valve stenosis have a 5 year event-free survival of less than 40%[10-13]. More than 25% of patients over the age of 65 have aortic valve calcification and sclerosis, which substantially increases risk for adverse cardiovascular events and all-cause mortality[7, 8]. Presently, no medical treatments have been shown to slow progression of aortic valve calcification or progression of stenosis in humans. Recent studies in pre-clinical models of disease, however, suggest that increasing nitric oxide signaling may be a useful intervention to attenuate osteogenic signaling and slow progression of CAVS[1, 2]. While attractive, harnessing NO signaling *in* vivo has proven to be logistically difficult due to elevated levels of oxidative stress and the depletion of NO synthase cofactors in end-stage CAVS[3, 4]. Furthermore, reductions in antioxidant capacity in CAVS strongly favor oxidation of soluble guanylate cyclase, thus rendering it insensitive to NO[5, 6]. To circumvent these problems, ***we propose that Ataciguat (HMR1766) will slow progression of calcific aortic valve disease,*** through preferential activation of oxidized soluble guanylate cyclase (sGC) and subsequent conveying of protective effects associated with NO signaling. More specifically, we propose that activation of oxidized sGC in CAVS will attenuate pro-osteogenic signaling, reduce pro-fibrotic signaling, and ultimately slow progression of aortic valve dysfunction in pre-clinical animal models of mild to moderate valve calcification, and ultimately, in humans with mild to moderate valve calcification and stenosis.

1.2 Investigational Agent

The investigational agent for this study is HMR1766 (ataciguat sodium) manufactured and supplied by Sanofi-Aventis Research and Development.

Drug Substance: Ataciguat

Drug substance used:		Ataciguat sodium
Chemical name according to IUPAC:		5-Chloro-2-(5-chloro-thiophene-2-sulfonylamino)-N-[4-(morpholine-4-sulfonyl)-phenyl]-benzamide, sodium salt
Laboratory code number:		HMR1766 S982766 Sodium salt of HMR1514
CAS registry number:		254976-06-2

Structural formula:		
Relative molecular mass:		576.50 (active moiety), 598.49 (sodium salt)
Structural similarities to other known compounds:		HMR1766 (ataciguat sodium) shows no structural similarities to other compounds approved as active pharmaceutical ingredient
Appearance:		White to pastel yellow powder
Solubility at 37°C:		Water (resulting pH: 11.1):Very slightly soluble

HMR1766 is a novel, nitric oxide (NO) independent activator of soluble guanylate cyclase (sGC) that preferentially activates the oxidized form of sGC, which is insensitive to NO. The clinical development of HMR1766 was initially proposed for the symptomatic treatment of peripheral arterial disease (PAD), Fontaine stage II (intermittent claudication). Currently, HMR1766 is being tested in the indication of neuropathic pain.

1.3 Pre-Clinical Data

Non-clinical pharmacology: Neuropathic pain is caused by tissue damage, especially direct injury of the nervous system and neuronal inflammation. It is characterized by increased sensitivity to painful stimuli (hyperalgesia), perception of innocuous stimuli as painful (allodynia) and spontaneous pain. Inflammatory as well as immune mechanisms play an important role. Schwann cells, satellite cells in the dorsal root ganglia, components of the peripheral immune system, spinal microglia and astrocytes are involved. HMR1766 – through activation of sGC and, consequently, elevation of cGMP levels – is expected to be effective in the treatment of neuropathic pain through blockade of glial cell activation and an increase of the nociceptive threshold through opening of K^+-ATP channels.

HMR1766 significantly and dose-dependently reduced tactile and cold allodynia in the spared nerve injury (SNI) induced mouse model of neuropathic pain. Furthermore HMR1766 significantly and dose-dependently reduced thermal hyperalgesia in the Carrageenan-induced mouse model of inflammatory triggered pain.

Effects on vasodilatation and endothelial function have been investigated in cardiovascular animal models. In a unilateral hind limb ischemia model in diabetic rats, ataciguat reduced muscle fatigue and increased perfusion pressure in the ischemic leg. In ApoE knockout mice on a high fat diet, HMR1766 inhibited the development of endothelial dysfunction and markedly reduced the development of atherosclerotic plaques. It induced concentration-dependent vasorelaxation in preconstricted aortic rings without tolerance development or induction of cross-tolerance to nitrates. In various animal models, HMR1766 positively effected on markers of coronary function, coronary thrombosis, and myocardial function without alteration of hemodynamic parameters.

HMR1766 blocked human ether-à-go-go related gene (hERG) currents at a concentration inhibiting 50% of an assay response (IC50) of 37 μM, which is ~100-fold higher than the concentration giving 50% of the maximum effect (EC50) for the activation of sGC. In Purkinje fibers, HMR1766 induced a concentration-dependent decrease of action potential duration at 3 and 10 μM, slightly decreased action potential amplitude and maximum rate of rise of the action potential (Vmax) at 10 μM, and caused pronounced modifications of all parameters associated with depolarization of the cellular membrane at 30 μM. Decreased blood pressure (BP), tachycardia with related electrocardiographic (ECG) changes (decreased PR-, PQ-, and QT-intervals), but no effects on QTc were observed after oral administration of single doses up to 400 mg/kg in dogs. Likewise, intravenous (IV) doses up to 30 mg/kg decreased BP and increased heart rate (HR), but had no significant effect on QTc in dogs. HMR1766 had no effect on general behavior, body temperature or locomotor behavior, respiratory parameters, blood glucose levels, and salidiuresis in rats.

Long-term treatment of low density lipoprotein receptor-deficient, apolipoprotein B100-only mice with HMR1766 significantly slowed progression of aortic valve dysfunction in female mice fed a Western diet for 9 months. Long-term treatment of male mice of the same strain (low density lipoprotein receptor-deficient, apolipoprotein B100-only) with HMR1766 did not slow progression of aortic valve dysfunction. Long-term treatment with HMR1766 did not significantly alter blood pressure in low density lipoprotein receptor-deficient, apolipoprotein B100-only mice of either sex, and did not significantly alter endothelium-dependent or endothelium-independent relaxation of conduit vessels in low density lipoprotein receptor-deficient, apolipoprotein B100-only mice of either sex.

Pharmacokinetics and product metabolism in animals: HMR1766 was rapidly absorbed following oral administration, with time to maximum concentration (t_{max}) at ~0.5 hour in rats and ~2.7 hours in dogs. Oral bioavailability was almost complete in both species. Following IV administration, HMR1766 was rapidly eliminated with a half-life of <0.5 hour in rat and ~2 hours in dog. After oral administration of [^{14}C] HMR1766, the compound and/or its metabolites showed a high plasma protein binding, were ubiquitously distributed, had a low central nervous system penetration, showed no significant retention in any tissues, and did not bind to formed blood elements. After oral administration to female rats or rabbits (dams), very low radioactivity levels were measurable in the fetuses. In a rat study, 74% of the plasma total radioactivity at t_{max} was accounted for by parent compound. Independent of the route of administration, HMR1766 was excreted almost completely via the feces in rats and dogs.

When administered orally, radiolabeled HMR1766 was primarily excreted as parent compound in rats, and primarily as metabolites in dogs.

Toxicology: Acute toxicity with HMR1766 revealed median lethal dose (LD_{50}) values of ~100 to 200 mg/kg after IV administration (rats and mice), ~250 (rats) and ~500 (mice) mg/kg after subcutaneous (SC) administration, and >5000 mg/kg body weight (rats and mice) after oral administration. In the 6-month oral toxicity study in rats, slight signs of anemia and slightly increased liver weights were observed at 500 mg/kg/day. In the 6 month oral toxicity study in dogs, increased bilirubin, alkaline phosphatase values, and myocardial steatosis were observed. The peripheral muscles also showed markedly increased lipid storage at 530 mg/kg/day. The no observed adverse effect level (NOAEL) was 100 mg/kg in 6-month studies with rats and dogs. No compound-related effects were observed in dogs after oral administration up to 200 mg/kg/day over a period of 12 months. Developmental toxicity was observed in rats at a very high dose, at which distinct maternal toxicity occurred (1500 mg/kg/day). In rabbits, an increased incidence of variations, which are known to occur spontaneously, was seen at the highest dose evaluated (1000 mg/kg/day), a dose which was maternally toxic. HMR1766 did not affect mating behavior and fertility or early embryonic development in rats. HMR1766 was not mutagenic in the Ames test or the unscheduled DNA synthesis (UDS) test ex vivo. Although HMR1766 was positive in a mammalian chromosome aberration test in vitro and in human lymphocytes in vitro at highly cytotoxic concentrations, it was negative in vivo in the micronucleus test in mouse bone marrow, the chromosome aberration test in rat bone marrow and the comet assay in rat livers.

Human exposure under steady state conditions in repeated administration of 200 mg/day reaches values that are still below those at the NOAEL in the 12-month dog study and at a dose level in the 6-month rat study, at which only minimal effects were observed.

1.4 Clinical Data to Date

Ataciguat is being developed as a novel, NO-independent activator of sGC that preferentially activates the oxidized form of sGC, which is insensitive to NO. Currently, ataciguat is being tested in the indication of neuropathic pain.

Sixteen clinical studies in healthy subjects have been clinically completed and 298 healthy subjects (245 men, 53 women) have been dosed with ataciguat. Four clinical studies in patients have been completed: a study in 23 patients with CAD, a study in 343 patients with stable angina, a study in 553 patients with PAD, and a study with 14 patients with mild-to-moderate calcific aortic valve stenosis. To date, oral dosages of 25 mg to 200 mg ataciguat have been given as single doses and oral dosages of 5 mg to 200 mg as repeated doses, the highest dosage regimen being 200 mg once daily for 26 weeks.

Ataciguat is to be administrated orally, once per day, with breakfast. (Under fasting conditions, the absorption was quicker [shorter tmax] and the exposure was greater [larger AUC]. The elimination half-life [t½,z], however, was unchanged [~22 hours].)

Effects in humans
Clinical studies conducted in healthy subjects and patients with acute or chronic CAD or mild-to-moderate calcific aortic valve stenosis have not identified any safety findings of clinical relevance for the ongoing clinical development program.

In addition, a good safety profile, comparable with placebo was found for all ataciguat dose groups (daily dose 25 mg, 100 mg, and 200 mg per day) in the 6-month, double-blind, placebo controlled Study DFI6174 (ACCELA) in patients with PAD (Fontaine II).

In study DFI10569 (SERENEATI) in patients with neuropathic pain, overall, ataciguat 200 mg was well tolerated. Study treatment was permanently discontinued due to a TEAE in one ataciguat patient only (transient hyperbilirubinemia likely related to Gilbert's syndrome). There were no SAEs or deaths reported in this study.

Possible risks: Ataciguat must not be given to pregnant and breast-feeding women, because no data are available in these subjects. Developmental toxicity was observed in rats at a very high dose, at which distinct maternal toxicity occurred. In rabbits, an increased incidence of variations, which are known to occur spontaneously, was seen at a high dose. A double-barrier method of contraception is required if ataciguat is administered to women of a child-bearing potential. It is not known whether ataciguat is excreted in human milk.

Drug interactions:
Based on current knowledge of the mechanism of action, ataciguat might potentiate the effect of vasodilators on BP.

Ataciguat is a moderate CYP2C9 inhibitor and might potentiate the effect of substrates of this isoenzyme, as confirmed in studies with tolbutamide and warfarin. The increase in concentration should be considered when administering ataciguat with drugs that have narrow therapeutic indices and which are metabolized by this pathway.

Ataciguat is a weak CYP3A inhibitor. The inhibitory potential was confirmed in a clinical study by a slightly increased exposure to midazolam given as a comedication. Levonorgestrel and ethinylestradiol exposures were also increased when coadministered with ataciguat.
These increased exposures should be considered when selecting doses of oral contraceptives.

Clearance of ataciguat is mediated in part by CYP3A, but it is not a sensitive CYP3A substrate. Strong inhibitors of CYP3A4 may increase the plasma concentration of ataciguat, which was shown upon comedication with the inhibitor ketoconazole. However, exposure to ataciguat was increased by only 1.4-fold.

In an ASA interaction study, platelet aggregation results were comparable between ataciguat + ASA and ASA alone. The findings of this study did not suggest that concomitant administration of ataciguat and ASA would pose a risk for the patient.

In the nitrate interaction study PDY6744, administration of ataciguat did not have any additional effect on hemodynamic responses to IV infusion of glyceroltrinitrate.

1.5 Dose Rationale and Risk/Benefits

Following close examination of the Investigator Brochure and extensive discussions with our Industry partner (Sanofi), it is evident that short-term or long-term administration of Ataciguat has not elicited vasodilation/hypotension in normal subjects or in patients with cardiovascular disease. This was confirmed by our initial safety study in patients with mild-to-moderate calcific aortic valve stenosis, in which treatment with HMR1766 (200 mg/day) for 14 consecutive days did not elicit baseline hypotension or exacerbate hypotension induced by orthostatic stressors (e.g., transition from seated to standing).

Ataciguat will be administered orally in the form of gelatin capsules containing 50 mg of drug. The capsules will be individually packaged in aluminum blister packets, and subjects will be instructed to take four capsules (corresponding to their appropriate dose of 200 mg). As noted in section 1.2, pharmacokinetics and bioavailability data suggest excellent absorption and near complete bioavailability following oral ingestion of ataciguat. Circulating levels of ataciguat reach steady state following 14 days of treatment. Thus, oral ingestion of the compound will be used for daily dosing with ataciguat to maintain steady state levels for the duration of the study.

Risk/Benefit ratio: The aim of this study is to determine whether Ataciguat effectively slows the progression of valve calcification and stenosis in patients with mild-to-moderate aortic valve stenosis. This study is the second step in determining whether Ataciguat will be a useful treatment to slow progression of aortic valve stenosis in humans. Participation in this study may directly benefit patients in the short term if they receive active compound and it is shown to protect against progression of aortic valve calcification and dysfunction.

The risks associated with participation in this study are very low given our previous experiences with this drug in this patient population in our recent Phase I clinical trial. Furthermore, our combination of study design (e.g., rigorous screening and close monitoring), stringent eligibility criteria, and safety profile/historical safety record of this drug provided by Sanofi makes the risk associated with participation in these studies very low.

Risk mitigation

1) Our Clinical Research and Trials Unit (CRTU)is staffed by registered nurses who have extensive experience monitoring patients with a variety of medical conditions, including end-stage cancer and severe cardiovascular disease. In order to mitigate risk associated with the current study, we plan to implement the following:
    a) Careful selection of patients by physicians who have extensive experience working with this patient population. Dr. Sarano will screen and make final selection decisions on all patients with calcific aortic valve stenosis who will participate in these studies. Thus, by careful clinical evaluation, we will be able to identify patients who are hemodynamically stable and at exceedingly low risk for participation in this study.

b) Each patient will have a physical examination prior to admission to the CRTU (and subsequent testing/drug administration). This will ensure that patients have not had significant alterations in their physiological/functional status since the time of their pre-surgical consultation.
c) We will conduct frequent monitoring of blood pressure and EKG following drug administration. A registered nurse will document blood pressures and electrocardiographic changes every 30 minutes for the first 2 hours following administration of the drug (while plasma concentrations are rapidly increasing). In brief, each patient's blood pressure will be measured using an automated arm cuff device (to detect sustained reductions in blood pressure) and beat-by-beat using a finger cuff device (to detect rapid reductions in pressure). It is important to note that the frequency and intensity of monitoring in the CRTU would exceed that which a patient would typically receive if he/she was admitted to the hospital the night before surgery.
d) We will ensure that a staff physician involved with this study is available for consultation for the full duration of each visit. In the unlikely event of a severe adverse reaction to the drug, patients will be stabilized by the RMH Rapid Response Team (which, under Joint Commission guidelines, will respond within 2-4 minutes) and appropriately trained CRTU nursing staff, and transferred by the Gold Cross Ambulance to SMH for admission to the hospital through the Emergency Department, if necessary.
e) As the drug used in the study has not undergone thorough testing for teratogenicity, all pre-menopausal women will need to complete point-of-care pregnancy testing prior to receiving the compound.

2 Study Objectives

Primary Objective: To determine whether Ataciguat increases systemic sGC signaling, and halts accumulation of aortic valve calcium in patients with mild to moderate CAVS.

Rationale: Progression of aortic valve calcification is the primary mechanism underlying progressive reductions in aortic valve area in aging humans. Recent work from our group and others support a model in which valvular calcium may accumulate via osteogenic mechanisms similar to those observed in bone, suggesting that progression of valve disease may be a modifiable process. Preliminary data for the UH2 phase of this application demonstrated that activation of soluble guanylate cyclase with HMR1766 abrogates BMP2-induced transcriptional responses, and that HMR1766 can slow accumulation of valvular calcium in an animal model of CAVS. The effect of sGC activation in humans with moderate CAVS, however, remains unknown.

Goal: Determine whether long-term treatment with HMR1766 will result in sustained increases in systemic sGC signaling and halt accumulation of aortic valve calcium in patients with mild to moderate CAVS. This will be done using computed tomography (CT) scanning

to evaluate aortic valve calcium levels, which is considered to be a "gold standard" for evaluating valvular calcium burden.

Quantitative criteria for success:

Key outcomes:

1) Quantiative outcomes:
    a. Changes in aortic valve calcium levels measured by computed tomography scanning every 6 months of treatment during the trial (minimum of 12 month treatment duration).
        i. Key comparision will be differences in the rate of change of calcium between patients receiving HMR1766 or placebo capsules

Secondary Objective 1: To determine whether Ataciguat reduces levels of circulating inflammatory cytokines in patients with mild to moderate CAVS.

Rationale. Pro-inflammatory cytokines are significantly increased in patients with moderate or severe CAVS. In experimental animals, interventions which reduce NO-sGC-cGMP signaling are known to significantly increase systemic inflammation in models of atherosclerosis and heart failure, and genetic or pharmacological interventions that increase NO-sGC-cGMP signaling in such models reduces local and systemic pro-inflammatory cytokine levels. The effects of activating sGC on circulating inflammatory cytokines in CAVS are unknown.

Goal: Determine whether long-term treatment with HMR1766 will result in sustained increases in systemic sGC signaling and reduce levels of circulating inflammatory cytokines in patients with mild to moderate CAVS. This will be done using ELISA-based measurements of interleukin-6 and tumor necrosis factor α in venous blood samples.

Quantitative criteria for success:

Key outcomes:

1) Quantiative outcomes:
    a. Change in levels of plasma interleukin-6 and plasma tumor necrosis factor α following 6 and 12months of treatment.
        i. Key comparisions will be between HMR1766-treated and placebo-treated groups, where we will examine the change in inflammatory cytokine levels from baseline in subjects receiving HMR1766 or placebo capsules

Secondary Hypothesis/Outcome 2: To determine whether HMR1766 will slow progression of aortic valve dysfunction in patients with mild to moderate CAVS.

Rationale*:* Progressive reductions in aortic valve area in CAVS are the result of progressive expansion of calcified lesions on the aortic valve. Our preliminary findings in mice suggest that reducing osteogenic signaling in experimental animals can slow or halt accumulation of calcification in early stages of CAVS, and that these molecular and histological changes result in significant reductions in progression of aortic valve dysfunction. Whether sGC activation with Ataciguat slows progression of aortic valve dysfunction in patients with CAVS, however, is unknown.

Goal: Determine whether long-term treatment with HMR1766 will result in sustained increases in systemic sGC signaling slow progression of aortic valve dysfunction in patients with mild to moderate CAVS. This will be done using echocardiography-based measurements of aortic valve function.

Quantitative criteria for success:

Key outcomes:

1) Quantiative outcomes:
    a. Change in aortic valve function following 6 and 12 months of treatment.
        i. Key comparisions will be between HMR1766-treated and placebo-treated groups, where we will examine the change in:
            1. aortic valve area over time (calculated from the continuity equation) in subjects receiving HMR1766 or placebo capsules
                a. AVA will be evaluated by both the absolute value and following normalization for body surface area
            2. mean transvalvular pressure gradient over time (calculated from the blood velocity trace using the Bernoulli equation) in subjects receiving HMR1766 or placebo capsules

Tertiary Hypothesis/Outcome: Ataciguat will slow progression of left ventricular dysfunction in patients with mild to moderate CAVS.

Rationale*:* Left ventricular dysfunction is a strong predictor of adverse events in patients with moderate to severe CAVS. Increasing NO-sGC-cGMP signaling has been shown to slow deterioration of left ventricular function in experimental animal models of pressure overload (e.g., transverse aortic constriction), and may even reverse mild ventricular dysfunction when initiated in early stages of such models. Whether activation of sGC by HMR1766 improves left ventricular function or slows progression of left ventricular dysfunction in patients with moderate CAVS is unknown.

Goal: Determine whether long-term treatment with HMR1766 will result in sustained increases in systemic sGC signaling slow progression of aortic valve dysfunction in patients with mild to moderate CAVS. This will be done using echocardiography-based measurements of aortic valve function.

Quantitative criteria for success:

Key outcomes:

1) Quantitative outcomes:
    a. Change in left ventricular function following 6 and 12 of treatment.
        i. Key comparisions will be between HMR1766-treated and placebo-treated groups, where we will examine the change in:
            1. Left ventricular systolic function (measured by echocardiographic measurement of left ventricular ejection fraction)
            2. Left ventricular diastolic function (measured using the E/A ratio derived from Doppler measurements)

3 Study Design

3.1 General Design

Study design. Patients will report to the Clinical Research and Trials Unit (CRTU) for tolerance testing. Patients will complete testing at one "baseline" visit and 2 "experimental" visits. In brief, patients will check in at the CRTU to have their height, weight and vital signs recorded. CRTU Nursing Staff will also perform a blood draw at this time. Patients will then lie quietly in a semi-recumbent position for 15 minutes, during which time baseline blood pressure will be measured using an automated blood pressure cuff around their upper arm to evaluate orthostatic tolerance. Patients with symptomatic hypotension upon standing during the "baseline" visit will be excluded from further participation.

Subjects will then undergo CT scanning (to evaluate aortic valve calcium levels), echocardiography (to evaluate aortic valve function and cardiac function), and DEXA scanning (to evaluate bone mineral density). Subjects will have blood samples taken for baseline levels of $pVASP^{239}$ levels and inflammatory cytokine levels. Subjects who meet all inclusion criteria will then be randomized to one of two treatment arms: placebo or Ataciguat (200 mg/day). Subjects will self-administer Ataciguat or placebo at home each morning with food for the duration of 12 months. Standing tests (orthostatic tolerance testing), CT Scanning, Echocardiography, DEXA scanning, and blood sampling will be repeated at 6 month intervals for the remainder of the study (12 minimum follow-up).

PRE-CLINICAL HUMAN STUDIES

Orthostatic tolerance

Standing test: To determine whether patients experience orthostatic intolerance during a transition from a standardized, semi-recumbent position to a standing position, patients will be placed in a semi-recumbent position on an adjustable chair (45° torso incline, 10% thigh incline, with lower limbs/calves parallel to the floor) for 15 minutes, during which blood pressure will be measured non-invasively using automated sphygmomanometry devices placed on the patient's upper arm (mean/steady-state blood pressure). Subjects will transition to a standing position at 15 minutes, allow for 2 minutes to elapse to reach a steady-state, and return to a semi-recumbent position. If necessary (e.g., due to technical problems, etc.), this maneuver may be repeated 15 minutes later. Key outcomes are the perception of light-headedness (i.e., symptomatic hypotension) and nadir blood pressures measured at the upper arm. Our previous experience with this test is that it is highly reproducible and useful for detecting subjects who may have a high propensity for experiencing orthostatic intolerance.

Echocardiography. After enrollment in the study, patients will have a standard, clinical echocardiographic evaluation every 6 months to determine whether aortic valve or cardiac function changes over the course of the study. This will include examination of cardiac function from short-axis, long-axis, apical 5-chamber, and parasternal views, as well as measurements/calculations of aortic valve area and mean transvalvular gradient. While this procedure will be performed in theClinical Research and Trials Unit (CRTU), the procedure and subsequent measurements will be performed by a clinical sonographer employed by Mayo Clinic. If enrollment in the study is greater than one month after the most recent

clinical echocardiogram, measurements of cardiac and aortic valve function will be repeated in the Clinical Research and Trials Unit (CRTU) for an optimal "baseline" measurement.

Computed tomography (CT) scanning. As described in detail previously, a standard, non-contrast chest CT will be used to evaluate aortic valve calcium levels every 6 months throughout the study (minimum duration of enrollment = 12 months).

Whole-body dual X-ray absorptiometry (DEXA) scanning. Whole-body DEXA scanning will be used to evaluate changes in bone mineral density every 6 months to ensure that Ataciguat does not accelerate age-associated bone loss over the course of the study. Changes in bone mineral density (or lack thereof) will be a core measurement evaluated by the DSMB every 6 months. These measurements will be performed in the Clinical Research and Trials Unit (CRTU) at Mayo Clinic.

Blood samples. Venous blood samples (approximately 3 tablespoons) will be acquired from an antecubital vein at baseline and every 6 months thereafter for measurement of pro-inflammatory cytokines, p-VASP^{239} levels, and NtBNP levels. We will also use this sample used to measure a number of markers of your general health and/or drug toxicity, including:

- Hemoglobin, hematocrit, red blood cell levels, white blood cell levels, white blood cell count with differential platelet count.
- Prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR)
- Glucose, sodium, potassium, chloride, bicarbonate, calcium, phosphorus
- Urea, serum creatinine, creatine phosphokinase, total cholesterol, low density lipoprotein levels, high density lipoprotein levels, triglycerides, total protein levels, bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase

3.2 Primary Study Endpoints

The primary endpoint of this study relates to efficacy. While we will document incidence of all adverse events resulting from ataciguat treatment in this study, our experimental visits focus primarily on detection of changes in aortic valve calcification and aortic valve function over time.

3.3 Primary Study Endpoints

The primary endpoints of this study are:

1) The change in aortic valve calcification over time (i.e., quantitative detection of changes in aortic valve calcification, which underlie subsequent changes in aortic valve function)

2) The change in systemic inflammation over time (i.e., quantitative detection of changes in circulating inflammatory cytokines).
3) The change in aortic valve function over time (i.e., quantitative detection of the changes in aortic valve area and the mean transvalvular pressure gradient).
4) The change in left ventricular function over time (i.e., quantitative detection of changes in left ventricular systolic and diastolic function)

4 Subject Selection Enrollment and Withdrawal

Our aim is to identify patients with mild-to-moderate CAVS who have well-preserved left ventricular function (the predominance of patients seen clinically at our institution) and are hemodynamically stable.

4.1 Inclusion Criteria

1. Age > 50 years
2. Male or female sex
3. Aortic valve area greater than 1.0 cm^2 but less than 2.0 cm^2
4. Aortic valve calcium levels greater than 150 AU from chest CT
5. Ejection fraction >50%

4.2 Exclusion Criteria

1. Orthostatic intolerance or symptomatic hypotension prior to study or during study visits
2. Positive pregnancy test during screening visit
3. Nitrate use within 24 hours
4. Systolic blood pressure <110 mm Hg
5. Mean systemic arterial pressure <75 mm Hg
6. Severe mitral or aortic regurgitation
7. Retinal or optic nerve problems
8. Recent (≤30 days) acute coronary syndrome
9. Oxygen saturation <90% on room air
10. Congenital valve disease
11. Hepatic dysfunction/elevated liver enzymes
12. Prescription of drugs known to alter NO-sGC-cGMP signaling (sildenafil, nitrates, etc.)
13. Prescription of Warfarin (Coumadin) for chronic anticoagulation
14. Concomitant participation in other trials at Mayo Clinic or elsewhere
15. Use of phenytoin or related compounds for any indication
16. Chronic midazolam treatment for any indication
17. Use of monoamine oxidase inhibitors for any indication
18. Use of anti-diabetic drugs in the sulfonylurea family
19. Use of fluvastatin (predominantly degraded by CYP2C9)

4.3 Subject Recruitment, Enrollment and Screening

Screening and selection of patients

Patients will be identified by daily screening of results from the Mayo Clinic Echocardiography Laboratory. As illustrated in Figure 1, study coordinators will identify patients meeting the criteria for moderate calcific aortic valve stenosis, and subsequently review the patient's medical record for evidence of additional exclusion criteria. Many patients with evidence of moderate calcific aortic valve disease are not referred for non-contrast chest CT scanning—as such we will work with our clinical staff to review echocardiographic images from patients and select subjects with significant echogenicity on the aortic valve cusps (indicative of valvular calcium). Our study coordinators will contact

these patients and schedule Visit #1 (which includes obtaining informed consent prior to CT scanning) to confirm that the subjects' valvular calcium levels are >150 AU and that they are eligible for participation in the study. Patients who receive a clinically-indicated CT scan and have evidence of valvular calcium levels >150 AU will not be required to undergo an additional CT scan as a part of Visit #1.

Orthostatic tolerance testing

Standing test: To determine whether patients experience orthostatic intolerance during a transition from a standardized, semi-recumbent position to a standing position, patients will be placed in a semi-recumbent position on an adjustable chair (45° torso incline, 10% thigh incline, with lower limbs/calves parallel to the floor) for 15 minutes, during which blood pressure will be measured non-invasively using automated sphygmomanometry devices placed on the patient's upper arm (mean/steady-state blood pressure). Subjects will transition to a standing position at 15 minutes, allow for 2 minutes to elapse to reach a steady-state, and return to a semi-recumbent position. If necessary (e.g., due to technical problems, etc.), this maneuver may be repeated 15 minutes later. Key outcomes are the perception of light-headedness (i.e., symptomatic hypotension) and nadir blood pressures measured at the upper arm. This testing will be performed prior to the CT scan to ensure subject eligibility and minimize radiation exposure to potential subjects.

4.4 Early Withdrawal of Subjects

There are three indications for early withdrawal of subjects:

1) Individual subject participation will be terminated by the Principal Investigator if at any time the Principal Investigator feels there is a medical indication to do so (e.g., episode of syncope/symptomatic hypotension, adverse event that would compromise patient care/well-being, etc.).
    a. Subjects that experience orthostatic intolerance/symptomatic hypotension during the initial "baseline" tests (i.e., Visit 1) will be excluded from further participation in the study.
2) Participation of all subjects will be terminated by the Principal Investigator if there is evidence that this pharmacological intervention unexpectedly accelerates progression of valve disease at any time point. These interim evaluations will be performed by the statistician and the DSMB, and the Principal Investigator will remain blinded.
3) At any point during the study, subjects retain the right to discontinue participation in the study. Subjects can discontinue their participation by notifying the Principal Investigator or a study coordinator through a phone conversation, email communication, or in writing. All contact information will be provided to subjects during the consent process.

4.4.1 Data Collection and Follow-up for Withdrawn Subjects

Data from subjects who elect to withdraw from this study for reasons unrelated to drug tolerance prior to the first follow-up visit (i.e., the 6 month time point) will be omitted from final analyses aimed at determining whether Ataciguat effectively slows progression of aortic

valve calcification/aortic valve function/left ventricular function in patients with mild to moderate aortic valve stenosis, as both pre-and post-testing is critical for quantitatively determining efficacy of the compound. For patients that withdraw due to an adverse event/side effect (or whose participation is terminated by a Principal Investigator for medical reasons), these data will be included in the calculation of proportion of patients who tolerate the drug.

5 Study Drug

5.1 Description

The following drug product presentation is used in clinical trials:

Capsules: Supplied for oral administration as soft capsules containing 50 mg of HMR1766 (both calculated with reference to the active moiety) and the following excipients: macrogols, macrogolglycerol hydroxystearate, medium-chain partial glycerides, and glycerol. The soft capsule shell contains the following excipients: gelatin, glycerol, and the commercially available Anidrisorb 85/70 (contains sorbitol, sorbitan, mannitol, and higher polyols). The soft capsules are packaged in blister packs.

Storage conditions and shelf life
Capsules: Stability studies of Ataciguat soft capsules are in progress to confirm that, throughout the duration of the clinical studies, clinical supplies remain within the acceptance criteria defined in the specifications. Based on available stability data, the soft capsules are stable for at least 24 months when stored according to conditions specified in the clinical supplies' labeling. As additional stability data become available, the shelf life may be extended.

Adventitious agents safety evaluation
Any animal- and/or human-derived material(s) used to manufacture the drug substance/drug product complies with the applicable BSE/TSE (Bovine Spongiform Encephalopathy/Transmissible Spongiform Encephalopathy) and viral safety regulations.

5.2 Treatment Regimen

Ataciguat will be administered orally in 50 mg gelatin capsules packaged in aluminum blister packs. A dose of 200 mg ataciguat or matching placebo (=4 capsules of 50 mg or matching placebo) will be given once daily in the morning, with food. This dosing will be continued for the duration of the study (i.e., daily dosing for 12 months). Participants should take the study medication in the morning with food/breakfast, and the subject is expected to take their medication at their normal time on the days they come to the CRTU.

5.3 Method for Assigning Subjects to Treatment Groups

Our industry partner (Sanofi) will provide us with pre-packaged boxes of Ataciguat or Placebo (each box containing sufficient capsules for 16 days of treatment at each dose). Subjects will be provided a 6 month supply of medication and will be given the additional medication as needed during interim or follow up visits. The Mayo Research Pharmacist will work with our statistician for the randomization and blinding of the order in which Ataciguat or Placebo boxes will be given to enrolled patients. A pharmacist from the Research Pharmacy at Mayo Clinic will give the supply of capsules to the CRTU nurse, who will subsequently bring the drug to each patient, confirm that the patient understands the risks and potential side effects associated with consuming the active compound (Ataciguat), and study team members will provide additional directions for taking the drug (i.e., with food, in the morning).

5.4 Preparation and Administration of Study Drug

Our industry partner, Sanofi, has prepared 50 mg gelatin capsules, packaged the capsules in aluminum blister packs, and will provide boxes which will contain 16 doses of the compound. Patients will be responsible for self-administration of the drug at home during the 6 month periods.

5.5 Subject Compliance Monitoring

On day 7 of dosing, a study coordinator will call patients to confirm that:

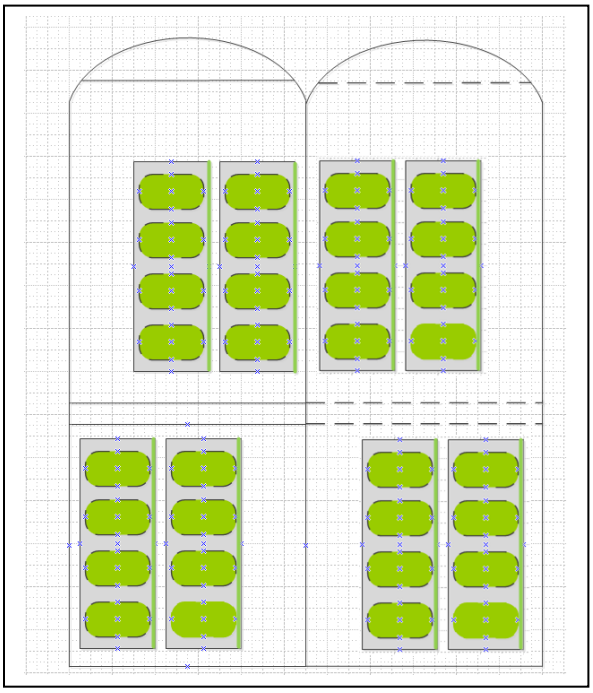
1) They are adhering to the treatment regimen for the study
2) They have not experienced significant adverse side effects.

A study coordinator will call patients every 30 days there-after, for the length of the study, to confirm that:

1) They are adhering to the treatment regimen for the study
2) They have not experienced significant adverse side effects.

5.6 Packaging

Our industry partner, Sanofi, has prepared 50 mg gelatin capsules, packaged the capsules in aluminum blister packs, and will provide numbered wallets which will contain 16 doses of the compound or 16 doses of placebo capsules.



5.7 Masking/Blinding of Study

Randomization will be done through a website and maintenance of the blind for the study will be the responsibility of the study team in cooperation with the Mayo Clinic Research Pharmacy according to the SOPs established.

5.8 Receiving, Storage, Dispensing and Return of study drug

Pre-packaged Ataciguat and Placebo capsules will be shipped from Sanofi to the Mayo Clinic Research Pharmacy, stored in a secure location within the research pharmacy between +2°C and +25°C, and protected from light. Both Ataciguat and Placebo will be stored in the original package. Each box containing Ataciguat or Placebo will be dispensed from the Research Pharmacy to the subject enrolled in the study, and at the completion of the study, subjects will return any unused compound to the Research Pharmacy.

5.8.1 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

OVERALL TIMELINE OF STUDY

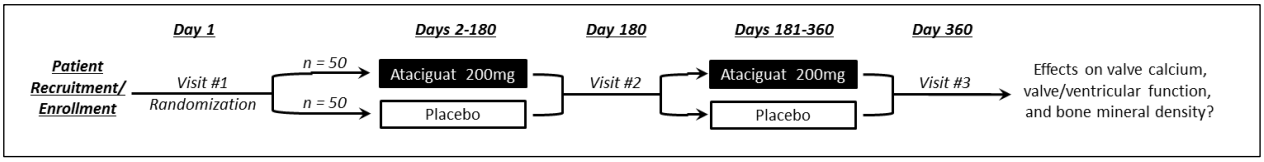
Day	Screening /Consent	Days 1-360	Day 7	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	Days 210/240/ 270/300/ 330	Day 360	
Month	0			1	2	3	4	5	6	variable	12	
Study Activity	Visit 1	Drug Treatment (at home)	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up	Visit 2	Follow up	Visit 3	
Informed consent	X											
History	X								X		X	
Physical exam (Ht, Wt, BSA, VS)	X								X		X	
Drug Treatment		X										
CT Scanning for valvular calcium levels	X								X		X	
Echocardiography for valvular/ventricular function	X								X		X	
Blood sample for p-VASP[239] and other clinical blood/biomarker testing	X								X		X	
DEXA scanning for bone mineral density	X								X		X	
Orthostatic Tolerance Testing	X								X		X	
POC Pregnancy testing	X											
History over the phone	X		X	X	X	X	X	X		X		
Phone follow-up for adverse events			X	X	X	X	X	X		X		

Summary of study/approach

Subjects will be identified from patients undergoing echocardiographic evaluation of aortic valve function for suspected calcific aortic valve disease at Mayo Clinic in Rochester, MN. Subjects who have mild/moderate aortic valve stenosis (aortic valve area greater than1.0 cm^2 but less than 2.0 cm^2 and valve calcium levels >150 AU from a chest CT scan) will be enrolled for participation in this study. The general design of the current study is a randomized, double-blinded, placebo-controlled protocol, in which patients will receive 200 mg per day of Ataciguat/HMR1766 for 12 months. Orthostatic tolerance, aortic valve calcium levels, aortic valve function, left ventricular function, bone mineral density, and markers of drug efficacy/treatment compliance will be measured pre- and post-treatment.

6 METHODS

General overview of visits



6.1 Screening and selection of patients

Patients will be identified by daily screening of results from the Mayo Clinic Echocardiography Laboratory. As illustrated in Figure 1, study coordinators will identify patients meeting the criteria for moderate calcific aortic valve stenosis, and subsequently review the patient's medical record for evidence of additional exclusion criteria. Many patients with evidence of moderate calcific aortic valve disease are not referred for non-contrast chest CT scanning—as such we will work with our clinical staff to review echocardiographic images from patients and select subjects with significant echogenicity on the aortic valve cusps (indicative of valvular calcium). Our study coordinators will contact these patients and schedule Visit #1 (which includes obtaining informed consent prior to CT scanning) to confirm that the subjects' valvular calcium levels are >150 AU and that they are eligible for participation in the study. Patients who receive a clinically-indicated CT scan and have evidence of valvular calcium levels >150 AU will not be required to undergo an additional CT scan as a part of Visit #1.

Key Inclusion Criteria	Key Exclusion Criteria
• Age > 50 years • Male or female sex • Aortic valve area greater than 1.0 cm^2 but less than 2.0 cm^2 • Aortic valve calcium levels greater than 150 AU from chest CT • Ejection fraction >50%	• history of orthostatic intolerance or symptomatic hypotension • Positive Pregnancy Test • nitrate use within 24 hours • systolic blood pressure <110 mm Hg, mean systemic arterial pressure <75 mm Hg • severe mitral or aortic regurgitation • retinal or optic nerve problems • recent (≤30 days) acute coronary syndrome • oxygen saturation <90% on room air • history/recent evidence of hepatic dysfunction from clinical record/blood testing • congenital valve disease • Hepatic Dysfunction/Elevated Liver Enzymes • prescription of drugs known to alter nitric oxide signaling (e.g., sildenafil, nitrates, etc.) • prescription of warfarin (coumadin) for chronic anticoagulation • concomitant participation in other trials at Mayo Clinic or elsewhere • use of phenytoin or related compounds for any indication • chronic midazolam treatment for any indication • use of monoamine oxidase inhibitors for any indication

	• use of anti-diabetic drugs in the sulfonylurea class • Use of fluvastatin (predominantly metabolized by CYP2C9)

After agreeing to participate in the study, subjects will report to the Clinical Research and Trials Unit (CRTU) for completion of Visit 1 (informed consent, confirmation of eligibility/review of medical history, physical examination/pre-screening to ensure subject does not have physical disabilities that would preclude completion of tolerance testing, etc.). At the end of Visit 1, subjects will schedule the follow-up visits for this study (i.e., experimental Visits 2 and 3).

Procedures for orthostatic tolerance testing (Visits 1, 2, and 3)

Standing test: To determine whether patients experience orthostatic intolerance during a transition from a standardized, semi-recumbent position to a standing position, patients will be placed in a semi-recumbent position on an adjustable chair (45° torso incline, 10% thigh incline, with lower limbs/calves parallel to the floor) for 15 minutes, during which blood pressure will be measured non-invasively using automated sphygmomanometry devices placed on the patient's upper arm (mean/steady-state blood pressure). Subjects will transition to a standing position at 15 minutes, allow for 2 minutes to elapse to reach a steady-state, and return to a semi-recumbent position. Key outcomes are the perception of light-headedness (i.e., symptomatic hypotension) and nadir blood pressures measured at the upper arm. This testing will be performed prior to the CT scan to ensure subject eligibility and minimize radiation exposure to potential subjects.

Flow of protocol and key blood pressure (BP) measurement points:

Time	15 minutes	2 minutes
Event	Acclimatization/ stabilization while sitting	Standing test

6.2 Overview of Visit 1

The purpose of Visit 1 is three-fold:

1) to confirm that patients do not experience orthostatic hypotension during any of the tests, which would preclude further participation in the study.
2) to confirm that subjects meet inclusion criteria for minimum valvular calcium levels via CT scanning and document baseline valvular calcium levels
3) to collect baseline data for valvular function, ventricular function, biomarker levels/inflammatory state, and bone mineral density prior to randomization to drug- or placebo-treated groups.

Admit to Clinical Research Unit/Acquire Blood Sample	Acclimatization to semi-recumbent position and subsequent "standing" test	CT Scan to evaluate aortic valve calcium levels	Echocardiography to evaluate aortic valve function and left ventricular function	DEXA scan to evaluate bone mineral density	Receive drug supply and directions for usage/administration and subsequent discharge
30 minutes	~45 minutes	~1 hour	~1 hour	45 minutes	2 hours
	Monitoring BP with automated arm cuff				Monitoring BP and ECG changes every 30 minutes

6.3 Overview of Visit 2

The purpose of Visit 2 is three-fold:

1) to confirm that patients do not experience newly documented orthostatic hypotension during any of the tests, which would warrant medical consultation and potential exclusion from participation in the study.
2) To document changes in aortic valve calcification following 6 months of treatment with either placebo or Ataciguat.
3) to determine whether there are changes in valvular function, ventricular function, biomarker levels/inflammatory state, and bone mineral density following 6 months of treatment with either placebo or Ataciguat.

Admit to Clinical Research Unit/Acquire Blood Sample	Acclimatization to semi-recumbent position and subsequent "standing" test	CT Scan to evaluate aortic valve calcium levels	Echocardiography to evaluate aortic valve function and left ventricular function	DEXA scan to evaluate bone mineral density	Receive drug supply and directions for usage/administration and subsequent discharge
30 minutes	~45 minutes	~1 hour	~1 hour	45 minutes	30 minutes
	Monitoring BP with automated arm cuff				

6.4 Overview of Visit 3

The purpose of Visit 3 is three-fold:

1) to confirm that patients do not experience newly documented orthostatic hypotension during any of the tests, which would warrant medical consultation and potential exclusion from participation in the study.
2) To document changes in aortic valve calcification following 12 months of treatment with either placebo or Ataciguat.
3) to determine whether there are changes in valvular function, ventricular function, biomarker levels/inflammatory state, and bone mineral density following 12 months of treatment with either placebo or Ataciguat.

Admit to Clinical Research Unit/Acquire Blood Sample	Acclimatization to semi-recumbent position and subsequent "standing" test	CT Scan to evaluate aortic valve calcium levels	Echocardiography to evaluate aortic valve function and left ventricular function	DEXA scan to evaluate bone mineral density	Return any left-over study medication/discharge
30 minutes	~45 minutes	~1 hour	~1 hour	45 minutes	30 minutes
	Monitoring BP with automated arm cuff				

7 Statistical Plan

The primary endpoint will be change in aortic valve calcification as assessed by computed tomography scanning. Secondary and tertiary endpoints are changes in valvular function, ventricular function, biomarker levels/inflammatory state, and bone mineral density following 12months of treatment with either placebo or Ataciguat.

7.1 Primary Hypothesis

Our primary hypothesis is:

1) Ataciguat will increase systemic sGC signaling and halt accumulation of aortic valve calcium in patients with mild to moderate CAVS.

7.2 Secondary Hypotheses

Our secondary hypotheses are:

1) Ataciguat will reduce levels of circulating inflammatory cytokines in patients with mild to moderate CAVS.
2) Ataciguat will slow progression of aortic valve dysfunction in patients with mild to moderate CAVS

7.3 Tertiary Hypothesis

Our tertiary hypothesis is:

1) Ataciguat will slow progression of left ventricular dysfunction in patients with mild to moderate CAVS

7.4 Subject Population for Analysis

The primary study evaluation will be an intent-to-treat (ITT) analysis. We will only perform statistical analyses on subjects that are randomized to treatment (they complete the baseline assessment and are not found to have orthostatic hypotension), and completed at least one post-treatment session. Subjects who do not complete at least one post-treatment session (orthostatic intolerance/adverse side effects, etc.) will not be included in the analysis. . We will also do a per protocol analysis. This patient population will consist of patients who were compliant with the treatment they received (even if it was not the treatment to which they were randomized). Compliance will be determined by self report and capsule blister pack counting at the end of the treatment period. To be included in the per-protocol analysis, patients need to be at least 60% or more compliant.

7.5 Sample size

This is a double-blinded, randomized, placebo-controlled study to determine whether Ataciguat administered at a dose of 200 mg/day effectively slows progression of aortic valve calcification in patients with mild-to-moderate CAVD. The intended analysis will be to compare the change in aortic valve calcium from baseline between the control group and the treatment group. A sample size of 50 in each group will have 80% power to detect an effect size of 0.57 using a two group t-test with a 0.05 two-sided significance level. The effect being compared between the two groups is the mean change in aortic valve calcium levels at 12 months (12 month aortic valve calcium level – baseline aortic valve calcium level). The effect size is the expected difference in the mean change from baseline between the two groups) divided by the within-group standard deviation of

the change from baseline. It is felt that for this drug to be of clinical interest in treating aortic value calcification, there would need to be a effect size of 0.50-0.60 standard deviations or more (i.e. moderate to large effect). A sample size of 100 patients (50 in each group) provides adequate power (80%) to detect such a difference at the 12 month time point (i.e., our minimum treatment duration).

7.6 Analysis

.

The primary analysis will be an intent-to-treat analysis where all randomized patients who underwent at least one post-treatment evaluation (e.g. at 6 months or 12 months); patients will be analyzed according to the treatment to which they were randomized. The continuous outcomes will be analysed with a mixed effects model to account for the correlation of measurements within the same patient. The effect of interest for the primary analysis is to determine whether the change in aortic valve calcium levels is the same between the control group (patient receiving placebo) and the treated group (patients receiving Ataciguat). An unstructured covariance matrix will be used in the analysis.A mixed effects model will also be used to evaluate the secondary and tertiary quantitative endpoints.

Secondary analysis will be on a per-protocol level where the patients included are those who were compliant with the treatment that they received. There will also be exploratory analyses done with respect to subgroups of patients (e.g. by gender) as well as any evaluation of patient/disease characterisitcs that are associated with changes in the endpoints.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that result in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in

this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB and the Data Safety Monitoring Board providing oversight for the study.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Unblinding Procedures

Following the completion of the first 25 patients in each arm at each time point (i.e., 25 placebo, 25 Ataciguat), we will count the total number non-tolerated adverse events and/or physiological changes indicating discontinuation of treatment due to patient safety. Our statistician will present the unblinded data to our Data Safety Monitoring Board (DSMB) for evaluation. Depending upon the number and magnitude of adverse events/adverse physiological changes in each group, we will abide by the recommendation of the DSMB to continue or discontinue the study.

In the unlikely event that there is a significant, unexpected, severe adverse event (e.g., myocardial infarction, sudden deterioration in clinical status, or death), we will immediately unblind investigators to determine whether the event was associated with Ataciguat treatment. In this unlikely event, we would also immediately report the event to our IRB, DSMB, and to NIH.

8.5 Stopping Rules

As noted in previous sections, we will stop these studies prematurely if:

1) There is evidence that patients will not tolerate the given dose of the drug (e.g., >30% of patients experiencing symptomatic/orthostatic hypotension following 6 months of treatment with Ataciguat).
2) There are serious/unexpected side effects in patients receiving Ataciguat.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events. Maurice Enriquez-Sarano, MD, is a cardiologist and Co-Principal Investigator in this study, has extensive experience in the medical management of this population, and will be providing regular medical oversight for this study.

8.6.1 Internal Data and Safety Monitoring Board

We have established an internal DSMB to provide oversight for this study. Membership of the DSMB is comprised of a minimum of five individuals from Mayo Clinic Rochester and includes;

- The chair of the DSMB (voting member), chosen from the physician representatives
- Additional three (3) or more physician representatives (voting members)
- Biostatistician representative (voting member)
- Representative from the Surgical Clinical Research Office (voting member)
- *Ad hoc* members from the Division of Cardiovascular Diseases at Mayo Clinic to be appointed to the committee by the chair for study-specific expertise.

The DSMB will meet at key decision points in the study, which include:

1) After 50 patients (25 patients per arm) complete Visit #2 (i.e., the first follow-up visit to evaluate changes in aortic valve calcification, aortic valve function, cytokine levels, and left ventricular function), unblinded data will be provided to the DSMB to determine whether there are any adverse physiological effects of the drug during the first 6 months of treatment.
2) After 50 patients (25 patients per arm) complete Visit #3 (i.e., the one year follow-up visit to evaluate changes in aortic valve calcification, aortic valve function, cytokine levels, and left ventricular function), unblinded data will be provided to the DSMB to determine whether there are any adverse physiological effects of the drug during the first 12 months of treatment.
3) At interim points, if necessary, when there is sufficient evidence suggesting that Ataciguat may elicit severe adverse events and/or have a poor tolerance profile in this patient population.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period. As clearly stated in the consent form, clincially-relevant information collected as a part of this study (e.g., blood testing results, results from echocardiographic testing, and results from CT scanning tests) will be added to the subject's medical record.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

Data Management
Initial data used for screening potential subjects will be derived from the electronic medical record and entered into electronic case report forms within Medidata Rave (a software package endorsed by Mayo Clinic's Clinical Trial Management System). Data collected on paper case report forms during screening visits (e.g., height, weight) will be transferred to electronic case report forms at the end of each session.

Data Processing
We will transcribe or directly enter subject source data into eCRFs using Medidata Rave at the time of or as soon as possible after the subject visit. All completed eCRFs will be reviewed by the study coordinator at regular intervals as the study progresses.

Data Security and Confidentiality

Database and Web servers will be secured through controlled physical access. For security reasons, and in compliance with regulatory guidelines of Medidata Rave, system access is granted to the user who owns the sign on identification and password in use. Access codes are non-transferrable. Site personnel who have not undergone training may not access the study eCRF's until appropriate training is completed and documented. The eCRF data elements do not reside on the users work station; they are transmitted to a secure central database (host site) as forms are completed or updated. Protocol-specified source documents (e.g. hospital discharge summaries, operative/procedural reports) will be retrieved as necessary. Copies of all study-related documentation will be retained at the site.

Data Quality Assurance/Clarification Process

The CTMS Medidata Rave database will have consistency checks programmed into the system to inform investigators of potential data issues as the data entry progresses. The exception log for entries will be reviewed by the study coordinator/monitor to identify potential training and/or data integrity issues. If data integrity issues are suspected, the study coordinator/monitor will perform site monitoring, including review of the eCRFs with verification to the source documentation. During monitoring visits, the site will make their computer and/or high speed internet access

available to the study coordinator/monitor so that he or she may verify the data entries with the source documentation.

9.4 Records Retention

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717

Whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is supported by National Institutes of Health Grant TR000954 (administered through the National Center for Accelerating Translational Sciences).

12.2 Conflict of Interest

Dr. Jordan D. Miller and Dr. Bin Zhang hold a provisional patent for harnessing sGC signaling to slow progression of calcification in humans with aortic valve stenosis. Drs. Miller and Zhang currently do not receive licensing royalties from these studies, but may at some point in the future.

12.3 Subject Stipends or Payments

Subjects will receive $125 upon completion of each experimental session (i.e., Visits 1, 2 and 3), for a total possible compensation of $375. This remuneration is for time and inconvenience, and is not based on risk or invasiveness of the proposed studies. Subjects will also be reimbursed for travel if they reside outside of Rochester, MN or its immediately surrounding communities.

13 Publication Plan

Data from these studies will be presented at national scientific meetings in the form of an abstract (which is frequently published as well), and will ultimately be published in a peer-reviewed scientific journal.

14 References

1. Kennedy JA, Hua X, Mishra K, Murphy GA, Rosenkranz AC, Horowitz JD. Inhibition of calcifying nodule formation in cultured porcine aortic valve cells by nitric oxide donors. *Eur J Pharmacol*. 2009;602:28-35
2. Rajamannan NM. Oxidative-mechanical stress signals stem cell niche mediated lrp5 osteogenesis in enos(-/-) null mice. *J Cell Biochem*. 2012;113:1623-1634
3. Miller JD, Chu Y, Brooks RM, Richenbacher WE, Pena-Silva R, Heistad DD. Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *J Am Coll Cardiol*. 2008;52:843-850
4. Miller JD, Weiss RM, Heistad DD. Calcific aortic valve stenosis: Methods, models, and mechanisms. *Circ Res*. 2011;108:1392-1412
5. Stasch JP, Schmidt PM, Nedvetsky PI, Nedvetskaya TY, H SA, Meurer S, Deile M, Taye A, Knorr A, Lapp H, Muller H, Turgay Y, Rothkegel C, Tersteegen A, Kemp-Harper B, Muller-Esterl W, Schmidt HH. Targeting the heme-oxidized nitric oxide receptor for selective vasodilatation of diseased blood vessels. *J Clin Invest*. 2006;116:2552-2561
6. Gladwin MT. Deconstructing endothelial dysfunction: Soluble guanylyl cyclase oxidation and the NO resistance syndrome. *J Clin Invest*. 2006;116:2330-2332
7. Volzke H, Haring R, Lorbeer R, Wallaschofski H, Reffelmann T, Empen K, Rettig R, John U, Felix SB, Dorr M. Heart valve sclerosis predicts all-cause and cardiovascular mortality. *Atherosclerosis*. 2010;209:606-610
8. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med*. 1999;341:142-147
9. Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M. Burden of valvular heart diseases: A population-based study. *Lancet*. 2006;368:1005-1011
10. Amato MC, Moffa PJ, Werner KE, Ramires JA. Treatment decision in asymptomatic aortic valve stenosis: Role of exercise testing. *Heart*. 2001;86:381-386
11. Rosenhek R, Zilberszac R, Schemper M, Czerny M, Mundigler G, Graf S, Bergler-Klein J, Grimm M, Gabriel H, Maurer G. Natural history of very severe aortic stenosis. *Circulation*. 2010;121:151-156
12. Briand M, Lemieux I, Dumesnil JG, Mathieu P, Cartier A, Despres JP, Arsenault M, Couet J, Pibarot P. Metabolic syndrome negatively influences disease progression and prognosis in aortic stenosis. *J Am Coll Cardiol*. 2006;47:2229-2236
13. Rosenhek R, Klaar U, Schemper M, Scholten C, Heger M, Gabriel H, Binder T, Maurer G, Baumgartner H. Mild and moderate aortic stenosis. Natural history and risk stratification by echocardiography. *Eur Heart J*. 2004;25:199-205