Hemodynamic Effects of a Novel Myeloperoxidase Inhibitor with Exercise in Heart Failure with Preserved Ejection Fraction – A Randomized, Double-Blind, Placebo Controlled Proof of Principle Study

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(Add additional amendment dates as protocol is modified and update the version number.)

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List of Abbreviations

A-V O_2 diff	Arteriovenous Oxygen content difference
AE	Adverse Event/Adverse Experience
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
CFR	Code of Federal Regulations
cGMP	cyclic Guanosine Monophosphate
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EMR	Electronic medical record
EndoPAT	Endothelial function peripheral arterial tonometry
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HIPAA	Health Insurance Portability and Accountability Act
HOCl	Hypochlorous Acid
IB	Investigator's Brochure
IMP	Investigational Medicinal Product
IND	Investigational New Drug Application
IRB	Institutional Review Board
MAD	Multiple Ascending Dose
MMP	Matrix Metalloproteinases
MPO	Myeloperoxidase
NFTF	Non Face to Face
NOAEL	No-observed-adverse-effect-level
NO	Nitric Oxide
O_2	Oxygen
PCWP	Pulmonary Capillary Wedge Pressure
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetic
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event/Serious Adverse Experience
SAD	Single Ascending Dose
SOP	Standard Operating Procedure
TAPSE	Tricuspid Annular Plane Systolic Excursion
VO_2	Oxygen consumption
Ve/VCO_2	Ventilatory efficiency

Study Summary

Title	Hemodynamic effects of a Novel Myeloperoxidase Inhibitor with Exercise in Heart Failure with Preserved Ejection Fraction – A Randomized, Double-Blind, Placebo Controlled Proof of Principle Study
Protocol Number	IRB #17-002907 AZ Study Code D6580C00005
Phase	Proof of Principle
Methodology	This study will be a Proof of Principle, randomized, blinded, placebo-controlled, clinical trial with all subjects undergoing exercise catheterization and randomized to either oral myeloperoxidase (MPO) inhibitor or placebo.
Overall Study Duration	1 year
Subject Participation Duration	9-14 days
Single or Multi-Site	Single site
Objectives	To determine the effect of a single dose of an oral acute MPO inhibitor on resting and exercise hemodynamics in patients with heart failure with preserved ejection fraction (HFpEF)
Number of Subjects	36
Diagnosis and Main Inclusion Criteria	HFpEF defined by clinical symptoms of HF (dyspnea, fatigue), normal EF (≥50%), and catheterization documented elevated left ventricular filling pressures with exercise (≥25 mmHg).
Study Product, Dose, Route, Regimen	Oral administration of MPO inhibitor (AZD4831 tablets 5 mg and matching AZD4831 Placebo tablets) at a dose of 30 mg during catheterization
Duration of Administration	Single administration
Reference therapy	Placebo
Statistical Methodology	The principal comparisons will be exercise hemodynamic parameters at 20 Watt workload after study drug relative to corresponding values at 20 Watts workload prior to study drug. Primary hypotheses will be tested using analysis of covariance.

Introduction

Heart failure (HF) with preserved ejection fraction (HFpEF) has become the most common cause of HF. Morbidity and mortality are high and there is no proven effective pharmacological treatment, making this a huge unmet public health need. Recently, abnormalities in Nitric Oxide (NO) signaling and Reactive Oxygen Species (ROS) generation have been implicated as a unifying mechanism by which aging and associated systemic comorbidities drive the myocardial and peripheral limitations that constrain exercise capacity in HFpEF. Myeloperoxidase (MPO) is a leukocyte enzyme that plays a key role in host defense but has also recently been implicated in the pathophysiology of incident cardiovascular diseases, including HF. MPO plays an important role in the regulation of vascular tone and arterial conduit function, since vascular MPO activity results in NO scavenging. Because loss of NO activity is heavily implicated in the pathogenesis of HFpEF, MPO inhibition may represent a novel therapeutic approach to target the NO imbalance and vascular dysfunction that exists in HFpEF.

Accordingly, **our guiding hypothesis** is that an oral MPO inhibitor (AZD4831) will improve cardiac hemodynamics at rest and during exercise in relation to enhanced NO bioavailability. This Proof of Principle study will assess the effect of a single dose of an oral acute MPO inhibitor on resting and exercise hemodynamics in patients with HFpEF referred to the catheterization lab. In addition, we will evaluate NO bioavailability, cyclic Guanosine Monophosphate (cGMP) response and forearm flow mediated vasodilation as surrogate markers for improving endothelial function with MPO inhibition.

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

1.1 Background

Heart failure (HF) with preserved ejection fraction (HFpEF) is the most common cause of heart failure, with both aging and comorbidity burden being directly proportional to risk of incident HFpEF [1]. Despite the high prevalence and socio-economic burden, no pharmacological treatment options are currently available. This may be due to phenotypic heterogeneity and failure to target the final common pathway in this syndrome [2]. Recently, abnormalities in Nitric Oxide (NO) signaling and Reactive Oxygen Species (ROS) generation have been implicated as a unifying mechanism by which aging and associated systemic comorbidities drive both the systemic inflammation and myocardial/peripheral limitations central to symptomatic HFpEF [3]. Current investigative strategies targeting this NO pathway focus on exogenous replacement but do not address the underlying systemic inflammatory process driving the endothelial dysfunction and effective NO deficiency.

MPO is a leukocyte enzyme involved in the generation of hypochlorous acid (HOCl) and ROS. While these actions are critical for host defense via beneficial bactericidal effects [4], recent evidence points to a deleterious role for excessive MPO activity in a number of cardiovascular diseases including HF [5], atherosclerosis [6, 7, 8, 9, 10, 11, 12] and endothelial dysfunction [13, 14, 15, 16]. In normal physiology, MPO also plays a role in the regulation of vascular tone and arterial conduit function [15, 17] by oxidizing and thereby inactivating NO [4, 13, 18, 19, 20, 21], and inhibiting endothelial NO synthase (Figure 1)[22]. Furthermore, MPO is also found in the subendothelial matrix and plays a role in tyrosine nitration with resultant adverse cardiac and tissue remodeling via modulation of matrix metalloproteinases (MMP) and other proteins [23, 24, 25, 26, 27, 28, 29]. Accordingly, MPO inhibition may represent a novel therapeutic approach not only addressing the NO imbalance and vascular dysfunction that exists in HFpEF, but may also directly suppress the inflammatory ROS cascade driving cardiac and peripheral adverse remodeling and functional NO/cGMP deficiency in this syndrome.

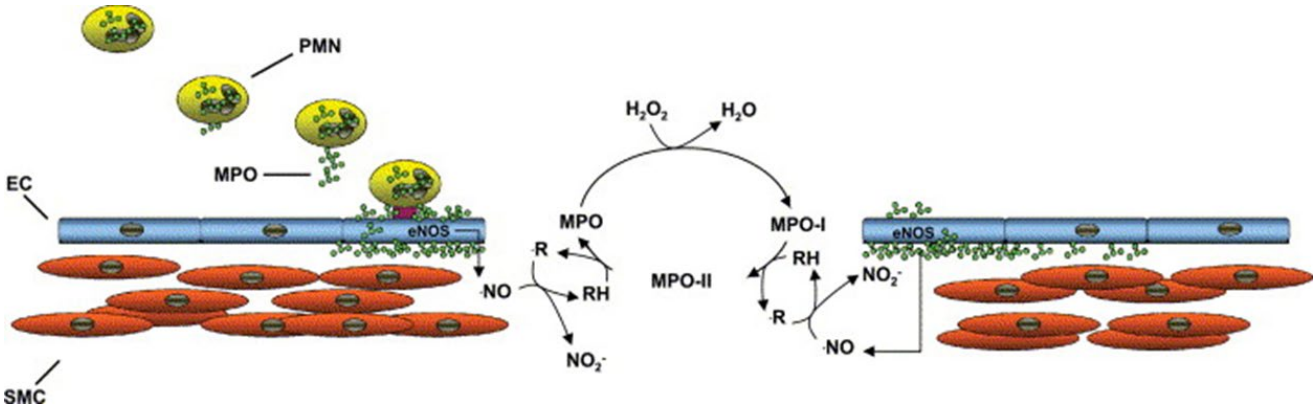


Figure 1. Scheme of the proposed mechanism of vascular distribution of MPO and MPO-dependent catabolism of NO.

In this study, we will evaluate whether an oral MPO inhibitor (AZD4831, currently under development by AstraZeneca) will exert beneficial effects on cardiac hemodynamics through improvement in NO bioavailability. This study will assess the effect of a single dose of an oral acute MPO inhibitor on resting and exercise hemodynamics in patients with HFpEF referred to the catheterization lab.

1.2 Investigational Agent

Code Name:	AZD4831
Molecular Formula:	C15H15ClN4OS
Molecular Weight:	335 g/mol

1.3 Preclinical Data

AZD4831 is a potent mechanism-based MPO-inhibitor, acting on purified MPO *in vitro* with an IC_{50} of approximately 1 nmol/L. In human PMA-stimulated neutrophils *in vitro*, AZD4831 inhibits extracellular peroxidase activity with an IC_{50} of 12 nmol/L, whereas the intragranular peroxidase activity is inhibited with an IC_{50} of 2.7 µmol/L. *In vitro*, AZD4831 dose-dependently inhibits MPO-driven formation of urate and allantoin from xanthine, whereas it does not inhibit xanthine oxidase activity (at concentrations up to 30 µmol/L) or URAT-1 mediated transport of urate (at concentrations up to 150 µmol/L). *In vivo*, AZD4831 dose-dependently reduces local peroxidase activity at doses down to 0.033 mg/kg (yielding a plasma exposure of 1.7 nmol/L at 4h) and systemic MPO levels at 17 and 83 mg/kg in mouse peritonitis models.

1.4 Clinical Data to Date

MPO has long been regarded as a mediator of bactericidal activity through its generation of HOCl and ROS. However, with recent evidence linking MPO to incident cardiovascular disease and NO catabolism with vasoconstriction, there has been renewed interest in exploring its role in various disease states. HFpEF is an enormous public health problem with no current treatment options. According to current disease paradigms, a primary driver for this syndrome is increased oxidative stress, ROS generation, endothelial dysfunction and relative NO deficiency. Thus HFpEF represents a high yield target for MPO inhibition; to not only improve exercise capacity through NO augmentation, but also to directly suppress the oxidative stress and endothelial dysfunction that promotes progression of the disease.

Our group at Mayo Clinic and others have demonstrated that endothelial function is impaired in HFpEF [30], [31] and is correlated with pulmonary vascular disease [32] as well as death and HF hospitalization [33]. Thus a therapy such as MPO inhibition that improves endothelial function is likely to be beneficial in preventing adverse outcomes, in addition to improving aerobic capacity and symptoms. Further supporting this approach of NO augmentation, recent trials of nitrite supplementation in HFpEF have demonstrated a reduction in exercise

filling pressures, ventricular performance, pulmonary vasodilation, exercise capacity and cardiac output response [34], [35], [36], [37] further supporting an approach of MPO inhibition to augment NO bioavailability.

There is preliminary evidence that MPO plays a role in vasoconstriction of muscular and conduit arteries [17] through a reduction in NO bioavailability. This appears to be mediated in part by vascular MPO scavenging of NO, since acute administration of heparin displaces subendothelial MPO and this results in enhanced NO activity [38]. Thus the development of an inhibitor of MPO represents a logical next step in the assessment of vascular and hemodynamic changes in humans, and HFpEF represents a high yield target to demonstrate potentially beneficial effects from these changes.

The presence of dynamic reserve limitations in HFpEF makes assessment of exercise hemodynamics a very sensitive and physiologically relevant way to evaluate pharmacologic effects. Our group has conducted and published numerous studies in HFpEF over the past 10 years using high fidelity micromanometer catheters. Specifically in the invasive hemodynamic laboratory, we have published numerous studies examining exercise hemodynamics and/or acute drug effects in patients with HFpEF [34, 35, 39]. Most recently, a similar trial design to the one proposed here was employed for the evaluation of sodium nitrite therapy demonstrating significant hemodynamic benefits [34, 35].

We have typically been able to enroll 2-3 patients per month over the past few years for these invasive hemodynamic exercise studies given the large volume of exercise right heart catheterizations performed at Mayo annually (>200 per year). The current proposal will enroll a much lower sample of 36 subjects over one year, further enhancing the likelihood of success.

Safety

AZD4831 has been administered to 59 healthy subjects in completed studies in single doses up to 405 mg, and in repeated doses up to 15 mg for up to 14 days. There were no deaths or SAEs and all subjects except one completed the studies. The most common adverse events were headache and generalized maculopapular rash. Dosing in 45 mg cohort in the Multiple Ascending Dose (MAD) study was stopped because of self-reported throat tightness in one subject that was considered intolerable by the Principal Investigator (PI) and Safety Review Committee (SRC). This subject also had a more long-lasting rash of moderate intensity. Otherwise AZD4831 has been well tolerated in healthy subjects. No clinically meaningful differences for changes over time in clinical laboratory tests, vital signs or ECGs were observed between subjects who received AZD4831 and those who received placebo.

Since the SAD and MAD studies conducted in healthy volunteers noted above, a new Phase 2a study (NCT03756285) has been undertaken in patients with HFpEF, as are being enrolled in this study. Thus far, 24 participants have been randomized to AZD4831 and 13 to matching placebo.

The safety results of this Phase 2a study in HFpEF are as follows:

- AZD4831 was generally well tolerated
- Of the 24 patients randomised to AZD4831, 23 completed 90 days treatment
- There were no deaths. Two SAEs (8.3%) were reported in AZD4831 group and one (7.7%) in the placebo group
- One patient (4.2%) in AZD4831 group was discontinued from treatment due to generalized maculopapular rash (CTCAE grade 3) as prespecified in the protocol. No patients on placebo were discontinued from treatment.
- AEs were balanced between groups and as expected for this patient population
- Overall, 16 (66.7%) patients experienced at least one AE after receiving treatment AZD4831 and 7 [53.8%] patients after receiving placebo.
- No clinically significant trends were observed in vital signs, clinical laboratory results, 12-lead ECGs

1.5 Dose Rationale

This is an oral single dose study in HFpEF patients and the first time AZD4831 will be dosed in patients with heart failure. Oral administration is the intended route of administration for AZD4831 at an expected dose of 7 mg.

In healthy subjects, when dosed from 5 to 405 mg AZD4831, a significant and dose dependent decrease in serum uric acid was observed at 135 mg and 405 mg AZD4831, respectively.

In addition, when dosed with 5 mg once daily for 10 days in healthy subjects, an approximate 30% reduction in MPO activity was observed at day 10 and steady state conditions.

In the same study, the pharmacokinetic analysis showed an accumulation of approximately 3. In other words, plasma levels of AZD4831 were approximately 3 fold higher at steady state with daily dosing as compared to single dose administration of AZD4831.

Assuming similar PK in healthy subjects and HFpEF patients, a single dose of 30mg AZD4831 is predicted to yield exposure seen at steady state following once daily administration of 10 mg AZD4831. Thus, a single dose of 30 mg provided in this acute, proof of concept trial, should replicate the degree of

MPO inhibition that will be seen with the predicted therapeutic dose of 7 mg AZD4831.

The single dose of 30mg AZD4831 in this study is predicted to yield an AUC and Cmax of 4.7 µmol*h/L and 0.24 µmol/L, respectively. These exposures are substantial lower than the AUC and Cmax achieved after a single dose of 405 mg AZD4831 in healthy subjects and 40-60 fold lower than the AUC and Cmax exposures achieved at the NOAEL (20 mg/kg) in the one-month dog toxicity study.

1.6 Risks and Benefits

While we expect AZD4831 to improve hemodynamics, the short term nature of the study does not provide a meaningful opportunity for clinical benefit.

A number of potential risks have been identified and are primarily based on preclinical safety studies with AZD4831 that include repeated dosing up to 1 month in rats and dogs.

The key adverse effects identified in the toxicology and safety pharmacology program for AZD4831 were:

- BP reduction and reflex tachycardia because of α1A adrenoceptor block, which can be monitored by frequent BP, HR and ECG examination.

- Inhibition of thyroid peroxidase: subjects with active thyroid disease will not be included in the study

Theoretical risks
The following risks are based on results from humans and animals with partial or total MPO deficiency. The relevance of the effects observed in genetic deficiency models as compared to administration of a MPO inhibitor is not known.

Host-defense impairment - infections
A theoretical risk is that treatment with an MPO inhibitor could impair host-defense mechanisms. The MPO in neutrophils generate ROS to fight infecting microorganisms. MPO deficient mice show impaired fungicidal activity but no other special abnormalities compared to wild type mice. Humans with total or partial MPO deficiency generally do not have an increased susceptibility to infections but the incidence of Candida infections may be increased. In studies with the other MPO inhibitors AZD3241 and AZD5904, no increased incidence of infections, severe infections or increased inflammation has been observed although treatment duration and number of patients have been limited.

Mitigation: Special considerations will be given to adverse events (AEs) related to frequency of infections, duration of infections, severity of infections and

inflammatory responses in clinical trials. The high-sensitivity C-reactive protein (hsCRP) will be measured as a general marker of inflammation prior to study drug and at the follow up visit.

Agranulocytosis and allergic reactions

Agranulocytosis is a serious side effect of drugs containing a thiourea motif that can be irreversible. Theoretical risks based on a thiourea motif of AZD4831 molecule are agranulocytosis and allergic reactions, which are known liabilities for registered anti-thyroid drugs with similar thiourea motifs as AZD4831 although the frequency is low (0.3 to 0.6%).

There are no good preclinical models available to study agranulocytosis. There were no consistent findings on hematological or bone marrow parameters in the 1 month studies in rats and dogs. In studies with the other MPO inhibitors AZD3241 and AZD5904 which also carry the thiourea motif, no cases of agranulocytosis were observed although treatment duration and number of patients were limited.

Mitigation: Hematological parameters including white blood cell (WBC) count will be monitored as safety laboratory parameter prior to receiving study drug and again at the follow up visit. Special considerations will be given to the AEs of fever, sore throat and flu-like symptoms. Agranulocytosis by antithyroid drugs may be responsive to treatment with granulocyte colony-stimulating factor but data suggests that there is no benefit to this approach (Yang et al., *Thyroid* 2016).

Identified risks from the SAD and MAD studies Maculopapular rash was an identified risk in the SAD study for AZD4831. Noteworthy, subjects in both SAD and MAD (multiple ascending dose) studies with a previous MPOi in development by AstraZeneca AZD5904, also developed maculopapular rash at about the same frequency as AZD4831 that resolved during continued dosing.

The maculopapular rash was self-limited in all cases and developed at 7-9 days post dose with AZD4831. The rash was generalized and classified as moderate. One rash occurred at the 45 mg, one at 135 mg and 2 at the 405 mg dose group. No rash was reported in the placebo, 5 mg or 15 mg dose groups.

As described above, a generalized maculopapular rash was also noted in 1/24 patients with HFpEF in the ongoing Phase 2a trial of AZD4831 (NCT03756285). This occurred 5 days after increasing the dose of AZD4831 from 2.5 to 5 mg daily, and resolved after discontinuation of study drug and symptomatic treatment using oral anti-histamines and corticosteroid.

Mitigation: A lower dose of AZD4831 will be used that in early dosing studies has not been associated with rash.

Full skin examination will be performed at the follow up videoconference visit in all participants to evaluate for rash. This will be performed remotely from the participants home, employing the clinical video platform (Zoom) that is currently used at the Mayo Clinic for non-face-to-face (NFTF) patient visits in our clinical practice. This system was developed to respond to travel restriction in the setting of the COVID19 pandemic and has proven very effective to manage symptoms and signs that are evident from video-conference evaluation, as with a skin rash.

If a concerning rash is identified, dermatology consultation will be obtained, with biopsy if deemed clinically appropriate by the consulting dermatologist. Patients will be instructed to report back immediately if they develop a rash prior to the follow up visit.

One subject self-reported throat tightness in the 45mg dose group which could not be verified by examination. .

2 Study Objectives

Aim 1: Determine whether acute administration of oral MPO inhibitor (AZD4831) at a dose of 30 mg attenuates the pathologic elevation in exercise Pulmonary Capillary Wedge Pressure (PCWP) in HFpEF.

*Our **primary hypothesis** is that acute administration of an oral MPO inhibitor will augment NO signaling and thereby attenuate the pathologic elevation in exercise PCWP in HFpEF, compared to placebo.*

Aim 2: Determine whether treatment with an oral MPO inhibitor (AZD4831) at a dose of 30 mg improves other resting and exercise hemodynamics, peripheral oxygen extraction, and endothelial function in HFpEF.

*Our **secondary hypotheses** are that acute administration of an oral MPO inhibitor will improve exercise cardiac output reserve, reduce biventricular filling pressures at rest, enhance peripheral oxygen extraction, improve endothelial function, and lower pulmonary arterial pressure in HFpEF, compared to placebo.*

3 Study Design

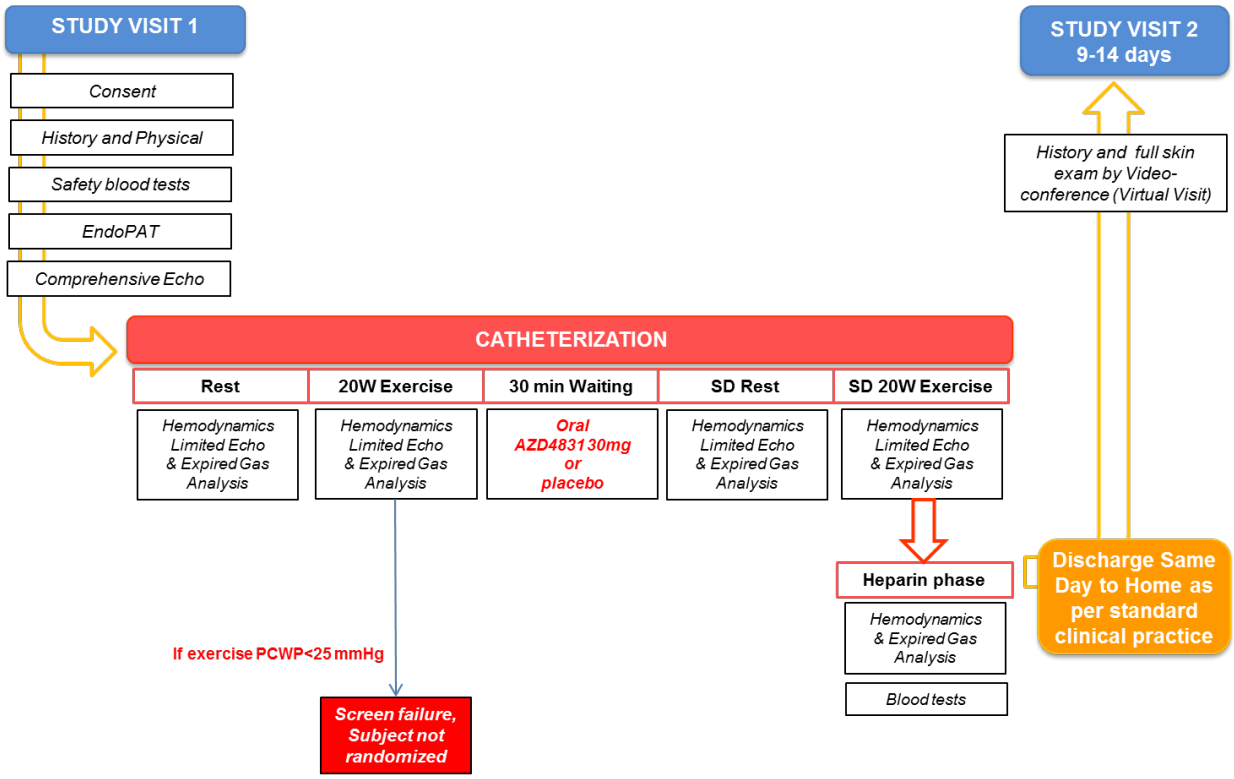
3.1 General Description

This is a Proof of Principle, randomized, double-blind, placebo-controlled trial testing whether an oral MPO inhibitor, as compared to placebo, can attenuate the pathologic elevations in PCWP during exercise in subjects with HFpEF.

Brief Synopsis of Methods (Figure 2):

1) Enroll subjects with normal EF referred to the cath lab for evaluation of dyspnea. Perform safety lab sampling (complete blood count, creatinine, blood urea nitrogen, sodium, potassium, TSH and T4, aspartate aminotransferase, alanine aminotransferase, total, direct and indirect bilirubin) hs c-RP, NTproBNP, echocardiography, arterial tonometry for pulse wave analysis, and assessment of endothelial function (EndoPAT) prior to administration of study drug.
2) Assess baseline exercise capacity and hemodynamics at rest and during exercise using gold standard invasive techniques in patients with HFpEF. Venous, arterial and coronary sinus blood samples obtained at rest and during exercise. Focused echocardiography examination with measurement of LV and RV tissue Doppler echocardiography and cardiac volumes will be performed.
3) Provide study drug (AZD4831 or matching placebo) orally following the invasive exercise test with the patient waiting for 30 minutes in the cath lab to facilitate therapeutic concentration. Vascular access will be maintained.
4) Repeat resting and exercise invasive hemodynamics with blood sampling and echocardiography exactly as in (2).
5) Measure biomarkers of oxidative stress, NO activity and PK before and after study drug (Soluble ST2, TnI, Gal-3, GDF15, NT-proCIII, C-telopeptide CI, Calprotectin, MMPx, hypoxanthine, xanthine, urate and allantoin, Arg/ADMA/SDMA).
6) At the conclusion of the second exercise test, when all primary and secondary endpoints have been assessed, a single bolus dose of heparin (70 units/kg body weight) will be given to measure plasma MPO before and after. The change in MPO (amount and activity) with heparin will then be related to hemodynamic effects of AZD4831 observed in the earlier part of the trial.
7) Subjects will be transferred to the catheterization lab recovery area, where sheaths will be removed and hemostasis verified according to our standard clinical practice.
8) Repeat assessment of endothelial function will be performed in the recovery area, prior to dismissal.
9) The primary analysis will compare resting and exercise hemodynamic response after AZD4831 or placebo relative to the initial assessment 2-way ANCOVA (using the baseline value as the covariate).
10) A follow up, non-face-to-face videoconference visit will be scheduled between days 9-14 after dosing. History and visual physical exam (including full skin exam) will be repeated. Patients will be instructed to report back immediately if they develop a rash prior to the follow up visit.

Figure 2: Study Flow Diagram



Innovation:

- The use of exercise hemodynamics to measure an intervention's effect is highly novel and clinically meaningful because exercise is the time where symptoms develop and hemodynamics are most perturbed in response to heightened physiological demand. Very few centers in the world could carry out this study.
- The hypothesis that NO availability is limited in part by increased vascular MPO activity is novel and has not been tested in HFpEF.
- No previous therapy in HFpEF has directly targeted the vascular processes that result in both decreased NO and increased ROS that are believed to be the underlying driver of global cardiac and peripheral reserve limitations in HFpEF.
- Myocardial efficiency, assessed by myocardial O2 consumption (using coronary sinus sampling) in relation to myocardial performance, is a highly sensitive method of detecting drug effect on myocardial function.

Significance:

- HFpEF afflicts millions worldwide, and with the aging population the prevalence is growing by 1% per year. This makes identification of effective treatments a major unmet public health need with the potential to change clinical practice.

- MPO inhibition has the potential to uniquely target the underlying hyperactive oxidative stress and inflammation that is believed to represent a common pathway driving the phenotypically diverse HFpEF syndrome.

- In addition, augmentation of NO bioavailability by decreasing its oxidation through MPO inhibition has the potential to improve exercise capacity. This is a clinically relevant end point in this population defined by exercise intolerance.

- Identification of a beneficial effect of MPO inhibition on hemodynamics would provide critical preliminary data to support a larger scale clinical trial in HFpEF focused on surrogate markers of HF severity, exercise tolerance or cardiac remodeling.

3.2 Primary Study Endpoints

The primary endpoint will be the PCWP at 20 Watts exercise after study drug relative to the PCWP at 20 Watts exercise in the initial assessment prior to study drug.

3.3 Secondary Study Endpoints

Secondary exploratory endpoints will be changes in resting PCWP after study drug as well as rest and exercise changes in right atrial pressure, pulmonary artery pressure, pulmonary vascular resistance and compliance, coronary sinus saturation, systemic BP, heart rate, cardiac output, A- V O2 content difference during exercise, VO_2, Ve/VCO_2 slope and Borg effort/dyspnea scores. Increase in cGMP in response to MPO inhibition along with changes in other biomarkers and MPO described above will also be assessed.

3.4 Primary Safety Endpoints

Safety and tolerability will be measured by the incidence of SAEs, AEs and clinical changes in ECG, vital signs and clinical laboratory evaluations.

A maculopapular skin rash can occur which will be monitored. The characteristics of the rash will be documented. Photos including a body overview photo plus a more detailed photo of a representative affected skin area will be taken, if rash occurs. Dermatologic evaluation with biopsy if deemed necessary will be obtained for significant rashes.

3.5 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF):

- Echo findings at baseline, during cath
- EndoPAT
- History and Physical Exam
- Hemodynamic data from all study phases
- Clinical data and Demographics

The following source data will not be directly collected in the Case Report Form (CRF), but will be captured in supportive documentation (study source documents, EMR):

- Laboratory results and clinical interpretation of the values.
- ECG records and clinical significance of observations (when applicable)

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

1. Males and females of non-childbearing potential confirmed at screening by fulfilling one of the following criteria:
   - Postmenopausal defined as amenorrhoea for at least 12 months or more following cessation of all exogenous hormonal treatments and follicle-stimulating hormone (FSH) levels in the postmenopausal range
   - Documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation
2. Age ≥ 30 years
3. Symptoms of dyspnea (II-IV) at the time of screening
4. EF ≥ 50% as determined on imaging study within 12 months of enrollment
5. Catheterization documented elevated filling pressures at rest (PCWP ≥15) or with exercise (PCWP ≥25)

4.2 Exclusion Criteria

1. Use of nitrates, phosphodiesterase 5 inhibitors or other NO-providing therapy in the past 24 hours of screening
2. Significant valvular disease (>moderate left-sided regurgitation, >mild stenosis)
3. Requirement of intravenous heparin at the start of case
4. Severe pulmonary parenchymal disease
5. Acute coronary syndrome or coronary disease requiring revascularization in the judgement of investigators
6. Resting systolic blood pressure < 100 mmHg
7. Constrictive pericarditis

8. Infiltrative, restrictive, or hypertrophic obstructive cardiomyopathies
9. Previous anaphylaxis to any drug
10. Pregnancy or breastfeeding mothers
11. High Output heart failure
12. Active thyroid disease
13. Treatment with a new chemical entity (defined as a compound which has not been approved for marketing) within the preceding 3 months
14. Patients with any prior allergy to propylthiouracil

4.3 Subject Recruitment, Enrollment and Screening

Eligible patients will be identified from screening of the patients listed for exercise right heart catheterization as well as patients with known HFpEF at the Mayo Clinic in Rochester, Minnesota.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Development of any condition that requires study withdrawal related to safety, disease progression, subject decision or failure to adhere to protocol requirements could qualify as reason to withdraw from the study.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Subjects withdrawing from the study will be contacted 2 weeks following study drug administration to ensure that there have been no adverse events. Participants that withdraw may be replaced to allow for achievement of target enrollment.

4.5 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to the sponsor and AstraZeneca.

4.5.1 Maternal Exposure

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

The outcome of all pregnancies (including spontaneous miscarriage, elective termination, and ectopic pregnancy) will be documented.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel informs the sponsor's representatives within 1 day of when he/she became aware of it.

4.6 Reproductive Restrictions

It is important that women of childbearing potential, who are the partners of male subjects, do not become pregnant during the study and for a total period of 3 months after subjects have taken the last dose of IMP.

Non-sterilized males who are sexually active with a female partner of childbearing potential must use condom and spermicide from Day 1 through and for a total period of 3 months after subjects have taken the last dose of IMP. Because male condom and spermicide is not a highly effective contraception method it is strongly recommended that female partners of male study subjects also use a highly effective method of contraception throughout this period.

Highly effective contraception is defined as having a low failure rate, i.e., less than 1% per year when used consistently and correctly. Highly effective contraception forms of birth control include:

Tubal occlusion

- Copper T intrauterine device
- Levonorgestrel-releasing intrauterine system (e.g., Mirena®)
- Medroxyprogesterone injections (e.g., Depo-Provera®)
- Etonogestrel implants (e.g., Implanon®, Norplan®)
- Combined pills
- Norelgestromin/ethinyl estradiol transdermal system (e.g., Ortho Evra®)
- Intravaginal device (e.g., NuvaRing®)
- Cerazette® pill

Male subjects who have been sterilized are required to use 1 barrier method of contraception (condom).

Sperm Donation
Male subjects should not donate sperm for the duration of the study and for at least 3 months after the last day of IMP administration.

Pregnancy
Subjects will be instructed that if their partner becomes pregnant during the study this should be reported to the Investigator. The Investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject's partner is subsequently found to be pregnant after the subject is included in the study, then consent will be sought from the partner and if granted any pregnancy will be followed and the status of mother and/or child will be reported to the sponsor after delivery.

A pregnancy notification form and follow-up will be completed.

5 Study Drug

5.1 Description

The IMP (Investigational Medicinal Product) is a white film-coated tablet containing 5 mg of AZD4831. A matched placebo (**PTM AZD4831 film-coated tablet 5 mg)** is also presented. The IMP will be manufactured by AstraZeneca in accordance with Good Manufacturing Practice (GMP).

5.2 Treatment Regimen

A single administration of MPO inhibitor at a dose of 30 mg or matched placebo, will be given orally following baseline exercise right heart catheterization, if patients meet the above eligibility criteria.

5.3 Method for Assigning Subjects to Treatment Groups

Patients will be randomized 1:1 in a blinded fashion to AZD4831 or placebo with blinding and randomization performed by the Mayo Clinic Research Pharmacy.

5.4 Preparation and Administration of Study Drug

The IMP will be provided in bulk from AstraZeneca, to be packed into study specific containers with study specific labels by the pharmacy, according to the bottling instruction provided by AstraZeneca.

The **AZD4831 film-coated tablet 5 mg** and **PTM AZD4831 film-coated tablet 5 mg** will be supplied in induction-sealed high-density polyethylene bottles with silica desiccant.

5.5 Subject Compliance Monitoring

This will be a single dose, directly observed dosing study so formal checks for long term compliance are not necessary

5.6 Prior and Concomitant Therapy

Participants' chronic medications will not be changed for the purposes of this study. They will continue taking their home medications as prescribed up until the morning of the procedure. Data for baseline medication usage will be obtained.

5.7 Packaging

AZD4831 film-coated tablet 5 mg and **PTM AZD4831 film-coated tablet 5 mg** will be delivered in bulk from AstraZeneca. Empty bottles, caps and desiccant will be delivered separately. Each bottle should be filled with 35 tablets, with 2 desiccant unit added, and be capped and labelled appropriately, including information of expiry date in accordance with bulk information.

5.8 Masking/Blinding of Study

Only research pharmacy staff will be aware of randomization scheme and all study personnel and subjects will remain blinded to the identity of study drug.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipping invoice. Any discrepancies, damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The sponsor-investigator must be notified immediately of any discrepancies, damaged or unusable products that are received.

5.9.2 Storage

The IMP will be stored in a secure facility under appropriate storage conditions. The IMP should be stored in room temperature (<30°C) protected from light prior to use. Details of storage conditions will be provided on the label of the IMP.

5.9.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.9.4 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.

6 Study Procedures

6.1 Visit 1

At Visit 1, subjects will provide written informed consent. Medications will be documented. Safety blood tests (complete blood count, creatinine, blood urea nitrogen, sodium, potassium, TSH and T4, aspartate aminotransferase, alanine aminotransferase, total, direct and indirect bilirubin) hs c-RP, NTproBNP, assessment of endothelial function, radial tonometry for pulse wave analysis, and comprehensive echocardiography will be performed before catheterization.

Invasive right heart catheterization with simultaneous limited echocardiography and expired gas analysis will then be performed at rest and during 20 watts of ergometer exercise as per standard practice for the evaluation of unexplained dyspnea (5 minute exercise duration). Subjects will be given study drug (AZD4831 or matching placebo) orally followed by 30 minutes waiting period in the cath lab to facilitate therapeutic concentration.

Patients will then repeat resting and exercise invasive catheterization at 20 W in exactly the same fashion as prior to study drug. Blood samples will be collected before and after study drug to measure biomarkers of oxidative stress, NO activity and PK.

Following completion of both rest-exercise runs, participants will receive a bolus of intravenous heparin (70 U/kg) to displace tissue MPO. By comparing MPO concentrations and activity prior to study drug, following study drug and then following heparin, we will be able to relate MPO activity to the pharmacodynamic effects of study drug on central hemodynamics.

Subjects will be transferred to the catheterization lab recovery area as per standard clinical practice, where intravascular sheaths will be removed and

hemostasis obtained. Participants will be monitored according to standard practice guidelines following exercise right heart catheterization procedures and discharged on the same day. A repeat EndoPAT test will be performed while the patients are in the recovery area.

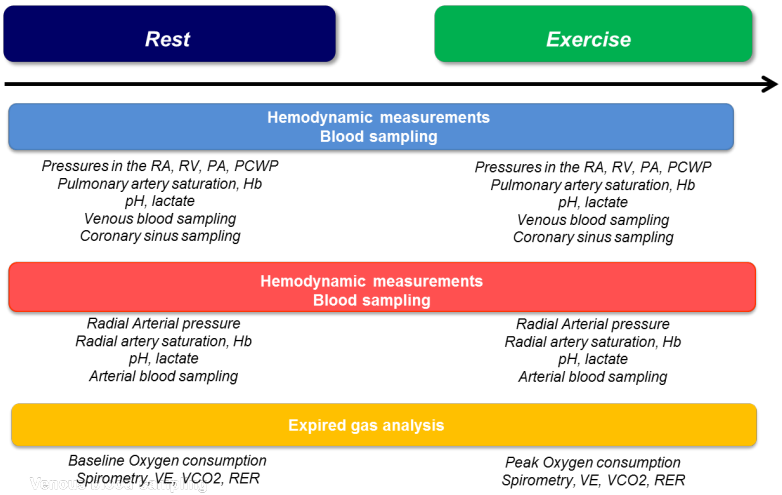
6.2 Visit 2

Visit 2 will be scheduled on day 9-14 after dosing and will be a virtual, NFTF (non-face to face) visit, performed through use of Zoom, similar to other NFTF visits performed clinically at Mayo Clinic. The focus at the virtual NFTF visit will be to assess for any symptoms or adverse events and to perform a full skin examination by video, given the potential for maculopapular rash. If a clinically significant rash is identified dermatologic consultation will be obtained with biopsy if deemed appropriate by the consulting dermatologist. Patients will be instructed to report back immediately if they develop a rash prior to the follow up visit.

Study Assessments (Figure 3):

Right heart catheterization will be performed using high fidelity micromanometers at rest and during supine exercise with simultaneous expired gas analysis (MedGraphics, St. Paul, MN, USA). A 9 Fr Internal jugular catheter for continuous right atrial pressure and to allow introduction of a balloon tipped fluid filled catheter will be placed in the right internal jugular vein. A high Fidelity micromanometer wire will be placed in the balloon wedge catheter to enable accurate pressure recording with exercise. A 4 Fr radial arterial cannula will also be placed for continuous arterial pressure measurement and arterial saturation with exercise (all standard care). The coronary sinus is cannulated at select times to measure myocardial biomarkers and indicators of metabolism. Figure 3 shows the assessments performed in the protocol. The protocol is rest—passive leg elevation—20 Watts exercise for 5 minutes. Hemodynamics, venous, arterial and coronary sinus blood samples, arterial and mixed venous blood gas and expired gas

Figure 3: Assessments During Testing



data are acquired at rest, and during exercise. Detailed list of assessments and timing is found in Appendix I.

Exercise is not performed to exhaustion, but we have previously shown that 80-90% of the increases in cardiac filling pressures that will occur with exercise occur at 20W workload in HFpEF, and the requirement to perform 2 studies in the same day dictates that this lower workload is used uniformly in both tests. Of note, this workload is close to maximal in most patients with HFpEF, and most relevant to the workload of activities of daily living with affected patients. Perceived symptoms of dyspnea and fatigue will be quantified using the Borg dyspnea and effort scores during exercise.

After the initial exercise study, the oral study drug (30 mg AZD4831 or placebo) will be provided. After a 30 minute waiting period for absorption and therapeutic concentration, subjects will repeat resting and exercise hemodynamics with expired gas data acquired exactly as in the initial run including venous, arterial and coronary sinus sampling at rest and exercise.

Heparin will not be used during the studies due to its known effect on liberating vascular MPO and increasing NO bioavailability.[38] At the conclusion of the study, a single bolus of heparin will be administered (70 units/kg) to evaluate vascular MPO levels and activity present in subjects. This will provide a more sensitive measure of tissue MPO activity after drug inhibition to correlate with hemodynamics. Both MPO level and activity will be measured, to allow quantification of specific activity. The blood sample collection for MPO assessment will be done at rest and following exercise, both before and after study drug. In addition following the exercise portion of the study it will be measured before and after heparin administration. Protamine will be given following final laboratory assessments prior to sheath removal to minimize risk of bleeding.

Comprehensive echocardiography will be performed before catheterization for descriptive purposes (appendix II).

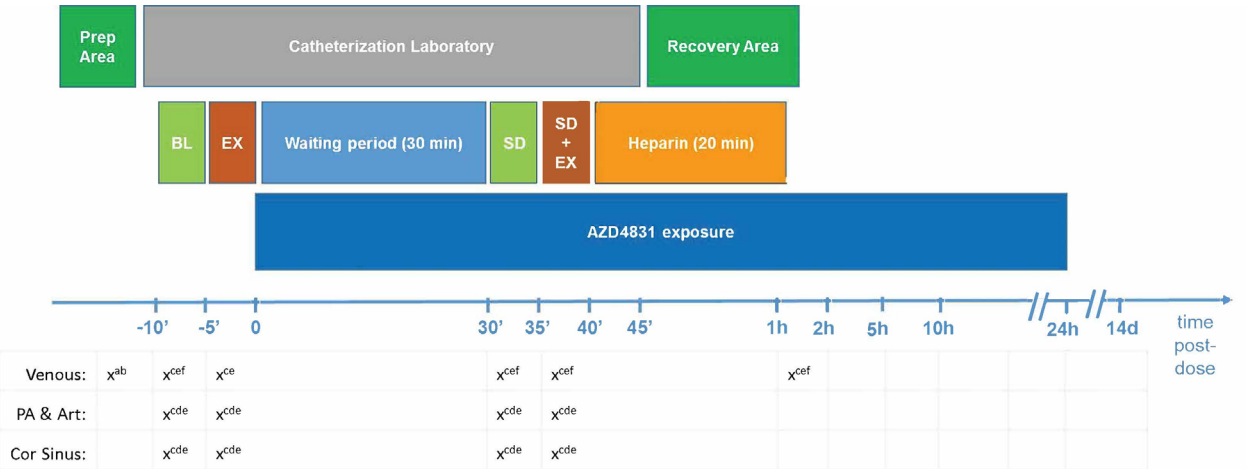
Aortic pulse wave analysis will be determined from invasive radial artery pressure waveforms obtained during the cardiac catheterization procedure.

Focused echocardiography will be done simultaneously with cardiac catheterization focusing on cardiac volumes and measures of biventricular systolic and diastolic function using strain and Tissue Doppler (appendix III). Measurements will be repeated at rest and during exercise before and after the study drug.

Blood samples will be collected during catheterization. Up to 150 cc of blood will be taken to measure arterial and venous blood gases, saturations, and cyclic guanosine monophosphate (cGMP) levels at rest and exercise. Baseline blood samples will be obtained for MPO activity (MPO concentration and activity, Arg/ADMA/SDMA) level and biomarkers of inflammation/oxidative stress (cRP, troponin I, GDF-15, NO metabolites), fibrosis/remodeling (C-

telopeptide CI, sST2, MMP, galactin 3, Pro-collagen III N-terminal peptide) and neurohormonal activation (NT proBNP).

Purine metabolites (hypoxanthine, xanthine, urate and allantoin) will also be quantified at these time points as this may provide important information regarding the mechanisms behind MPO-driven urate reduction. High sensitivity troponin will be assessed as an indicator of myocardial stress. The metabolites and troponin levels will be quantified in samples drawn from the radial artery, superior vena cava, the coronary sinus and from the main pulmonary artery to determine impact of AZD4831 on the different vascular beds. All metabolites should increase in the circulation at exercise. If MPO is a "vasomotor controller, as is believed, then pretreatment with AZD4831 should decrease the concentration of all metabolites. If MPO inhibition only affects the oxidation of hypoxanthine and xanthine, the concentration of these metabolites should be unaffected/increased and urate and allantoin decreased. If AZD4831 unloads the heart, this may reduce troponin levels in the circulation, particularly in the coronary sinus samples. A detailed list of blood sample testing is included below.



a = Safety blood tests + NT-proBNP. Analyzed at Mayo (hematology, liver, kidney, thyroid status).
b = HF-related biomarkers analysed by TATAA Biocenter (possibly a combination of 3 ProSeek panels from OLINK). 5 mL blood. Plasma divided into 0.5 mL aliquots
c = Biomarkers related to MPO biology analysed by AZ (purine metabolites, MPO concentration and activity, Arg/ADMA/SDMA).
d = Blood gases
e = cGMP
f = PK sampling. Exposure analyzed by AZ. 2 mL blood/timepoint.

BL, baseline; EX, exercise at 20W; PA, pulmonary artery; Art, radial artery; Cor Sinus, coronary sinus

Endothelial function testing will be assessed by pulse wave analysis using the Endo PAT (Peripheral Arterial Tone) [40] system beforestudy drug. Indirect serological surrogates of endothelial function including plasma cGMP and NO metabolites will also be assessed before and after study drug. The Endo PAT will be performed for baseline values before catheterization in preparation room and for follow-up values in the recovery area following the procedure, but prior to discharge.

7 Statistical Plan

7.1 Sample Size Determination

No data is available on the effect of an oral MPO inhibitor on exercise PCWP in humans. Based on our previously published data on IV sodium nitrite, the decrease in exercise PCWP was 2±5 with placebo and 11±5 with sodium nitrite [35]. To detect a decrease in PCWP magnitude of this magnitude or greater compared to placebo with 80% power at an α=0.05, only 6 subjects would be required in each group.

However, since we expect the magnitude of hemodynamic effect of an oral MPO inhibitor to be at least 25% less than that of IV sodium nitrite (which would still be a meaningful decrease in PCWP); we will enroll 18 patients in each arm for a total of 36 patients. This will allow for 80% power to detect a reduction in exercise PCWP of 5.3 mmHg or greater, which would be considered to be clinically significant.

7.2 Primary Outcome Analysis

Primary Statistical Hypotheses:

Ha: Exercise PCWP values at 20 Watt workload after study drug relative to corresponding values at 20 Watts workload prior to study drug will be lower after study drug administration compared to placebo

Analysis Summary:
The effect of AZD4831 on the primary endpoint of exercise PCWP will be assessed by analysis of covariance, using the initial exercise PCWP measured before study drug infusion and exercise PCWP following study drug as the covariates. Statistical testing for the primary analysis will be two-sided at the alpha=0.05 level of significance. Other details of the statistical approach will be included in the Statistical Analysis Plan (SAP).

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 disability or incapacity
- 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

For this study, the study treatment follow-up period is defined as the period between Visit 1 and Visit 2. Visit 2 should occur 9 to 14 days after Visit 1. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

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.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

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 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. In addition to this, all SAEs are to be submitted to the AstraZeneca Product Safety mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com The investigator ensures that all the necessary information is provided to the AstraZeneca Product Safety mailbox **within 1 calendar day** of initial receipt for fatal and life threatening events and **within 5 calendar days** of initial receipt for all other SAEs. 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 investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Information collected on the adverse event worksheet

- Subject's name:
- Medical record number:
- Disease (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event:
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs will be reported to the IRB.

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.

8.3.3 Sponsor-Investigator reporting: Notifying AstraZeneca

AstraZeneca will be notified of any maculopapular rash on an ongoing basis, within 7 days of each occurrence is known.

8.4 Unmasking/Unblinding Procedures

The investigators will be given access to the treatment code for their Participants for emergency un-blinding. Any suspected study drug-related events should be treated as though the Participant received active therapy.

Randomization data are kept strictly confidential, accessible only to authorized persons, until the time of un-blinding.

8.5 Stopping Rules

The study may be terminated prematurely if:

- The PI (sponsor) and AstraZeneca assess that the number and/or severity of AEs justify discontinuation of the study. For instance if there were a case of fatal SAE or 2 cases of other SAEs that were considered related by the Investigator and AstraZeneca.
- AstraZeneca considers the applied doses of the study drug to be no longer relevant.
- The PI (sponsor) decides to discontinue the study.
- Data not known before become available and raise concern about the safety of study drug so that continuation would pose potential risks to the subjects.

Premature termination of the study must be mutually agreed upon by the PI (sponsor) and AstraZeneca and must be documented. However, study results will be reported according to the requirements outlined in this clinical study protocol as far as applicable.

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.

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.

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

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

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 –"Retention of and Access to 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

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide periodic study monitoring as a service for the sponsor-investigator, to verify the validity and integrity of the data and protection of human research subjects. Written monitoring reports will be provided to the Sponsor-Investigator and should be provided to the IRB at the time of continuing review. This will assist with compliance with Food and Drug Administration regulations and GCP Guidelines.

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 financed through AstraZeneca. Study drug including MPO inhibitor and placebo will be provided by AstraZeneca.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

12.3 Subject Stipends or Payments

$300 per subject and up to $200 for subject's family/caregiver that may need to stay longer in town for hotel and meal expenses for the extra overnight stay requirement.

13 Publication Plan

The primary responsibility for publication of the results lies with the principal investigator. The trial will be registered on ClinicalTrials.gov.

14 References

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15 Attachments

Appendix I: Schedule of Assessments

	Visit 1 (Day 1)									NFTF Visit 2 (Day 9-14)
	Baseline (Before Cath)	Baseline Cath		Study drug Cath		Heparin phase Cath				
		Rest	20 Watts	Rest	20 Watts	Recovery After Heparin				
Time relative to study drug	-20 min	-10 min	-5 min	30 min	35 min	60 min				
Eligibility Review and Consent	X									
History and physical exam	X									X
Venous Blood samples										
Safety Labs#	X*									
High sensitive CRP	X*									
NT proBNP	X*									
FSH for female, if not previously done	X									
ProSeek panel†		X**								
AZD4831 exposure		X**		X**	X**	X**				
MPO biomarkers ‡		X**	X**	X**	X**	X**				
cGMP and NO metabolites and hs troponin levels		X**	X**	X**	X**	X**				
Pulmonary artery sample										
Pulmonary artery saturation		X	X	X	X					
MPO biomarkers ‡		X**	X**	X**	X**					
cGMP and NO metabolites and hs troponin levels		X**	X**	X**	X**					
Pulmonary capillary sample										
Pulmonary capillary saturation		X	X	X	X					
MPO biomarkers ‡		X**	X**	X**	X**					
cGMP and NO metabolites and hs troponin levels		X**	X**	X**	X**					
Coronary sinus samples										

	Visit 1 (Day 1)									NFTF Visit 2 (Day 9-14)
	Baseline (Before Cath)	Baseline Cath		Study drug Cath		Heparin phase Cath				
		Rest	20 Watts	Rest	20 Watts	Recovery After Heparin				
Coronary sinus blood gas		X	X	X	X					
MPO biomarkers ‡		X**	X**	X**	X**					
cGMP and NO metabolites and hs troponin levels		X**	X**	X**	X**					
Arterial blood samples										
Arterial blood gas		X	X	X	X					
MPO biomarkers ‡		X**	X**	X**	X**					
cGMP and NO metabolites and hs troponin levels		X**	X**	X**	X**					
Endopat	X									
Comprehensive echocardiogram	X									
Limited echocardiography*		X	X	X	X					
Arterial Pulse Wave Analysis	X									
PA pressure		X	X	X	X					
PCWP		X	X	X	X					
RA pressure		X	X	X	X					
A-V O2 content difference		X	X	X	X					
O2 consumption		X	X	X	X					
VE/VCO2, RER		X	X	X	X					
Systemic BP		X	X	X	X					
Heart rate/ ECG/telemetry		X	X	X	X	X				

* Measured at Mayo Clinic
** Measured by/at AstraZeneca
***.
† Soluble ST2, TnI, Gal-3, GDF15, NT-proCIII, C-telopeptide CI, Elastase, Calprotectin, MMPx
‡ Purine metabolites (hypoxanthine, xanthine, urate and allantoin), MPO concentration and activity, Arg/ADMA/SDMA
# Safety labs include: complete blood count, creatinine, blood urea nitrogen, sodium, potassium, TSH and T4, aspartate aminotransferase, alanine aminotransferase, total, direct and indirect bilirubin. TSH and T4 will only be assessed at baseline.

Appendix II: Comprehensive Echocardiogram Data at Baseline

	Value
LV Septal wall thickness	
LV posterior wall thickness	
LV End Diastolic Dimension	
LV End Systolic Dimension	
LVEDV	
LVESV	
LVEF	
LA Volume	
RV dimension base	
RV dimension mid	
Mitral E (m/sec)	
Mitral A (m/sec)	
Early diastolic mitral annular velocity (medial) (medial e')	
Systolic mitral annular velocity (medial) (medial s')	
Early diastolic mitral annular velocity (lateral) (lateral e')	
Systolic mitral annular velocity (lateral) (lateral s')	
RV early diastolic lateral annular velocity (RV e')	
RV systolic lateral annular velocity (RV e')	
TAPSE (mm)	
TR velocity (m/sec)	
RA Pressure estimate (mmHg)	
4 chamber, 2 chamber and 3 chamber view of LV for retrospective LV strain calculations	
4 chamber, 2 chamber and 3 chamber view of LA for retrospective atrial strain calculations	

Appendix III: Echocardiography Data with Exercise

	Value
LVEDV	
LVESV	
LVEF	
Mitral E (m/sec)	
Mitral A (m/sec)	
Early diastolic mitral annular velocity (medial) (medial e')	
Systolic mitral annular velocity (medial) (medial s')	
Early diastolic mitral annular velocity (lateral) (lateral e')	
Systolic mitral annular velocity (lateral) (lateral s')	
RV early diastolic lateral annular velocity (RV e')	
RV systolic lateral annular velocity (RV e')	
TAPSE (mm)	
RV Fractional area change	