Does Sildenafil Improve Endothelial Dysfunction in Rheumatoid Arthritis?

Short Title: SEDRA Study

NCT number	NCT02908490
Test drug	Sildenafil
Study purpose	Efficacy and Safety
Clinical study phase	Phase II
Funding agency	University of Pittsburgh National Institutes of Health / NIAMS Study Drug provided by Pfizer, Inc.

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Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

The Lead Principal Investigator (Protocol Chair) should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of the Regulatory documents and a copy should be maintained at the site.

PRINCIPAL INVESTIGATOR

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Contents

1. PROTOCOL SYNOPSIS

Title	**Does Sildenafil Improve Endothelial Dysfunction in Rheumatoid Arthritis?**
Short title	SEDRA
Clinical study phase	II
Study objective(s)	The primary objective of this study is to determine whether sildenafil use in rheumatoid arthritis (RA) patients leads to improvement in parameters of vascular function and to confirm its safety profile.
Test drug(s)	Sildenafil
Name of active ingredient	Sildenafil
Dose(s)	50 mg once daily
Route of administration	Oral
Duration of treatment	3 months
Reference drug(s)	Placebo
Name of active ingredient	Not applicable
Dose(s)	Matching placebo tablets to sildenafil 50 mg once daily
Route of administration	Oral
Duration of treatment	3 months
Background treatment	Not applicable
Indication	RA patients with no known history of cardiovascular disease(CVD), but at least one traditional cardiovascular (CV) risk factor in addition to their RA

Diagnosis and main criteria for inclusion	a. Meets 2010 American College of Rheumatology (ACR) classification criteria[1] for diagnosis of RA b. Aged 18 years or older c. No known history of CVD (see Exclusion Criteria) d. At least one traditional CV risk factor (i.e., older age [men ≥45 years, women ≥55 years], obesity [defined as body mass index (BMI) >30 kg/m2], smoking, hypertension, hyperlipidemia, diabetes mellitus, family history of premature [defined as diagnosed at <65 years old] CVD in first-degree relative) e. On stable baseline doses of RA medications, defined as no change in dose within past 4 weeks and no anticipated changes over the next 6 months f. On no higher than 10 mg per day of prednisone or prednisone-equivalent within past 4 weeks g. RA disease duration (from symptom onset) of more than 6 months h. Having clinical disease activity index (CDAI) of >2.8 but ≤22 (i.e., either low or moderate disease activity), within 30 days of study enrollment
Study design	6-month prospective phase II, single-center, randomized double-blind, placebo-controlled crossover single-center efficacy trial
Methodology	This study is designed to investigate the efficacy and safety of sildenafil 50 mg once daily in RA patients with no known history of CVD, but at least one traditional CV risk factor. Patients will be randomized 1:1 to receive either sildenafil or placebo for 3 months, then after a 2-week washout, crossed over to each respective group for an additional 3 months. Vascular studies validated in assessing endothelial dysfunction (brachial artery flow-mediated dilation [FMD][2-4] and peripheral arterial tone [PAT])[5] and laboratory studies for selected atherosclerosis biomarkers will be performed at baseline, 3 months pre- and post-washout, and 6 months. Adverse events will be collected to assess sildenafil's safety.

Type of control	Placebo
Number of subjects	Up to 80 randomized subjects (to achieve final total of 60 subjects after expected ~20% drop-out)
Primary variable	Vascular function, determined through brachial arterial flow-mediated dilation (FMD) and peripheral arterial tone (PAT)
Plan for statistical analysis	For the **primary variable**, treatment group (sildenafil vs. placebo) is the *exposure* of interest, with vascular function measures: (1) percent increase in mean maximum brachial artery diameter (%BAD) using FMD (continuous), and (2) mean PAT ratio (continuous) using EndoPAT 2000, as the *primary outcomes* of interest (measured at 0, 3 months pre- and post-washout, and 6 months). Given the cross-over design, analyses will focus on within-subject comparisons of sildenafil versus placebo periods. All tests will be 2-sided with α=0.05. The analysis will use linear mixed models[6], with a term for treatment order (i.e. randomized initially to sildenafil or placebo), to assess whether %BAD and mean PAT ratio differ significantly between the sildenafil and placebo periods within subjects, adjusting for treatment order and CVD risk factors, baseline RA and CV medication use, disease activity (CDAI) and duration, and baseline levels of %BAD and PAT. *Secondary outcomes* of interest for Specific Aim 1 are the frequencies of AEs and SAEs. Since the absolute numbers of these events is anticipated to be small, Fisher's exact tests will be used to compare these frequencies between the initial sildenafil and placebo groups.

List of abbreviations

ACE	angiotensin-converting enzyme
ACR	American College of Rheumatology
AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase (also known as SGPT, *qv*)
AST	aspartate aminotransferase (also known as SGOT, *qv*)
ARB	angiotensin receptor blocker
AUC	area under the plasma concentration versus time curve
BAD	brachial artery diameter
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
cAMP	cyclic adenosine monophosphate
CCP	anti-cyclic citrullinated peptide antibody
CDAI	Clinical Disease Activity Index
CD40L	CD40 ligand
cGMP	cyclic guanosine monophosphate
CI	confidence interval
CRF	case report form
CRP	C-reactive protein
CV	cardiovascular
CVD	cardiovascular disease
DAS28	Disease Activity Score-28
DM	diabetes mellitus
DMARDs	disease modifying antirheumatic drugs
ECG	electrocardiogram
ED	erectile dysfunction
ELISA	enzyme linked immunosorbent assay
ESR	erythrocyte sedimentation rate
FMD	flow-mediated dilation
g	gram
GFR	glomerular filtration rate
HDL	high density lipoprotein
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	human immunodeficiency virus
HOMA-IR	homeostatic model assessment of insulin resistance
HR	heart rate
HRQoL	health-related quality of life
hsCRP	high-sensitivity C-reactive protein
HTN	hypertension
ICAM	intracellular adhesion molecule
IDS	Investigational Drug Service
IL	interleukin
IRB	Institutional Review Board
ITT	intent to treat

kg	kilogram
L	liter
LDL	low density lipoprotein
LFTs	liver function tests
Lp(a)	lipoprotein(a)
MD	medical doctor
mg	milligram
ml	milliliter
mmHg	millimeters of mercury
MMP	matrix metalloproteinase
MOOP	manual of operations and procedures
MPO	myeloperoxidase
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases
NO	nitric oxide
NSAIDs	nonsteroidal anti-inflammatory drugs
OHRP	Office of Human Research Protections
PAH	pulmonary arterial hypertension
PAT	peripheral arterial tone
PDE	phosphodiesterase
PDE5	phosphodiesterase 5
RAPID-3	Routine Assessment of Patient Index Data-3
RDMS	Rheumatic Disease Management System
RF	rheumatoid factor
RHI	reactive hyperemia index
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
UPMC	University of Pittsburgh Medical Center
VCAM	vascular cell adhesion molecule

2. INTRODUCTION

2.1 Background

2.1.1 CARDIOVASCULAR RISK AND ENDOTHELIAL DYSFUNCTION IN RA

- Cardiovascular Risk in RA is Increased, Yet Primary CVD Preventive Care in RA is Lacking

We and others have previously shown that rheumatoid arthritis (RA) is independently associated with an approximately 2-fold increased risk of cardiovascular disease (CVD). Indeed, RA itself is deemed to impart a cardiovascular (CV) risk equivalent to diabetes mellitus (DM). However, unlike in DM, little is known about optimal adjunctive primary and secondary CV prevention strategies for the RA population. Inflammation is known to contribute to CVD risk in RA. Multiple studies show that disease modifying anti-rheumatic drugs (DMARDs) and biologic agents (e.g., tumor necrosis factor [TNF]-α inhibitors) can partially mitigate the increased CVD risk in RA patients. The importance of using these drugs to control inflammation early in RA is well recognized, both for reducing joint damage and long-term CVD risk. However, despite early use of these drugs to treat RA, the mortality gap in RA patients compared to the general population is still widening, in part due to suboptimal primary and secondary CVD preventive care in RA. Furthermore, negative side effects of DMARDs and biologic agents hinder their use as adjunctive primary CVD prevention strategies.

- RA Patients Experience Unique Barriers to Traditional CVD Preventive Care Strategies

In a review of statins and CVD risk in RA, it was noted that statins may be beneficial in primary (but not secondary) CVD prevention in RA. However, they cause more musculoskeletal and gastrointestinal side effects in RA patients than the general population, which limits their routine use. Similarly, pain and fatigue in RA patients limit their abilities to enact lifestyle changes (e.g., exercise) that are well-known to improve CV risk.

- Endothelial Dysfunction: An Important "First Step" in Atherosclerosis and CV Risk in RA

One of the most important early steps of atherogenesis is endothelial dysfunction, which also contributes to the progression of atherosclerotic plaque. The fundamental feature of endothelial dysfunction is impaired nitric oxide (NO) bioavailability, resulting in an impaired ability of the artery to dilate in response to physical and chemical stimuli. NO exerts cardioprotective roles by relaxing smooth muscle cells of the vessel wall, preventing leukocyte migration, adhesion molecule (e.g., vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1) expression, and platelet aggregation. The degree of endothelial dysfunction has been shown in numerous clinical studies in other

high-risk populations to be closely associated with increased risk of future CVD events and adds prognostic value beyond traditional CV risk factors. Specifically, a recent prospective study of 528 stable patients at high risk of CVD events showed that advanced endothelial dysfunction significantly correlated with near future CVD events, and adding a physiological measure of endothelial dysfunction to other risk classification models (e.g., Framingham score, angiographic scoring system, etc.) could be clinically valuable in identifying vulnerable patients.

- Endothelial Dysfunction is Increased in RA Patients

Recent studies have shown that atherosclerosis lesions occur earlier and have a more rapid evolution in RA patients than the general population. In mouse models of collagen-induced arthritis, the severity of endothelial dysfunction mirrored the arthritis severity. In observational human studies, subclinical atherosclerosis and endothelial dysfunction increased in early RA patients and RA patients without CVD risk factors or complications compared to controls.

- Endothelial Dysfunction in RA: Both Inflammation and Traditional CVD Risk Factors Are Important

In RA, both traditional CVD risk factors and markers of RA severity contribute to models predicting CVD events. Recent studies showed abnormal endothelium-independent microvascular function that correlates with inflammation, but is not altered by short-term anti-inflammatory therapy. Classical CVD risk factors and anti-tumor necrosis factor (TNF)-α medications have different effects on micro- and macrovascular endothelial function, suggesting combined CVD prevention approaches (i.e., treating both inflammation and classical CVD risk-related endothelial dysfunction) may be necessary.

- Biomarkers of Endothelial Dysfunction in RA Correlate with Subclinical Atherosclerosis

Biomarkers of endothelial dysfunction are significantly higher in RA patients than controls, even after controlling for medication use or traditional CVD risk factors. VCAM-1 has been associated with common carotid artery intima-media thickness (cIMT) as determined by carotid ultrasonography (US), a measure of subclinical atherosclerosis. In preliminary studies from my current NIH K23 award (K23 AR061407), we have similarly found in a cross-sectional analysis that markers of endothelial dysfunction (ICAM-1, VCAM-1, and E-selectin) were significantly positively associated with cIMT in 46 RA subjects and 70 controls, both overall and in the RA group alone.

- Predictors of Endothelial Dysfunction in RA - Inflammatory Markers and Higher Disease Activity

Sarli et al investigated predictors of endothelial dysfunction (as measured by brachial artery FMD) in patients with RA in a case-control study, and found RA patients had significantly more endothelial dysfunction than controls. Erythrocyte sedimentation rate (ESR), disease duration, and disease activity score in 28 joints (DAS28) were independent predictors of impaired FMD in RA.

2.1.2 SILDENAFIL

- Sildenafil is a Well-Suited Agent Targeting Endothelial Dysfunction

Drugs that improve endothelial dysfunction hold promise for preventing CVD. Sildenafil, which has been used to treat erectile dysfunction (ED) and pulmonary arterial hypertension (PAH), enhances NO signaling by inhibiting phosphodiesterase-5 (PDE5). (**Figure 1**) In a mouse model of atherosclerosis, sildenafil restored endothelial function by reducing oxidative stress and plaque deposition in the aorta. Sildenafil has a shorter half-life (4 hours) than other PDE5 inhibitors (e.g., tadalafil or vardenafil), making it well-suited for a crossover study by minimizing potential carryover effects. In pharmacodynamics studies, the effect of sildenafil on erectile response showed diminished response at 4 hours compared to 2 hours, and the effect on blood pressure in healthy volunteers was not different than placebo at 8 hours, irrespective of dosage. Notably, in previous PAH trials, there was significant clinical worsening in PAH after a 2-week washout of sildenafil.[51] It is likely that sildenafil's effects on the brachial vasculature would be similar to other vascular beds. Hence, the short drug activity effects (up to 8 hours) is unlikely to pose significant carryover effects.

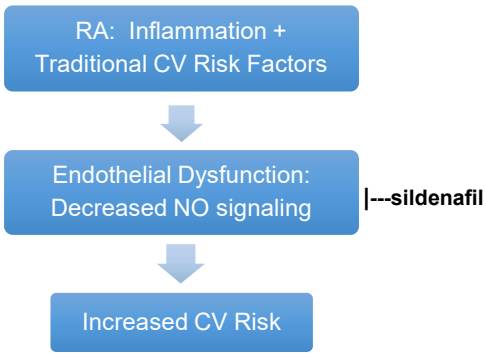

Figure 1

- PDE Inhibitors Have Pleiotropic Beneficial Effects and Immunomodulatory Properties

PDE5 inhibitors like sildenafil have been shown to improve endothelial function in patients with PAH and DM. They have also been found to have pleiotropic beneficial effects and were safe and well-tolerated over long-term use in patients with ED coexistent with multiple other CV comorbidities. In studies of patients with ED and stable CAD, sildenafil at doses of 50 mg to 100 mg while off nitrates improved cardiac function significantly more than placebo. Phosphodiesterases (PDE) and their substrates (e.g., cAMP and cGMP) are ubiquitously expressed in the immune system. PDE inhibition exerts anti-inflammatory activity by increasing intracellular cAMP. PDE4 inhibitors have efficacy in

experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis. Dual PDE4 and PDE7 inhibitors decreased the severity of collagen induced arthritis (CIA) in a rat model of RA. The PDE4 inhibitor apremilast was shown in a phase 2 clinical trial in Behcet's disease and phase 3 human randomized controlled clinical trials in psoriatic arthritis to be effective, safe, and well-tolerated. Both psoriatic arthritis and Behcet's are also systemic autoimmune diseases like RA. PDE5 inhibitors like sildenafil also have immunomodulatory actions, though human studies to date have primarily focused on end-organ manifestations of autoimmune diseases like PAH, Raynaud's phenomenon, renal disease (e.g., IgA nephropathy), and pulmonary fibrosis. Although sildenafil is much more selective for PDE5 (4,000-fold), it also has >700-fold selectivity for PDE4 and other PDEs; hence, it is promising as a potential agent to target not only endothelial dysfunction but inflammation in patients with RA.

3. Study Drug

3.1 Mechanism of Action

Sildenafil is a phosphodiesterase-5 (PDE5) inhibitor. PDE5 normally breaks down the intracellular second messenger of NO, cGMP. Hence, PDE5 inhibition by sildenafil results in increased cGMP and improved endothelial function, with vascular smooth muscle cell relaxation. Sildenafil has a shorter half-life (4 hours) than other PDE5 inhibitors (e.g., tadalafil or vardenafil), making it well-suited for a crossover study by minimizing potential carryover effects. In pharmacodynamics studies, the effect of sildenafil on erectile response showed diminished response at 4 hours compared to 2 hours, and the effect on blood pressure in healthy volunteers was not different than placebo at 8 hours, irrespective of dosage. Notably, in previous PAH trials, there was significant clinical worsening in PAH after a 2-week washout of sildenafil. It is likely that sildenafil's effects on the brachial vasculature would be similar to other vascular beds. **Hence, the short drug activity effects (up to 8 hours) is unlikely to pose significant carryover effects.**

3.2 Rationale

Rheumatoid arthritis (RA), a chronic inflammatory disorder affecting the joints, is associated with a 2-fold increased risk of cardiovascular disease (CVD) and sudden death, which is not explained by traditional cardiovascular (CV) risk factors alone. CVD is a strong contributor to excess morbidity and

mortality in RA, and is likened to an extra-articular disease manifestation akin to a "rheumatoid vasculopathy." Indeed, RA itself imparts a CV risk equivalent to diabetes mellitus (DM). It is well-recognized that chronic inflammation contributes to CVD risk in RA patients. Studies show that early tight control of disease activity in RA reduces disability and long-term CVD risk. Yet, despite early treatment for RA, the mortality gap in RA compared to the general population is still widening, in part due to suboptimal primary and secondary CVD preventive care in RA. Hence, besides early tight disease control in RA, **attention must now shift to adjunctive primary prevention strategies to mitigate CVD risk in these high-risk patients**. To date, there are no published controlled intervention trials for primary CV prevention in RA, despite this clearly urgent unmet need. The only such trial, TRACE-RA (a placebo-controlled double-blind primary CVD prevention trial in RA with atorvastatin) was stopped prematurely due to low numbers of CVD events that occurred, underscoring the need for use of subclinical CVD endpoints rather than hard CVD events in such trials.

One of the early stages of atherosclerosis is endothelial dysfunction, which is closely associated with risk of future CVD. Using drugs to target endothelial dysfunction is a promising novel strategy for CVD prevention. In RA, inflammation mediates premature endothelial dysfunction by activation of endothelial cells. The fundamental feature of endothelial dysfunction is impaired nitric oxide (NO) bioavailability. NO exerts cardio-protective roles by relaxing vascular smooth muscle cells and preventing leukocyte adhesion. Sildenafil, which is used to treat erectile dysfunction (ED) and pulmonary arterial hypertension (PAH), enhances NO signaling by inhibiting phosphodiesterase-5 (PDE5). In an experimental mouse model of atherosclerosis, sildenafil restored endothelial function, with a 40% decrease in aortic plaque. In human studies, PDE5 inhibitors including sildenafil improved endothelial function in PAH and DM, and were safe and well-tolerated in those with CV co-morbidities. Sildenafil's favorable safety profile has been studied longer than any other PDE5 inhibitor. Furthermore, PDE inhibitors have immunomodulatory properties that hold promise in treating autoimmune conditions like RA.

Predictors of endothelial dysfunction in RA include markers of inflammation and higher disease activity. In RA, abnormal microvascular endothelial function correlated with inflammation, but was not altered by short-term anti-inflammatory therapy; hence, combined CVD-prevention approaches beyond anti-inflammatory therapy may be necessary. In light of this background, **our central hypothesis is that**

sildenafil, by targeting endothelial dysfunction, may be a uniquely suited agent and novel adjunctive primary CVD prevention strategy in RA. Specifically, our goal is to determine if sildenafil use in RA patients improves endothelial dysfunction, inflammation, and atherosclerosis biomarkers.

4. Risk-Benefit assessment

4.1 Benefits of Study in General:

The benefits from the results of this study will assist in determining whether sildenafil improves endothelial function in RA, which may be important in arresting one of the first steps in the pathogenesis of atherosclerosis and development of future CVD events in RA. Such knowledge may result in a better understanding of the disease process and ultimately may lead to new adjunctive primary preventive and therapeutic approaches for cardiovascular risk associated with RA.

4.2 Potential Risks of Study in General:

Common side effects of the vascular studies (occurring in less than 25% of patients) include discomfort, numbness, or pain from the blood pressure cuff occluding the subject's arm. The EndoPAT and flow mediated dilation tests will be done together at the same time. The subjects will be asked to lie still on their back during the tests; this could cause stiffness, back pain or dizziness. During the tests, the occlusion cuff that will be placed on the subject's arm will stay inflated for 5 minutes. The cuff will squeeze the arm tightly; this could cause minor pain, possible bruising, numbness, tingling and irritation to the skin. If the subject experiences pain or numbness that is bothersome, s/he will be asked to alert the technician performing the test. During the EndoPAT test the finger probes will fit snugly over the index fingers and stay on for about 20 minutes. This could cause numbness, tingling and possible irritation to the skin. Again, if the subject experiences pain or numbness that is bothersome, s/he will be asked to alert the technician performing the test.

Rare study risks (occurring in less than 1% of patients) include minor skin irritations from the ECG leads.

To minimize these risks, a physician and emergency drugs and equipment will be readily available at the VCTRC site where the vascular studies will be performed. Subjects will be asked during and after the

testing how they are feeling and their responses will be noted. The test will be stopped at any point as per the subject's discretion. Subjects will be monitored for gross ECG changes and arrhythmias during the study at all times. Continuous monitoring by pulse oximetry will be maintained during the study. Subjects may be monitored for 30 to 90 minutes after completion of the vascular studies.

There is a small risk that confidentiality may be breached. Data will be kept confidential in a secure database. All forms will be stored in locked filing cabinets. Electronic data will be identified by patient ID only. All computer files will be protected from unauthorized access.

5. STUDY OBJECTIVES

5.1 Primary endpoint

- Specific Aim 1: To determine whether sildenafil use in RA patients leads to improvement in parameters of vascular function; and to confirm its safety profile.

5.2 The Primary Efficacy Outcome measures will be:

5.2.1 Brachial Artery Flow Mediated Dilation (FMD) Without Nitroglycerin

The methods of assessment of endothelial function via FMD will be performed following guidelines. Vascular reactivity will be measured noninvasively using vascular ultrasound imaging. Flow and diameter changes of the brachial artery will be assessed by Duplex ultrasound using a high-resolution linear array transducer. The difference between the maximum brachial artery diameter (BAD) post-occlusion and the baseline diameter is expressed as a percentage (%BAD). Generally, %BAD values below 5-7% represent endothelial dysfunction, which is associated with CV risk factors, future CVD and mortality. Effective treatment of risk factors may improve endothelial function. Given its reversibility and non-invasiveness, brachial artery FMD is attractive as an assessment tool to monitor strategies to reduce CVD.

5.2.2 Peripheral Arterial Tone (PAT)

PAT measured by the EndoPAT 2000 device is a non-invasive method to assess endothelial function. It is a standardized, rapid, and easy to apply method, and has been found to correlate with multiple traditional CV risk factors and to be responsive to interventions. PAT is a validated alternative measure to brachial arterial FMD in assessing endothelial function, and is less operator-dependent than FMD. FMD directly measures the dilation capability of the large-conduit artery, whereas PAT measures flow response hyperemia, which is related to endothelial function of small arteries of microcirculation. PAT measures endothelium-mediated changes in vascular tone using bio-sensors placed on fingertips. Changes in arterial tone are elicited by creating a down-stream hyperemic response of the brachial artery from arm occlusion. Contra-lateral arm measurements are used to control for concurrent non-endothelial dependent changes in vascular tone. The semi-automatically calculated result is an index of endothelial function.

5.2.3 Safety Profile: Adverse Event Outcomes:

All adverse event (AE) and serious adverse event (SAE) data will be collected by separate study personnel from those collecting other outcomes; we will document AE and SAE diagnosis, date of onset and resolution, severity, whether it was unexpected or related to intervention; whether the blind was broken; and outcome. Specific AE data will include all common side effects of sildenafil as per Micromedex.

5.3 Secondary endpoint

- Specific Aim 2: To determine whether sildenafil use in RA patients is associated with improvement in serum atherosclerosis biomarkers.

Biomarkers for endothelial dysfunction, atherosclerosis and inflammation will be measured as follows:

High-sensitivity CRP (hsCRP), ESR, rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibody (CCP) will be measured in all patients, using standard clinical laboratory protocols. Blood nitrite levels will be measured using chemiluminescence. The following additional selected biomarkers

involved in endothelial dysfunction and atherosclerosis will be measured in all subjects using enzyme linked immunosorbent assay (ELISA): leukocyte adhesion molecules (E-selectin, ICAM-1, VCAM-1), CD40 ligand (CD40L), interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), and myeloperoxidase (MPO).

6. INVESTIGATORY TEAM

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7. STUDY DESIGN

7.1 Overview

After obtaining written informed consent, eligible participants will be randomized in a 1:1 fashion to receive either sildenafil 50 mg orally once daily or placebo orally once daily for 3 months. After 3 months, all subjects will undergo a 2-week washout period. Then, subjects in each group will cross over to the respective group (i.e., those receiving sildenafil will receive placebo, and vice versa), for an additional 3 months. This cross-over design will aid in recruitment feasibility and was based in part on known patient preferences from other trials, since all subjects will receive a potentially beneficial drug at some point during the study. It also allows for up to 80 recruited subjects to contribute safety data for Specific Aim 1. Since sildenafil has a half-life of only 4 hours and pharmacodynamics effects of only 8 hours, and there will be a 2-week washout period, there is unlikely to be a substantial carry-over effect of the drug during the cross-over phase. Any potential for carry-over effect will also be accounted for by including treatment order in our statistical models. We will use Stata statistical software to randomly assign treatment status using randomized blocks of size 6.

7.2 Phase one

7.2.1 Screening phase (up to 4 weeks)

After signing the informed consent document, subjects will undergo a screening to include baseline labs (creatinine and LFTs and HIV and urine pregnancy test) and review of the inclusion/exclusion criteria. This will include the Consent & HIPAA process.

Note: Screening and baseline can occur on the same day if clinical labs (creatinine and LFTs) have been drawn as part of routine care within 90 days of the baseline.

7.3 Phase two

7.3.1 Initial Study Treatment phase (Month 0 to Month 3)

Once Screening is fully satisfied, participants will proceed to the Baseline visit. At the Baseline study staff will dispense Study Drug.

Note: Screening and baseline can occur on the same day if clinical labs (creatinine and LFTs) have been drawn as part of routine care within 3 months of the baseline.

Participants will begin use of study drug the day after baseline visit and will remain on that regimen (changes notwithstanding) for 3 months (+/- 7 days).

7.4 Phase three

7.4.1 Washout phase (Month 3 to 2 weeks-post Month 3)

At this visit participants will surrender their study drug supply and begin a two-week long washout period, after which they will return for the Crossover visit, a.k.a., 3-months plus 2-weeks, a.k.a. Visit Four.

In disputes about the timing of visits the priority must go to ensuring the participant is washed-out of the first phase of study drug use for no less than 14 days. If that gap, for any reason, must be longer, justification should be provided in the study participant's progress notes.

7.4.2 Crossover Treatment phase (Month 3 post 2-week-washout to Month 6)

At this visit, the Month 3 plus 2-weeks post-washout visit, participants will be switched to whatever study drug they did not utilize in the first three months (Sildenafil to placebo or placebo to Sildenafil).

Participants will remain in this phase, taking the crossover treatment for 3-months (+/- 7 days)

7.4.3 Termination Visit and Safety Follow-up Visit

This is the final visit of the study. It marks the 6-month time point which is the culmination of the first 3-month phase, the 2-week washout phase, and the second 3-month phase.
There are no planned visits beyond the Termination visit.

8. STUDY POPULATION

8.1 Inclusion criteria

Subjects must meet the following criteria to be eligible for enrollment in the study:

1) Meets 2010 American College of Rheumatology (ACR) classification criteria for diagnosis of RA (see MOOP, Appendix 2)

2) Aged 18 years or older

3) No known history of CVD (see Exclusion Criteria)

4) At least one traditional CV risk factor (i.e.,
    a. older age [men ≥45 years, women ≥55 years],
    b. obesity [defined as body mass index (BMI) >30 kg/m2],
    c. smoking,
    d. hypertension,
    e. hyperlipidemia,
    f. diabetes mellitus,
    g. family history of premature [defined as diagnosed at <65 years old] CVD in first-degree relative)

5) On stable baseline doses of RA medications (defined as no change in dose within past 4 weeks and no anticipated changes over the next 6 months)

6) On no higher than 10 mg per day of prednisone or prednisone-equivalent within past 4 weeks (however, intra-articular corticosteroids are permitted)

7) RA disease duration (from symptom onset) of more than 6 months

8) Having clinical disease activity index (CDAI) of >2.8 but ≤22 (i.e., either low or moderate disease activity), within 30 days of study enrollment

8.2 Exclusion criteria

Subjects who meet any of the following criteria will be excluded from enrollment in the study:

1) Aged <18 years old
2) Pregnant women
3) Known personal history of CVD: clinical diagnoses of
    a. stroke,
    b. transient ischemic attack,
    c. myocardial infarction,
    d. acute coronary syndrome,
    e. peripheral arterial disease,
    f. percutaneous coronary intervention
    g. coronary bypass graft surgery
4) Use of high-dose statins (i.e., atorvastatin 40-80 mg/day or rosuvastatin 20-40 mg/day) currently or within past 3 months, or any dose changes of statins or of blood pressure medications that may affect endothelial function (i.e., angiotensin-converting-enzyme [ACE] inhibitors or angiotensin receptor blockers [ARBs]) within past 3 months. If on statin or an ACE-inhibitor or ARB, there should be no anticipated dose changes over the next 6 months.
5) Persons with intra-cardiac and intra-pulmonary shunts, unstable cardiopulmonary conditions, or anyone on chronic oxygen therapy
6) Persons taking nitric oxide donors, organic nitrites and nitrates, such as glyceryl trinitrate (nitroglycerin), sodium nitroprusside, amyl nitrite ("poppers")

7) Severe hepatic impairment (liver function tests >1.5 times upper limit of normal) within past 4 weeks

8) Severe impairment in renal function (serum creatinine ≥1.5 mg/dL) within past 4 weeks

9) Hypotension (defined as blood pressure [BP] <90/60)

10) Hereditary degenerative retinal disorders (including genetic disorders of retinal phosphodiesterases)

11) Persons already taking (or taken within 3 months) sildenafil or other PDE inhibitors (i.e., tadalafil, vardenafil)

12) Persons unable to provide voluntary written informed consent

13) Severe hypertension (BP >170/110)

14) Persons with HIV/AIDS

8.3 Justification of selection criteria

The selection criteria were carefully selected to exclude subjects from the study who may potentially be exposed to specific risks after administering the study drug as well as subjects with conditions that may have an impact on the aims of this study.

Subjects over 65 years old will be excluded due to safety concerns regarding use of the 50 mg daily dose of sildenafil in this age group, and inability to achieve blinding with 25 mg daily dose due to altered pharmacokinetics of the drug if over-encapsulated.

8.4 Withdrawal of Subjects

Subjects *must* be withdrawn from the study for the following reasons:

- At their own request or at the request of their legally acceptable representative

  At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.

- Development of any of the exclusion criteria except for #4 (new or changed doses of CV and RA medications will be documented and addressed via statistical adjustment).

- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being

- Occurrence of AEs or inter-current diseases which the investigator judges unacceptable for continuation of participation in the study

- Pre-specified stopping rules for adverse events: Participants will be automatically withdrawn from the trial if any of the following adverse events occurs: hypotension with BP < 80/50, myocardial infarction, non-arteritic ischemic optic neuropathy, retinal hemorrhage, sudden hearing loss, priapism (prolonged penile erection), and pregnancy.

- Occurrence of adverse drug reactions, which in the investigator's opinion have a negative impact on the subject's individual risk-benefit ratio.

- Non-compliance with the conditions for the trial or instructions by the investigator

- Although not preferred, subjects may interrupt their intake of study medication for reasonable circumstances/reasons at any time (e.g., hospitalization in a remote hospital without study medication access, safety reasons, and side effects). If study medication nonadherence is >50% by pill counts, or if the nonadherence is for 14 or more consecutive days before the study visit, the subject must be withdrawn. In case treatment requires interruption for> 3 days and ≤ 14 consecutive days before the study visit, it is at the discretion of the investigator if the study medication can be restarted. However, any study medication nonadherence will be documented, and interruption of medication use for 3-45 days will be recorded as a protocol deviation.

- In case of pregnancy or breast feeding.

- In case the subject does not tolerate the drug.

8.5 Replacement of Withdrawn Subjects

There will be no replacement of randomized subjects who withdraw from the study.

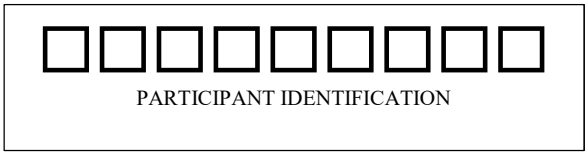
For withdrawn subjects, certified return receipt of drug will be requested by mailing postage-paid mailers to the subjects, after at least three call attempts.

8.6 Subject Identification

After a subject signs the informed consent form, a subject identification number will be created. This will be a unique code composed of study name and subject number

First 5 letters = SEDRA (to identify with this trial)

Last 4 digits = Unique subject number (chronologically entered in Recruitment and Screening Log)



Within the Rheumatic Disease Management System (RDMS) electronic research database, each study subject will be assigned a random 9-digit study ID number, which will be used for source documents, blinded study drug dispensation, etc.

8.7 Study Population Demographics

The following demographic data will be recorded:

- Date of birth (month and year) (age)
- Sex
- Race/Ethnicity
- Socioeconomic data
- Education level
- Emergency Contact
- Mailing address

- Contact information
- Medical History - A medical history will be collected by study personnel including all active conditions (and corresponding organ systems) and non-active conditions (and corresponding organ systems) going back one year.

9. TREATMENT

9.1 Study drug

Test Drug:	Sildenafil
Dosage:	50 mg
Route of administration:	oral
Time and frequency of administration:	once daily

9.1.1 Sildenafil

Sildenafil will be supplied at 50 mg as film-coated, immediate-release tablets. Beginning May 1, 2019, sildenafil will be supplied as two 20-mg and two 5-mg tablets to equal a 50 mg dose. All study drug and placebo tablets will be provided through the UPMC Investigational Drug Service, who will also be handling patient randomization.

9.1.2 Matching placebo

Matching placebo tablets will appear identical to active sildenafil tablets but will not contain active study drug.

9.2 Justification of study drug selection

This dose was chosen because it is the usual dose used and FDA-approved for the treatment of erectile dysfunction (ED), with a known favorable safety profile at that dose. Furthermore, this was the dose used in a double-blind, placebo-controlled, prospective trial of 24 men with type 2 diabetes with ED, wherein it was shown that daily use of sildenafil 50 mg/day for 10 weeks resulted in improved endothelial function as measured by brachial arterial FMD.

Sildenafil 50 mg orally once daily for 3 months will be provided in bottles during the sildenafil treatment periods.

Placebo pills with the same size, shape, color, and texture as sildenafil will also be provided in bottles, to be taken orally once daily for 3 months during the placebo periods.

9.3 Assignment – Randomization

This is a randomized, double-blind, placebo-controlled, crossover, single-center study. Subjects who complete all screening procedures and meet all the eligibility criteria are to be randomized in a 1:1 ratio by the UPMC IDS.

9.4 Blinding

The study will be conducted in double-blind fashion. Active sildenafil and placebo tablet formulations will be identical in appearance (size, shape, color) and smell. The packaging and labeling will be designed to maintain blinded conditions for the investigator and the study team. The study data will remain blinded until database lock.

9.4.1 Allocation Concealment and Blinding

The study will be blinded such that neither the investigator(s) nor the subject(s) will know the allocation assignment. To ensure enforcement of this, a biostatistician not involved with patient care will generate the treatment status randomization list using Stata (Stata Corp, USA). The randomization sequence will be kept confidential by an independent data handler. A secure password-protected electronic database will be used for eligibility confirmation and allocation assignment. Different study personnel will collect adverse event data vs. outcome data, to minimize risk of unblinding. Criteria for breaking the blinding will include adverse risk benefit profiles in either group, as determined by the Institutional Review Board and/or safety monitor.

9.4.2 Emergency Unblinding

In case of emergency, the investigator is permitted to unblind individual cases. However, this will be restricted to cases of emergency where the unblinding result is of importance for the acute treatment strategy. The occurrence of an SAE should not routinely precipitate the immediate unblinding of the

label. Date, time, and reason for unblinding will be captured. Unblinding will be coordinated through the Investigational Drug Service.

9.5 Drug logistics and Accountability

Drug logistics and accountability

9.5.1 Institutional level accountability

Management of Study Drug, its ordering, receipt, storage, maintenance, dispensing, destruction and record-keeping will be maintained by the UPMC Investigational Drug Service (UPMC IDS). This will include a complete record of batch numbers and expiration dates of all study treatment, as well as the labels, will be maintained by the UPMC IDS.

9.5.2 Participant Level accountability

A drug dispensing log will be completed for each subject. Subjects will receive all study medication dispensed at the baseline visit and again at the Month 3 post-washout visit. At months 3 and 6, subjects will be instructed to bring all study drug packaging, including unused study drug and empty packaging, to the investigative site. Tablets will be counted for a compliance check and drug accountability. When Study Drug is returned to the site, study staff will return all study drug related materials back to the UPMC IDS for management.

- <u>If the participant misses a dose</u>

If a dose of study drug is missed, the subject should take a dose immediately if on the same day. Otherwise, they can resume normal once daily dosing the following day. The dose should not be doubled to make up for a missed dose within the same day.

9.6 Concomitant Therapies

The following must be avoided during the timeframe in which subjects are taking the study drug therapy.

9.6.1 Off limits

Nitrates or NO donors (such as amyl nitrate) in any form, including topical; phosphodiesterase (PDE) 5 (PDE5) inhibitors (such as sildenafil, tadalafil, vardenafil); and nonspecific PDE inhibitors (theophylline, dipyridamole) are prohibited.

Glucocorticoid maintenance dose increases to higher than 10 mg/day for past 4+ weeks and/or any change or initiation of new DMARDs or biologic agents between study visits will be collected. Subjects with either of these medication changes will be withdrawn. We anticipate the number of subjects requiring RA medication changes to be small, since the inclusion criteria mandates that subjects be on stable doses of RA medications with no anticipated changes over the next 6 months.

9.7 Premature Discontinuation of Study Drug

In the event of premature discontinuation of study drug treatment, subjects must undergo the same procedures as outlined for the Month 6 end of study visit.

10. STUDY PROCEDURES

10.1 Timing of procedures

Screening and baseline visits can occur on the same day.

- Visit Windows

There are windows of time specific to any given visit in which the visit may take place without deviating from the protocol by being “Late” or “Early”.

- Baseline Visit must occur within 28 days of Screening, otherwise, Screening must be repeated.
- Three Month Visit must occur three months +/- 7 days after Baseline
- The Two Week Washout Visit must take place between fourteen and seventeen days after Three Month Visit
- Six Month Visit must occur three months +/- 7 days after the Two Week Washout Visit

10.2 Visit Breakdown

10.2.1 Visit 1 – Screening

Screening evaluations will occur after the subject has provided written informed consent. The following evaluations will be performed and information obtained up to 28 days before baseline randomization and the start of study drug treatment:

- Patient information and obtaining of written informed consent
- Eligibility: Assessment of inclusion and exclusion criteria
- Demographic data, including age, gender and ethnicity
- Complete medical history (obtained through patient interview and medical record review)
- Liver function tests and creatinine with GFR. Labs obtained as part of routine clinical care up to 3 months prior to screening will be accepted.
- HIV 1/2 test (results within 3 months prior to screening will be accepted)

If re-screening is required, the liver function tests, creatinine, HIV 1/2 test results, and urine pregnancy test (if applicable) from the previous screening visit may be used if no longer than 3 months prior.

10.2.2 Visit 2 – Baseline

The following assessments will be performed at the Baseline visit (Day 0 of study drug treatment):

- Reconfirmation of eligibility (assessment of inclusion/exclusion criteria), if performed at separate visits
- Cardiovascular history and assessment of CV risk factors
- Assessment of RA Disease Activity (questionnaires)
    - Routine Assessment of Patient Index Data (RAPID-3)
    - DAS28
    - CDAI (including tender and swollen joint counts, patient and assessor VAS)
- Additional RA Questionnaires
    - RA medications
    - RA disease duration (calculated from date of symptom onset)
- Vital signs (blood pressure x3, heart rate, height, weight, waist:hip circumference)

- Vascular Imaging (to be performed simultaneously)
    - Brachial FMD
    - EndoPAT
- Pregnancy test (urine) for all women of childbearing potential
- Blood sample (at least 4-hr fasting)
    - Biomarkers (hsCRP, ESR, RF, CCP, E-selectin, ICAM-1, VCAM-1, CD40L, IL-6, MMP-9, and MPO)
    - Nitrite levels
    - Total cholesterol, LDL, HDL, Triglycerides, Homocysteine, Lipoprotein A
    - Glucose and insulin
- Recording and assessment of AEs
- Recording of Concomitant therapy (including CV medications)
- Randomization to initial sildenafil or placebo
- Dispense study drug

10.2.3 Visit 3 – 3months

- Reconfirmation of eligibility (assessment of inclusion/exclusion criteria), if performed at separate visits
- Cardiovascular history and assessment of CV risk factors
- Assessment of RA Disease Activity (questionnaires)
    - Routine Assessment of Patient Index Data (RAPID-3)
    - DAS28
    - CDAI (including tender and swollen joint counts, patient and assessor VAS)
- Additional RA Questionnaires
    - RA medications
    - RA disease duration (calculated from date of symptom onset)
- Vital signs (blood pressure x3, heart rate, height, weight, waist:hip circumference)
- Vascular Imaging (to be performed simultaneously)
    - Brachial FMD
    - EndoPAT

- Pregnancy test (urine) for all women of childbearing potential
- Blood sample (at least 4-hr fasting)
    - Biomarkers (hsCRP, ESR, RF, CCP, E-selectin, ICAM-1, VCAM-1, CD40L, IL-6, MMP-9, and MPO)
    - Nitrite levels
    - Total cholesterol, LDL, HDL, Triglycerides, Homocysteine, Lipoprotein A
    - Glucose and insulin
- Recording and assessment of AEs
- Recording of Concomitant therapy (including CV medications)
- Study Drug Compliance
- Two-week study drug washout period begins the day after the last pill was taken

10.2.4 Visit 4 – 3months post washout

- Reconfirmation of eligibility (assessment of inclusion/exclusion criteria), if performed at separate visits
- Cardiovascular history and assessment of CV risk factors
- Assessment of RA Disease Activity (questionnaires)
    - Routine Assessment of Patient Index Data (RAPID-3)
    - DAS28
    - CDAI (including tender and swollen joint counts, patient and assessor VAS)
- Additional RA Questionnaires
    - RA medications
    - RA disease duration (calculated from date of symptom onset)
- Vital signs (blood pressure x3, heart rate, height, weight, waist:hip circumference)
- Vascular Imaging (to be performed simultaneously)
    - Brachial FMD
    - EndoPAT
- Pregnancy test (urine) for all women of childbearing potential
- Blood sample (at least 4-hr fasting)

- Biomarkers (hsCRP, ESR, RF, CCP, E-selectin, ICAM-1, VCAM-1, CD40L, IL-6, MMP-9, and MPO)
- Nitrite levels
- Total cholesterol, LDL, HDL, Triglycerides, Homocysteine, Lipoprotein A
- Glucose and insulin

- Recording and assessment of AEs
- Recording of Concomitant therapy (including CV medications)
- Dispense Study Drug

10.2.5 Visit 5 – 6months – END OF STUDY

- Reconfirmation of eligibility (assessment of inclusion/exclusion criteria), if performed at separate visits
- Cardiovascular history and assessment of CV risk factors
- Assessment of RA Disease Activity (questionnaires)
  - Routine Assessment of Patient Index Data (RAPID-3)
  - DAS28
  - CDAI (including tender and swollen joint counts, patient and assessor VAS)
- Additional RA Questionnaires
  - RA medications
  - RA disease duration (calculated from date of symptom onset)
- Vital signs (blood pressure x3, heart rate, height, weight, waist:hip circumference)
- Vascular Imaging (to be performed simultaneously)
  - Brachial FMD
  - EndoPAT
- Pregnancy test (urine) for all women of childbearing potential
- Blood sample (at least 4-hr fasting)
  - Biomarkers (hsCRP, ESR, RF, CCP, E-selectin, ICAM-1, VCAM-1, CD40L, IL-6, MMP-9, and MPO)
  - Nitrite levels
  - Total cholesterol, LDL, HDL, Triglycerides, Homocysteine, Lipoprotein A
  - Glucose and insulin

- Recording and assessment of AEs
- Recording of Concomitant therapy (including CV medications)
- Study Drug Compliance

11. Summary of Tests

11.1 FMD

Brachial Artery Flow Mediated Dilation (FMD) Without Nitroglycerin

The methods of assessment of endothelial function via FMD will be performed following guidelines. Vascular reactivity will be measured noninvasively using vascular ultrasound imaging. Flow and diameter changes of the brachial artery will be assessed by Duplex ultrasound using a high-resolution linear array transducer. The difference between the maximum brachial artery diameter (BAD) post occlusion and the baseline diameter is expressed as a percentage (%BAD). Generally, %BAD values below 5-7% represent endothelial dysfunction, which is associated with CV risk factors, future CVD and mortality. Effective treatment of risk factors may improve endothelial function.[17] Given its reversibility and non-invasiveness, brachial artery FMD is attractive as an assessment tool to monitor strategies to reduce CVD.

11.2 PAT

Peripheral Arterial Tone (PAT)

PAT measured by the EndoPAT 2000 device is a non-invasive method to assess endothelial function. It is a standardized, rapid, and easy to apply method, and has been found to correlate with multiple traditional CV risk factors and to be responsive to interventions. PAT is a validated alternative measure to brachial arterial FMD in assessing endothelial function, and is less operator-dependent than FMD. FMD directly measures the dilation capability of the large-conduit artery, whereas PAT measures flow response hyperemia, which is related to endothelial function of small arteries of microcirculation. PAT measures endothelium-mediated changes in vascular tone using bio-sensors placed on fingertips. Changes in arterial tone are elicited by creating a down-stream hyperemic response of the brachial artery from arm occlusion. Contra-lateral arm measurements are used to control for concurrent non-endothelial

dependent changes in vascular tone. The semi-automatically calculated result is an index of endothelial function.

11.3 Cardiovascular Risk Factors:

Information will be obtained on

- age
- sex
- race/ethnicity
- socioeconomic data
- smoking
- family history of premature CVD
- physical activity level
- menopausal status
- use of estrogen replacement therapy
- diabetes
- fasting glucose and insulin levels measured to calculate the homeostatic model assessment of insulin resistance (HOMA-IR)
- Body mass index (BMI)
- waist-to-hip (W:H) ratios
- Blood pressure at each of the four Study Visits #2-5 will be determined using an average of 3 consecutive sitting blood pressure readings
- Blood tests:
    - Levels of total cholesterol
    - low density lipoprotein (LDL) (LDL will be estimated with the Friedewald equation)
    - high density lipoprotein (HDL)
    - triglycerides, homocysteine
    - lipoprotein (a)

11.3.1 Justification of Cardiovascular Risk Factors:

Information will be obtained on age, sex, race/ethnicity, socioeconomic data, smoking, family history of premature CVD, physical activity level, menopausal status, use of estrogen replacement therapy, diabetes, and fasting glucose and insulin levels measured to calculate the homeostatic model assessment of insulin resistance (HOMA-IR). Body mass index (BMI) and waist-to-hip (W:H) ratios will be

obtained. Blood pressure at each of the four Study Visits #2-5 will be determined using an average of 3 consecutive sitting blood pressure readings. Levels of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, homocysteine, and lipoprotein (a) will be measured. LDL will be estimated with the Friedewald equation.

Use of statins, ACE-inhibitors, and ARBs including dosages and any dose changes will be recorded. Subjects with any of these medication changes will be recorded as a procotol deviation but will not be withdrawn from the study. We anticipate the number of subjects requiring CV medication changes to be small, since the inclusion criteria mandates that subjects be on stable doses of these CV medications with no anticipated changes over the next 6 months.

11.4 **RA Disease-Specific Risk Factors:**

The following validated RA disease activity data will be collected at each study visit:

- Routine Assessment of Patient Index Data (RAPID-3) (a modified health assessment questionnaire and patient visual analogue scales)
- DAS28
- CDAI
- RA medication use will be collected at each study visit, including any new medications or dose adjustments made. A complete list of these medications can be found on the RA Medications List CRF. They include
    - DMARDs
    - biologic agents
    - glucocorticoids
    - non-steroidal anti-inflammatory drugs (NSAIDs)
    - Cox-2 inhibitors

Glucocorticoid maintenance dose increases to higher than 10 mg/day for past 4+ weeks and/or any change or initiation of new DMARDs or biologic agents between study visits will be collected. Subjects with either of these medication changes will be recorded as a procotol deviation but will not be

withdrawn from the study. We anticipate the number of subjects requiring RA medication changes to be small, since the inclusion criteria mandates that subjects be on stable doses of RA medications with no anticipated changes over the next 6 months.

RA disease duration from symptom onset will be collected.

11.4.1 Justification of RA Disease-Specific Risk Factors:

The following validated RA disease activity data will be collected at each study visit: (a) Routine Assessment of Patient Index Data (RAPID-3) (a modified health assessment questionnaire and patient visual analogue scales); (b) DAS28; and (c) CDAI. RA medication use will be collected at each study visit, including any new medications or dose adjustments made. These include DMARDs, biologic agents, glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs), and Cox-2 inhibitors. Glucocorticoid maintenance dose increases to higher than 10 mg/day for past 4+ weeks and/or any change or initiation of new DMARDs or biologic agents between study visits will be collected. Subjects with either of these medication changes will be recorded as protocol deviations but will not be withdrawn. We anticipate the number of subjects requiring RA medication changes to be small, since the inclusion criteria mandates that subjects be on stable doses of RA medications with no anticipated changes over the next 6 months. RA disease duration from symptom onset will be collected.

11.5 Urine Pregnancy Test

Urine Pregnancy tests will be performed on women of child bearing potential at each study visit. Participants in this category will be advised to use at least one verifiable form of birth control during the course of the study to prevent pregnancy and for two weeks after Termination whether termination is early or on schedule.

- Note: if Screening and Baseline are performed simultaneously, only one Urine Pregnancy Test and verification of birth control check are necessary.

11.6 Serum Biomarkers

High-sensitivity CRP (hsCRP), ESR, rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibody (CCP) will be measured in all patients, using standard clinical laboratory protocols. Whole

blood nitrite levels (a measure of NO) will be measured using chemiluminescence. The following additional selected biomarkers involved in endothelial dysfunction and atherosclerosis will be measured in all subjects using enzyme linked immunosorbent assay (ELISA): leukocyte adhesion molecules (E-selectin, ICAM-1, VCAM-1), CD40 ligand (CD40L), interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), and myeloperoxidase (MPO).

12. EFFICACY

For more details about the Efficacy measures see 11 above

12.1 The Primary Efficacy Outcome measures will be:

- Brachial Artery Flow Mediated Dilation (FMD) Without Nitroglycerin
- Peripheral Arterial Tone (PAT)

12.2 Secondary Efficacy measurements

- Serum Biomarkers

13. PHARMACOKINETICS

DOES NOT APPLY TO THIS STUDY

14. SAFETY

14.1 Definition of adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

In the following differentiation between medical history and AEs, the term "condition" may include abnormal e.g., physical examination findings, symptoms, diseases, laboratory, ECG.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent will be recorded as medical history (e.g., seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, will be recorded as medical history (e.g., allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as AEs.

14.2 Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned
  (i.e., elective or scheduled surgery arranged prior to the start of the study)
- The admission is not associated with an AE
  (e.g., social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect

f. Is another medically important serious event as judged by the investigator

14.3 Category

Adverse Events will be categorized by body system utilizing the NIH category system found at https://safetyprofiler-ctep.nci.nih.gov/

14.4 Outcome

The outcome of the AE is to be documented as follows:

- Recovered, without treatment
- Recovered, with treatment
- Still present, no treatment
- Still present, being treated
- Residual effect(s) present, no treatment
- Residual effect(s) present, being treated
- Subject died

14.5 Severity / Intensity

The intensity of an AE is classified according to the following categories:

- Mild
- Moderate
- Severe

Mild is defined as asymptomatic or mild symptoms; intervention not indicated.

Moderate is defined as moderate symptoms present; only minimal intervention indicated.

Severe is defined as severe or medically significant symptoms requiring hospitalization or urgent intervention.

14.6 Relationship to Study

The assessment of the causal relationship between an AE and the administration of treatment is a clinical-decision based on all available information. The assessment is based on the question whether there was a "reasonable causal relationship" to the study treatment in question.

An assessment of "not related" is defined as an adverse event being clearly not related or unlikely to be related. Adverse events can also be attributed as "possible/probable" defined as reasonably possible or likely to be related, or "definite" defined as the adverse event is clearly related.

Important factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge):
- Subject's response after de-challenge or subjects response after re-challenge should be considered in the view of the usual clinical course of the event in question.

- Underlying, concomitant, intercurrent diseases:
  Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment:
  The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study treatment:
  The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual subject's pharmacodynamics should be considered.

14.7 Impact on Study Drug Administration

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

- Drug withdrawn
- Drug interrupted
- Not applicable
- Unknown

14.8 Serious Adverse Events

14.8.1 Reporting

- Sponsor

Pfizer is the provider of the study drug Sildenafil and its placebo ("drug sponsor"). Serious Adverse Events must be reported to the drug sponsor utilizing the forms provided by Pfizer and in accordance with the guide they have provided. These items can be found in the Manual of Operations and Procedures (MOOP) under Safety Reporting.

- Independent Safety Officer

Investigator's notification to the Independent Safety Officer

All SAEs occurring during the observation period defined in Section 14.8 must immediately (within 48 hours of the investigator's awareness) be reported to KAI, NIAMS and the independent Safety Officer. An SAE form must also be completed within 48 hours of the investigator awareness and forwarded. Each SAE must be followed up until resolution or stabilization by submission of updated reports.

- IRB

Notification of the IRB

Notification of the University of Pittsburgh IRB about all relevant events (e.g., SAEs, suspected, unexpected, serious adverse reactions [SUSARs]) will be performed according to IRB regulations.

14.8.2 Unanticipated Problems

The phrase "unanticipated problems involving risks to subjects or others" is found but not defined in the HHS regulations at 45 CFR part 46. OHRP considers *unanticipated problems*, in general, to include any incident, experience, or outcome that meets **all** of the following criteria:

1. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

2. related or possibly related to participation in the research (in this guidance document, *possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

- Reporting
- Independent Safety Officer

Investigator's notification to the Independent Safety Officer

All Unanticipated Problem occurring during the observation period defined in Section must immediately (within 48 hours of the investigator's awareness) be reported to KAI, NIAMS and the independent Safety Officer. An Unanticipated Problem form must also be completed within 48 hours of the investigator awareness and forwarded. Each Unanticipated Problem must be followed up until resolution or stabilization by submission of updated reports

- IRB

Notification of the IRB

Notification of the University of Pittsburgh IRB about all relevant events (e.g., SAEs, suspected, unexpected, serious adverse reactions [SUSARs*]) will be performed according to IRB regulations.
*A SERIOUS UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR) is an unexpected adverse reaction (UAR) is an adverse reaction that is not consistent with the product information. A suspected unexpected serious adverse reaction (SUSAR) is any UAR that at any dose:
• results in death;
• is life threatening (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
• requires hospitalization or prolongation of existing hospitalization;
• results in persistent or significant disability or incapacity;
• is a congenital anomaly or birth defect.

14.8.3 Subsequent Change to Study Treatment

If changes to the administration of the study drug occur (e.g. changes in response to an Adverse Event, poor compliance with study drug, missing/lost study drug, etc) these are to be reported to the drug sponsor and noted in the appropriate form (Adverse Events, Serious Adverse Event, Unanticipated Problem, etc).

- Reporting to INDEPENDENT SAFETY OFFICER

Any such changes to Study Treatment that involve unfavorable changes to the risk benefit ratio should be reported to the Independent Safety Officer.

14.9 Expected adverse events

As per the Sildenafil package insert:

"When VIAGRA was taken as recommended (on an as-needed basis) in flexible-dose, placebo-controlled clinical trials, the following adverse events were reported:

TABLE 2. ADVERSE EVENTS REPORTED BY ≥2% OF PATIENTS TREATED WITH VIAGRA AND MORE FREQUENT ON DRUG THAN PLACEBO IN PRN FLEXIBLE-DOSE PHASE II/III STUDIES

Adverse Event Percentage of Patients Reporting Event

	VIAGRA N=734	PLACEBO N=725
Headache	16%	4%
Flushing	10%	1%
Dyspepsia	7%	2%
Nasal Congestion	4%	2%
Urinary Tract Infection	3%	2%
Abnormal Vision†	3%	0%
Diarrhea	3%	1%
Dizziness	2%	1%
Rash	2%	1%

†Abnormal Vision: Mild and transient, predominantly color tinge to vision, but also increased sensitivity to light or blurred vision. In these studies, only one patient discontinued due to abnormal vision.

Other adverse reactions occurred at a rate of >2%, but equally common on placebo: respiratory infection, back pain, flu syndrome, and arthralgia."

These common events will be noted as Adverse Events if they occur, but may be noted as "EXPECTED" on the Adverse Events Log.

14.9.1 Pregnancy testing

Pregnancy testing is to be performed at the Screening visit. In the event of pregnancy, a referral to a gynecologist for confirmation of pregnancy will be promptly organized. Further consequences with regard to ongoing participation in the study must be discussed with the patient.

Women of childbearing potential and non-vasectomized male participants **must agree to use adequate contraception when sexually active**. 'Adequate contraception' is defined as any combination of at least 2 effective methods of birth control, of which at least one is a physical barrier (e.g. condoms with

hormonal contraception or implants or combined oral contraceptives, certain intrauterine devices). The exception will be women who have undergone tubal ligation and men who have undergone a vasectomy.

15. STATISTICAL METHODS

General considerations

Baseline variables will be summarized with appropriate descriptive statistics within each starting treatment group. Significance of any differences will be assessed using the Wilcoxon rank sum (non-normal data) or t-test for continuous data, and the chi-square test for categorical data.

15.1 Statistical Methods for Specific Aims

For **Specific Aim 1**, treatment group (sildenafil vs. placebo) is the *exposure* of interest, with vascular function measures: (1) percent increase in mean maximum brachial artery diameter (%BAD) using FMD (continuous), and (2) mean PAT ratio (continuous) using EndoPAT 2000, as the *primary outcomes* of interest (measured at 0, 3 months pre- and post-washout, and 6 months). Given the cross-over design, analyses will focus on within-subject comparisons of sildenafil versus placebo periods. All tests will be 2-sided with α=0.05.

The analysis will use linear mixed models, with a term for treatment order (i.e. randomized initially to sildenafil or placebo), to assess whether %BAD and mean PAT ratio differ significantly between the sildenafil and placebo periods within subjects, adjusting for treatment order and CVD risk factors, baseline RA and CV medication use, disease activity (CDAI) and duration, and baseline levels of %BAD and PAT. Model fit, appropriate assumptions (e.g. normality, linearity, homoscedasticity), and potential collinearity, will be assessed with standard diagnostic plots and variance inflation factors (VIF). If needed, we will explore corresponding transformations of the covariates, and/or (in the case of collinearity) dropping variables from the model. We will address any non-compliance using an intention-to-treat strategy and will conduct a sensitivity analysis to conservatively assess observing how the worst outcome would affect results. We do not anticipate many will have RA medication changes but those participants will be included in the analysis; a secondary sensitivity analysis will add a covariate (at the corresponding time point(s)) to denote (yes or no) whether medication changed during the course of the study. Although we recognize that different types of medication changes may have

different effects on the outcome, we will not have sufficient power to estimate heterogeneity of effects across different types of medication changes.

Secondary outcomes of interest for Specific Aim 1 are the frequencies of AEs and SAE. Since the absolute numbers of these events is anticipated to be small, Fisher's exact tests will be used to compare these frequencies between the initial sildenafil and placebo groups.

For **Specific Aim 2**, treatment group is again the *exposure* of interest, with multiple continuous biomarkers of atherosclerosis as the *outcomes* of interest. Using the same analysis strategy as Specific Aim 1, linear mixed models will be used for each of these atherosclerosis biomarkers as functions of treatment group status, treatment order, clinically important covariates, and any significant baseline factors. Model fit and assumptions will again be checked via standard diagnostic plots and VIF.

To inform future trials, we will summarize differences in outcome measures from the beginning to end of the washout period, and compared to baseline, with mean (standard deviation) and median (range). P-values will be calculated with paired t-tests or Wilcoxon signed-rank tests for non-normal distributions.

15.2 Sample Size Justification

We aim to recruit up to 80 subjects to achieve a final sample size of 60 evaluable subjects. For Aim 1, the **sample size of 60** (30 in initial sildenafil group and 30 in initial placebo group), gives 80% power for a relatively small effect size of 0.37 for the change in %BAD in the sildenafil versus placebo periods (using a paired t-test, although we will use a linear mixed model for the analysis). Based on previous studies in diabetes, with equivalent CV risk, the estimated standard deviation (SD) of %BAD is approximately 4. The effect size of 0.37 thus corresponds to a % increase of 1.5%, which is clinically meaningful. We will analyze drop-ins, drop-outs, withdrawals, and non-adherences according to intention-to-treat principles.

For Aim 2, given that we are using the same analysis strategy as Aim 1, the sample size of 60 (30 in each group) again yields 80% power to detect an effect size of 0.37 for the change in a given biomarker. The estimated power (in either aim) corresponding to the final mixed model is difficult to estimate more

specifically since it is unclear how much residual variability will remain after adjusting for other covariates, and/or whether a stronger association will be evident after model adjustment.

15.3 POTENTIAL PITFALLS AND SOLUTIONS

15.3.1 How will we account for the potential variability in results of brachial artery FMD measurements?

A number of factors may potentially influence the results of brachial artery FMD. To account for this, subject-specific factors will be minimized by requesting subjects to limit antioxidant vitamin and/or antioxidant-rich diet within 72 hours; document use of CV medications (e.g., β-blockers, calcium channel blockers, NSAIDs, and aspirin); limit tobacco and caffeine use within 12 hours; document the phase of menstrual cycle for premenopausal women (since optimum time for FMD studies is days 1-7 of menses); abstain from exercise within 12 hours; and be fasting and rested before the FMD study. Finally, since we recognize there is diurnal variation in the FMD response, we will attempt to schedule each subject's visits at consistent times of the day.

15.3.2 What if study subject recruitment and retention rates are slower and/or less than anticipated?

We will increase advertising efforts and may open the study at other community rheumatology clinics, with over 6,000 active RA patients. We may include those on statins, ACE-i or ARB (on stable dose x 4+ months), and retain subjects with RA medication changes. We will address these factors statistically, if needed.

15.3.3 How will the effect of comorbidities and RA disease-related factors that affect endothelial function be mitigated so as not to confound the results of sildenafil's effect on endothelial function?

A potential confounder is the effect of traditional CVD risk factors (e.g., DM), CV medications, RA medications, disease activity and duration on potential change in endothelial function over time. As described in Section 4.4, we plan to adjust for these through multivariable linear mixed models. Furthermore, subjects who require a new start or change in RA medication or glucocorticoid maintenance dose increase to 10 mg/day or greater for 4+ weeks during the trial will be withdrawn from the study. Since this is a randomized placebo-controlled study, we anticipate the frequency of these potential confounders will be similar between groups.

15.3.4 Will potential effects of sildenafil risk un-blinding due to altered erectile function or headaches?

Potential effect on erectile function is unlikely to be recognized by male subjects unless they already suffer from ED, in which they will likely already be taking PDE inhibitors and will thus be excluded. Further, most RA patients are women. Per Micromedex, headaches were only seen in 21% of ED subjects taking the 50 mg daily dose (vs. 7% in placebo). Although these potential side effects may unblind subjects, the outcome measures in this study (vascular function and serum biomarkers) are unlikely to be affected by subject un-blinding. Different study personnel will collect AE data vs. other outcome data, to minimize risk of un-blinding.

15.4 Interim Analyses

Potential non-compliance (assessed via pill counts) will be analyzed using intention-to-treat strategy, and for missing data, we will conduct a sensitivity analysis to conservatively assess observing how the worst outcome (of %BAD) would affect results. The test of a treatment × period interaction is not possible in the given cross-over design since it is confounded by the group effect.

We do not anticipate many subjects with RA or CV medication changes, since inclusion criteria state no expected therapy changes over next 6 months. Moreover, such changes are unlikely to occur systematically more often in the initial sildenafil vs. placebo groups. However, in the event there are many with such changes, we will also conduct a secondary sensitivity analysis including them to assure this does not affect results. The %BAD was chosen as the primary outcome on which to calculate sample sizes based on clinical considerations. Specifically, %BAD has been validated longer and in more populations than PAT.

16. QUALITY ASSURANCE

16.1 Data recording

All data will be entered into the secure Rheumatic Disease Management System (RDMS), which is a Division-wide system for rheumatologic research. A source document checklist will be used at the site to identify the source data for all data points collected.

16.2 Archiving

Documents shall be archived safely and securely in such a way that ensures that they are readily available if required, and in compliance with the University of Pittsburgh guidelines. According to the University of Pittsburgh IRB, all study-related documents will be stored for 7 years. The investigator site file is not to be destroyed without approval by NIAMS or the Safety Monitor.

16.3 Publication Policy

Following completion of the SEDRA study, the investigators will publish the results of this research in scientific journals. The International Committee of Medical Journal Editors (ICMJE) member journals has adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. The SEDRA study is registered with ClinicalTrials.gov, with number NCT02908490.

As this is a single-center study, there will be no publications committee. Authorship determination will be made in consultation with the co-investigators of the study.

Publication of the results of this trial will be governed by NIAMS publication policies. Any presentation, abstract, or manuscript will be made available for review by the NIAMS supporters prior to its submission. Dr. Liang will maintain control of all SEDRA-generated specimens and will decide on the scientific merit of any future sub-studies. They will adhere to the letter and the spirit of the prevailing NIH and NIAMS guidelines on the sharing of research resources produced from federally-funded research. Specifically, any publications will be submitted, as required, to the NIH National Library of Medicine PubMed central for archiving upon acceptance for publication. These policy details (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-05-022.html) will be adhered to.

16.4 Confidentiality / HIPAA

This is a single-center study, with a relatively small number of subjects. All personnel involved in this study have undergone training and certification on the protection of human subjects, confidentiality, HIPAA compliance and research integrity. This training is conducted regularly by the University of Pittsburgh via an internet-based system of education in research.

The only individuals with access to identifiable information and treatment assignment will be the data manager and study statistician. Access to study data will be password protected. All linkage materials are stored separately and maintained in a secure area.

All blood samples will be collected in a de-identified manner using labels generated through the RDMS system. Linkage codes will be maintained by the data manager in the event of emergencies or future need, and these will be kept in a locked filing cabinet located in the Division of Rheumatology research office.

ETHICAL AND LEGAL CONSIDERATIONS

16.5 Ethical and legal conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to abide by GCP guidelines and under the guiding principles detailed in the Declaration of Helsinki. IRB approval has been obtained. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IRB approval. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment will then be submitted to the University of Pittsburgh IRB. Any deviations from the protocol will be explained and documented by the investigator. Protocol deviations will also be reported biannually to the Safety Monitor.

16.6 Subject information and consent

The investigator will discuss that written approval of the IRB has been obtained. Consent forms, describing in detail the therapies, procedures, and risks, will be given to the participant, and written documentation of informed consent is required prior to starting the study agent(s). Consent forms will be IRB-approved, and the participant will be asked to read and review the document. Upon reviewing the document, the study coordinator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants will have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. Only if the subject voluntarily agrees to sign the informed consent form and has done so, may s/he enter

the study. Additionally, the PI will personally sign and date the form. The subject will receive a copy of the signed and dated form. The signed informed consent statement is to remain in the investigator site file. In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject´s clinical record must clearly show that informed consent was obtained prior to these procedures.