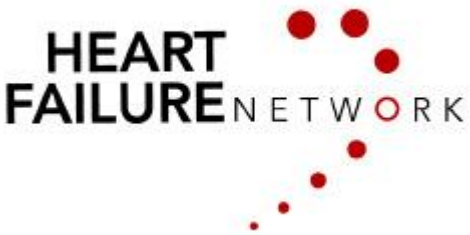

Heart Failure Network

Protocol for the Heart Failure Clinical Research Network:

PhosphodiesteRasE-5 Inhibition to Improve CLinical Status And EXercise Capacity in Diastolic Heart Failure

Study acronym: RELAX

Compiled by:
The Heart Failure Network Research Group
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Amendment 3

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1. LIST OF ABREVIATIONS

ACE	angiotensin converting enzyme inhibitor
AE	adverse event
ANP	Atrial natriuretic peptide
ARB	angiotensin receptor blocker
DIG	digitalis investigators group
BNP	Brain (or B-type)natriuretic peptide
cAMP	cyclic adenosine monophosphate
CCB	calcium channel blockers
cGMP	cyclic guanosine monophosphate
DCC	data coordinating center
DHF	diastolic heart failure
Ea	effective arterial elastance
Ed	LV diastolic elastance
Ees	LV end-systolic elastance
EF	left ventricular ejection fraction
eNOS	endothelial nitric oxide synthase
GC	guanylyl cyclase
GFR	glomerular filtration rate
HF	heart failure
HFN	heart failure network
iNOS	inducible nitric oxide synthase
LV	left ventricular
LVEDP	LV end-diastolic pressure
MLWHFQ	Minnesota living with heart failure questionnaire
MOP	manual of procedures
NO	nitric oxide
NP	natriuretic peptide
NT-proBNP	N-terminal proBNP
PASP	pulmonary artery systolic pressure
PDE-5	type v phosphodiesterase
Peak VO_2	peak oxygen uptake during exercise
pGC	particulate guanylyl cyclase
PKG	cGMP-dependent protein kinase
RAAS	renin-angiotensin-aldosterone system
RCC	regional clinical center
SAE	serious adverse event
sGC	soluble guanylyl cyclase
SHF	systolic heart failure (EF<50%)
SNS	sympathetic nervous system
SVR	systemic vascular resistance
t.i.d.	three times a day
VAT	ventilatory anaerobic threshold

2. EXECUTIVE SUMMARY

Study Design: This is a double-blind, placebo controlled study testing the hypothesis that chronic PDE-5 inhibition (Sildenafil) improves exercise capacity and clinical status in patients with heart failure (HF) and normal ejection fraction (diastolic HF, DHF). The study also measures the effect of this therapy on key pathophysiological parameters which are postulated to impact clinical status and exercise performance in DHF. Approximately 215 patients with DHF will be studied.

Intervention: PDE-5 inhibition (Sildenafil) vs Placebo (20 mg tid for 12 weeks followed by 60 mg tid for 12 weeks).

Enrollment period: Patients will be enrolled over a 3.25 year period.

Rationale for this study is provided by proven benefits of PDE-5 inhibition in patients with pulmonary hypertension, emerging evidence of benefit in HF with systolic dysfunction in pre-clinical and early clinical studies, seminal studies in animal models of pressure overload suggesting unique activation of cardiac PDE-5 in pressure overload hypertrophy and animal studies suggesting that PDE-5 up-regulation mediates hypo-responsiveness to natriuretic peptides and contributes to sodium retention in HF. In aggregate, these studies suggest PDE-5 inhibition may provide beneficial effects on the heart, peripheral vasculature, pulmonary vasculature, kidney and neuroendocrine function. Such multi-system effects suggest the potential for PDE-5 inhibition to ameliorate several key pathophysiological perturbations and thus, improve exercise capacity and clinical status in DHF.

Primary Endpoint: The primary endpoint will be the change in exercise capacity as assessed by peak VO_2 after 24 weeks of double-blinded treatment with PDE-5 inhibitor or placebo.

Secondary Endpoints:
1. Change in a composite score reflective of clinical status after 24 weeks of therapy.
2. Change in submaximal exercise capacity at 12 and 24 weeks as assessed by 6 minute walk test
3. Change in peak VO_2 after 12 weeks of therapy

Tertiary Endpoints
1. Change in exercise time at 12 and 24 weeks
2. Change in ventilatory anaerobic threshold (VAT) at 12 and 24 weeks
3. Change in MLWHFQ at 12 and 24 weeks
4. Change in LV mass (assessed by cardiac MRI) and serological markers of extracellular matrix metabolism at 24 weeks
5. Change in LV diastolic function (LV relaxation, diastolic elastance and filling pressure by Doppler) at 24 weeks
6. Change in peripheral vascular function (effective arterial elastance (Ea), systemic vascular resistance (SVR) and aortic thickness and distensibility) at 24 weeks
7. Change in pulmonary hemodynamics (Doppler estimated pulmonary artery systolic pressure (PASP)) at 24 weeks
8. Change in neuroendocrine function (Cyclic guanosine monophosphate (cGMP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and aldosterone) at 24 weeks
9. Change in renal function (creatinine, estimated glomerular filtration rate (GFR), cystatin C and diuretic dose) at 24 weeks

Pre-specified subgroup analysis will include comparison of the effect of therapy in patients with:
1. LV mass index above and below the median at entry into the study.
2. Estimated PASP above and below the median at entry into the study
3. Dose of sildenafil achieved
4. Presence of atrial fibrillation
5. Differences in background therapy

Primary Hypothesis: As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in exercise performance as measured by peak VO_2 in DHF after 24 weeks of double blinded therapy.

Secondary Hypotheses:

1. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in clinical status at 24 weeks.
2. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in submaximal exercise capacity as assessed by 6 minute walk distance at 12 and 24 weeks.
3. As compared to placebo, chronic PDE-5 inhibition will result in improved exercise performance as measured by peak VO_2 after 12 weeks.
4. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in other measures of exercise capacity (exercise time and VAT) at 12 and 24 weeks.
5. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in quality of life as assessed by the MLWHFQ score at 12 weeks and 24 weeks.
6. As compared to placebo, chronic PDE-5 inhibition will result in greater reduction in LV mass, serological markers of LV fibrosis, diastolic dysfunction, peripheral vascular dysfunction, pulmonary vascular dysfunction, humoral activation and renal dysfunction at 24 weeks.
7. Increasing the dose of sildenafil from 20 mg tid to 60 mg tid will result in further improvement in exercise performance as measured by peak VO_2 and 6 minute walk distance.

Relevance to the goals of the HFN: This proposal is highly responsive to the scientific aim of the HFN which is "to translate new basic science findings, or novel uses for known therapeutic agents or interventions, into clinical testing". The multicenter design of the network will enhance the speed with which the study can be conducted and the generalization of results to DHF as it presents across regions/centers. Quality of data assessment will be enhanced through the use of core laboratories.

3. SPECIFIC AIMS AND OBJECTIVES / HYPOTHESES

This is a double-blind, placebo controlled study testing the hypothesis that chronic PDE-5 inhibition (sildenafil 20 mg tid for 12 weeks followed by 60 mg tid for 12 weeks) improves exercise capacity (primary endpoint) and clinical status (secondary endpoint) in patients with DHF. The study also measures the effect of this therapy on key pathophysiological parameters which are postulated to impact clinical status and exercise performance in DHF. Approximately 215 patients will be enrolled over a planned 3.25 year enrollment period.

Primary Hypothesis: As compared to placebo, chronic PDE-5 inhibition will result in improved exercise performance as measured by peak VO_2 in DHF after 24 weeks of double blinded therapy.

Secondary Hypotheses:

1. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in global clinical status at 24 weeks.
2. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in submaximal exercise capacity as assessed by 6 minute walk distance at 12 and 24 weeks.
3. As compared to placebo, chronic PDE-5 inhibition will result in improved exercise performance as measured by peak VO_2 in DHF after 12 weeks.
4. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in other measures of exercise capacity (exercise time and VAT) at 12 and 24 weeks.
5. As compared to placebo, chronic PDE-5 inhibition will result in greater improvement in quality of life as assessed by the MLWHFQ score at 12 weeks and 24 weeks.
6. As compared to placebo, chronic PDE-5 inhibition will result in greater reduction in LV mass, serological markers of LV fibrosis, diastolic dysfunction, peripheral vascular dysfunction, pulmonary vascular dysfunction, humoral activation and renal dysfunction at 24 weeks.
7. Increasing the dose of sildenafil from 20 mg tid to 60 mg tid will result in improvement in exercise performance as measured by peak VO_2 and 6 minute walk distance.

4. BACKGROUND AND SIGNIFICANCE

4.1 Diastolic HF is a growing public health problem

Between 30 and 50% of patients with clinical HF have preserved ejection fraction (EF) [1-4]. Patients with DHF are older and more likely female than patients with HF and reduced EF(systolic HF, SHF). Indeed, the prevalence of DHF increases far more steeply with age than does the prevalence of SHF particularly in women [5]. DHF is characterized by chronic exercise intolerance, progressive functional decline and a high rate of readmission[6-8]. Mortality for DHF has been reported as similar to or slightly lower than that associated with SHF[1,2,6-11]. Importantly, a recent study suggests that the prevalence of DHF is increasing and while survival among patients with SHF has improved in recent years, survival for patients with DHF has not[12]. The absence of proven therapy for DHF likely contributes to the lack of improvement in survival.

4.2 Diastolic HF pathophysiology

Impaired relaxation and increased LV diastolic stiffness (diastolic elastance, Ed) have been reported in patients with DHF[13-16]. Other studies suggest that increased vascular (Ea) and LV systolic (Ees) elastance are also present in DHF[17-19]. Increased large artery stiffness enhances the oscillatory component of Ea and leads to systolic hypertension, widened pulse pressure, impairment in coronary perfusion, increased transmission of pulsatile flow to the microvasculature and increased end-organ damage[20-24]. In order to maintain optimal interaction with the arterial system, the LV itself must develop greater systolic stiffness (Ees) in the presence of increases in Ea. Increases in Ees lead to increased volume sensitivity and load dependent diastolic dysfunction[18,20,22]. Studies in a novel canine model of DHF[25,26] and population based studies[27] support a role for impaired relaxation and generalized cardiovascular stiffening

(increases in Ea, Ees and Ed) in the pathogenesis of DHF. The proven and theoretical mechanisms underlying cardiovascular stiffening are likely multiple including increased vascular and ventricular fibrosis, altered calcium handling, perturbations in myocardial energetics, vascular wall thickening, endothelial dysfunction, advanced glycation endproduct induced collagen cross-linking, titin isoform switches and altered titin phosphorylation status and alterations in cardiomyocyte microtubules[3,4,22,28,29]. Increased relative wall thickness with or without increased LV mass has been reported in DHF and while most studies report normal LV volume on average, a subset of patients may have LV dilatation with increases in "operant" (preload dependent) Ed[17].

A growing number of studies have confirmed evidence of diastolic dysfunction at rest in patients with DHF as noted above. Few studies have assessed mechanisms mediating reduced exercise capacity in DHF patients. While diastolic dysfunction with impaired filling and inability to augment preload (LV diastolic volume) despite marked increases in LV diastolic pressures may play a key role in mediating exercise intolerance in patients with DHF[30], a recent study found evidence for autonomic dysfunction with chronotropic incompetence, impaired vasodilatatory response to exercise and impaired contractile reserve in DHF patients as compared to age and disease matched controls[31]. Further, Hundley et al demonstrated that aortic stiffness is increased in DHF and correlates with reduced exercise capacity[32] and previous studies have demonstrated that vasodilator therapies improve exercise capacity in patients with diastolic dysfunction and exercise induced hypertension[33,34]. These studies suggest that in addition to diastolic dysfunction, vascular function may influence clinical status in DHF.

Neurohumoral activation is key to the pathogenesis of HF with reduced EF but has not been as well characterized in DHF. Activation of natriuretic peptide (NP) and the sympathetic nervous system (SNS) has been reported but no large studies have characterized neurohumoral function in relation to clinical status and prognosis as has been done for the SNS, the renin-angiotensin-aldosterone system (RAAS), endothelin and mediators of inflammation in SHF. DHF patients have lower natriuretic peptide levels (atrial NP (ANP), brain NP (BNP) and N terminal proBNP) than observed in SHF, suggesting a relative natriuretic peptide deficiency in DHF which may be related to impaired production or release (no LV dilation) or increased metabolism[35].

Renal dysfunction is a key determinant of clinical stability and a powerful prognostic factor in HF regardless of EF[36]. The prevalence and severity of renal dysfunction is equivalent in patients with HF of either type[12,37-39]. The need for renal protective therapies in HF is well recognized[40].

Chronic pulmonary venous hypertension whether occurring in the presence of preserved or reduced systolic function leads to pulmonary arterial hypertension via multiple mechanisms. In one study reporting pulmonary pressures in DHF, 44% of patients had at least moderate PH and the average pulmonary artery systolic pressure was 47 mmHg[41]. Severe pulmonary hypertension associated with DHF has been reported[42] and exercise induced pulmonary hypertension has also been reported[43].

4.3 "Standard therapy" for Diastolic HF

Current guidelines for management of DHF stress control of hypertension, avoidance of tachycardia, maintenance of sinus rhythm, use of diuretics and revascularization if diastolic dysfunction is thought related to ischemia[44]. These recommendations are based on consensus opinion as there have been few clinical trials in DHF. These recommendations have not changed significantly since 1990 [45]. The role of neurohumoral antagonists in DHF patients in whom blood pressure is controlled is unproven. Indeed, in two large clinical trials in DHF, angiotensin receptor blockade (ARB) and angiotensin converting enzyme inhibitors (ACE) showed no benefit in unadjusted analysis [46,47] although cross-over may have limited power in the ACE study. A second study of ARB in DHF is ongoing (I-PRESERVE). Patients with DHF are usually hypertensive and often already being treated with angiotensin converting enzyme inhibitors (ACE), ARB and/or beta blockers, a factor which complicates trials involving these agents. While digoxin reduced HF hospitalizations in DHF in the Digitalis Investigators Group (DIG) Ancillary Trial, equal increases in hospitalizations for unstable angina negated this beneficial effect[48]. The SENIORS trial tested beta blockade in elderly patients with HF of either type and showed benefit of beta blockers but included relatively few patients with EF>50%[49]. A smaller unblinded trial suggested po-

tential for benefit of collagen cross link breakers [50] and a trial with this agent is planned in diabetics with DHF. There is strong support for potential benefit of aldosterone antagonism in DHF and a trial is underway (TOPCAT). An exploratory study of endothelin antagonism in DHF is also underway.

4.4 Phosphodiesterases

Both the NP (via particulate guanylyl cyclase (pGC)) and nitric oxide (NO, via soluble guanylyl cyclase, sGC) stimulate production of cGMP, an intracellular second messenger which exerts effects via cGMP effector proteins including cGMP-dependent protein kinase (PKG) I and II, cyclic nucleotide-regulated ion channels and PDE's which hydrolyze cGMP and/or cyclic adenosine monophosphate (cAMP). There are now known to be 11 different PDE gene families expressed in mammalian tissues. Most families contain more than one gene and most genes code for more than one mRNA (by alternative splicing or alternative transcriptional start sites). Splice variants may mediate tissue specificity. Various PDEs do not necessarily share the same subcellular localization and therefore often subserve, at least in part, different functional compartments in the cell. PDE1 (calcium-calmodulin dependent), PDE2 (cGMP stimulated), PDE3 (cGMP inhibited), PDE10 and PDE11 hydrolyze both cAMP and cGMP while PDE5, PDE6 and PDE9 hydrolyze only cGMP. PDE4, PDE7 and PDE8 hydrolyze only cAMP. While PDE 1-4 are well recognized to be present in the heart, recent studies suggest that PDE5 may also be present in the heart and specifically in cardiomyocytes and that its production or activity may be up-regulated in pressure overload hypertrophy[51]. Further studies suggest that although present at low levels in normal myocardium, PDE-5 modulates response to sympathetic stimulation [52] or ischemia[53]. However, another study suggested a down regulation of PDE5 activity in the rapid ventricular pacing model of systolic dysfunction [54].

5. PRELIMINARY STUDIES

Several lines of evidence support the hypothesis that PDE-5 inhibition may improve exercise performance and clinical status in DHF.

5.1 PDE-5 inhibition improves ventricular structure and function in pressure overload and ischemia/reperfusion

In a seminal study, Takimoto et al found that PDE-5 inhibition ameliorated cardiac hypertrophy and fibrosis and associated reduction in ventricular function induced by marked pressure overload in mice[51]. These investigators provided evidence that PDE-5 is upregulated in the heart in response to pressure overload, and that the effects of PDE-5 inhibition were not mediated by an effect on blood pressure. Further, they provided evidence that PDE-5 selectively degrades cGMP produced by nitric oxide activated sGC rather than NP activated pGC. This finding is consistent with a recent in vitro study using isolated rat myocytes transfected with cGMP gated ion channels to assess the role of PDE-5 and PDE-2 in regulating cGMP production resulting from NP or NO stimulation [55]. While myocardial and vascular effects of PDE-5 inhibition may be mediated in large part or in whole by modulation of sGC derived cGMP, this is not likely to be the case in the kidney, where evidence for PDE-5 in the degradation of cGMP related to NP activated particulate GC exists[56] (see below). Further, more recent studies show that PDE-5 inhibition preserves myocardial function after ischemia/reperfusion injury in inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) gene deletion models[53], alters myocardial function in response to β-adrenergic signaling in normal humans[52] and has blunted effects on right ventricular remodeling in pulmonary hypertension in NP receptor null mice[57]. Thus, whether PDE-5 inhibition exerts beneficial effects exclusively by modulating sGC exclusively is unclear but the evidence for beneficial effects on myocardial structure and function remains regardless of this issue.

As compared to SHF, DHF may be uniquely sensitive to modulation of myocardial and vascular function by PDE-5 inhibition as PDE-5 activity may be down regulated in advanced systolic dysfunction[54]. Additional myocardial effects of PDE-5 inhibition which may translate to clinical benefit include protec-

tion against necrosis and apoptosis demonstrated in in vivo [53,58] and in vitro studies [59] and potential for direct beneficial effects on LV relaxation rate if cGMP actions are potentiated [60,61]. Finally, acute PDE-5 inhibition reduced filling pressures and chronic PDE-5 inhibition increased survival in cardiomyopathic hamsters[62]. Similarly, chronic PDE-5 inhibition decreased filling pressures, norepinephrine levels, systemic vascular resistance and pulmonary vascular resistance and increased cardiac output in the canine rapid ventricular pacing model of HF[57]. While only a few studies are available to suggest potential beneficial myocardial effects of PDE-5 inhibition in DHF, those available provide strong rationale and build the case for early translation to the human, consistent with the goals of the HFN.

5.2 PDE-5 inhibition improves peripheral vascular function

The impaired vasodilatory response to exercise and impaired contractile reserve in DHF patients as compared to age and disease matched controls[31] suggests that therapies which enhance endothelial function may enhance exercise capacity. Katz et al as well as others have documented improvements in flow mediated vasodilatation with PDE-5 administration in patients with HF and systolic dysfunction[63-65]. Further, Mahmud et al reported decreases in measures of large artery stiffness and reflected wave with acute administration of a PDE-5 inhibitor in hypertensive men[66]. While PDE-5 inhibitors generally have only a very modest anti-hypertensive effect, effects to decrease large artery stiffness and improve flow mediated vasodilatation with exercise may improve exercise capacity and clinical status in DHF.

5.3 PDE-5 inhibition improves pulmonary vascular function

Chronic pulmonary venous hypertension whether occurring in the presence of preserved or reduced systolic function leads to pulmonary arterial hypertension via multiple mechanisms. In one study reporting pulmonary pressures in DHF, 44% of patients had at least moderate PH and the average pulmonary artery systolic pressure was 47 mmHg[41]. Severe pulmonary hypertension associated with DHF has been reported[42] and exercise induced pulmonary hypertension has also been reported[43]. PDE-5 is abundantly expressed in the lungs and PDE-5 inhibition has potential to enhance the optimization of regional distribution of blood flow in the lung which is provided by activation of endogenous NO in areas of optimal ventilation.

As recently reviewed, numerous studies have demonstrated beneficial effects (including amelioration of right ventricular hypertrophy) of PDE-5 inhibition in experimental and human pulmonary hypertension[67] and a seminal randomized clinical trial has confirmed sustained beneficial effects[68]. Acute administration of a PDE-5 inhibitor has also been shown to ameliorate adverse pulmonary hemodynamics and improve exercise and lung in human heart failure[64,65,69] and chronic PDE-5 inhibitor therapy improved exercise tolerance in patients with HF and systolic dysfunction[70]. Thus, as patients with DHF have resting and exercise induced pulmonary hypertension, favorable effects on right ventricular load may enhance exercise performance and clinical status as well as retard long term progression of HF by ameliorating pulmonary hypertension.

5.4 PDE-5 inhibition may improve volume status and renal function by restoring responsiveness to natriuretic peptides

The natriuretic actions of the NP are attenuated in overt HF as compared to normal organisms or those with mild/early HF[71]. Indeed, renal resistance to NP is a hallmark of states of pathological sodium retention including HF, cirrhosis and the nephrotic syndrome[56]. The mechanisms which mediate the attenuated response to NP in overt HF and other sodium retaining states include impaired delivery of NP to intrarenal target sites (decreases in renal blood flow and GFR), increased intrarenal degradation of natriuretic peptides due to up-regulation of neutral endopeptidases, decreased number of or reduced affinity of biological receptors, post receptor events leading to reduced production of cGMP or increased cGMP degradation by phosphodiesterases. Indeed, both the NP/cGMP and NO/cGMP signaling pathways are impaired in overt HF[72]. Furthermore, renal cGMP generation is a marker of renal responsiveness to the NP and NO and is attenuated in overt HF[73]. PDE metabolizes cGMP and is abundant in the

vasculature and the kidney. Studies by Haneda et al.[74] and Kim et al.[75] suggest that angiotensin II and other calcium stimulating peptides play an important role to upregulate PDE-5 in both vascular smooth muscle cells and the glomeruli.

One of the mechanisms for the development of renal dysfunction in overt HF may be upregulation of PDE-5 in the glomeruli leading to increased intra-renal clearance of cGMP. Indeed, Valentin et al. demonstrated that PDE-5 was upregulated in experimental nephrotic syndrome and that acute PDE-5 inhibition reversed the renal resistance to exogenous NP associated with nephrotic syndrome[76]. Subsequent studies also demonstrated that PDE-5 activation mediates renal resistance to NP in several other states of pathological sodium retention including cirrhosis, pregnancy and Heynam nephritis [77-80]. In experimental systolic HF, recent studies show that PDE-5 inhibition markedly restores renal responsiveness to low dose BNP [81] and improves renal function and decreases renal endothelin generation in experimental HF[82]. As renal function is a key determinant of clinical status and prognosis in HF, beneficial effects on renal function represent an additional mechanism where by chronic PDE-5 inhibition may improve exercise performance and clinical status in DHF.

In summary, there are multiple "signals" suggesting the potential for benefit with PDE-5 inhibition in DHF. There are no preliminary human studies in DHF although there are published and ongoing studies in human SHF. The proposed study thus seeks to extend promising basic and pre-clinical laboratory research to a "proof of concept" study in human DHF, consistent with the goals of the HFN. At least one PDE-5 inhibitor (sildenafil) will soon go off patent and there has been no interest in a DHF program by pharmaceutical companies marketing PDE-5 inhibitors.

6. BASIC STUDY DESIGN

Study Design: Randomized (1:1), double-blind, placebo controlled treatment study

Intervention: PDE-5 inhibition with sildenafil (20 mg tid for 12 weeks followed by 60 mg tid for 12 weeks) or placebo for 24 weeks

Study population: Approximately 215 patients with a clinical diagnosis of HF and normal EF (≥ 50%) enrolled over a planned 3.25 year enrollment period.

Primary outcome: The primary endpoint will be exercise capacity as assessed by the change in peak peak VO_2 at 24 weeks of double blinded therapy compared to the baseline peak VO_2.

Secondary outcomes:
1. Change in a composite score reflective of clinical status after 24 weeks of double-blinded treatment with PDE-5 inhibitor or placebo.
2. Change in submaximal exercise capacity at 12 and 24 weeks as assessed by 6 minute walk test
3. Change in peak VO_2 at 12 weeks

Additional tertiary endpoints will explore potential mechanisms whereby PDE-5 inhibition may exert benefit on exercise capacity and clinical status.

1. Change in exercise time at 12 and 24 weeks
2. Change in ventilatory anaerobic threshold (VAT) at 12 and 24 weeks
3. Change in MLWHFQ at 12 and 24 weeks
4. Change in LV mass and serological markers of extracellular matrix metabolism at 24 weeks
5. Change in LV diastolic function (LV relaxation, diastolic elastance and filling pressure by Doppler) at 24 weeks
6. Change in peripheral vascular function (effective arterial elastance (Ea), systemic vascular resistance (SVR) and aortic thickness and distensibility) at 24 weeks

7. Change in pulmonary hemodynamics (Doppler estimated pulmonary artery systolic pressure (PASP) at 24 weeks
8. Change in neuroendocrine function (Cyclic guanosine monophosphate (cGMP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and aldosterone) at 24 weeks
9. Change in renal function (creatinine, estimated glomerular filtration rate (GFR), cystatin C and diuretic dose) at 24 weeks

Duration:

Consent, Screening studies (up to 2 weeks)
Baseline studies and Randomization (up to 2 weeks)
Treatment phase (24 weeks)

Frequency of study related visits:

1. Consent
2. Screening labs and cardiopulmonary exercise test (if needed)
3. Baseline studies (in patients meeting eligibility requirements)
4. Randomization and initial dosing (wk 0, 20 mg tid)
5. Week 3 study visit and safety labs (+/- 7 days)
6. 12 week visit, studies and dose escalation (60 mg tid) (+/- 7 days)
7. 24 week visit and studies (+/- 7 days)
8. Phone visits (at 1 and 2 days after initiation and dose escalation and at week 1, 8, 13, 16 and 20 wks (+/- 5 days)

7. PREPARATORY STUDIES

Clinical studies in erectile dysfunction, pulmonary hypertension and SHF have provided important data regarding dosing, side effects and safety. Thus preparatory studies are not indicated to address these issues. Similarly, there are a large number of previous trials in SHF establishing the ability to measure the endpoints tested here in a reproducible manner.

8. STUDY POPULATION AND ELIGIBILITY CRITERIA

8.1 Study Population and Source of Participants

Study population: Patients with DHF who meet inclusion and exclusion criteria as listed below are eligible for participation.

Source of participants: DHF patients cared for at any site participating in the HFN are eligible for participation although the complexity of testing may require that patients be seen at the primary RCC site in some cases. While a very large number of clinical and epidemiology studies have established that 30-50% of patients with HF have normal EF, patients with DHF are often not cared for by cardiologists, particularly at academic institutions. Thus, close collaboration with non-cardiologist care providers at each site will be important to insure adequate enrollment. Suggested strategies for identification of DHF patients are elaborated on in section 10; Recruitment Procedures.

8.2 Inclusion Criteria

1. Age > 18 years
2. Previous clinical diagnosis of HF (see Appendix 22.2) with current NYHA Class II-IV symptoms
3. Must have had **at least one** of the following within the **12 months prior** to consent
    a. Hospitalization for decompensated HF
    b. Acute treatment for HF with intravenous loop diuretic or hemofiltration

c. Chronic treatment with a loop diuretic for control of HF symptoms + chronic diastolic dysfunction on echocardiography as evidenced by left atrial enlargement
d. Mean pulmonary capillary wedge pressure > 15 mmHg or LV end diastolic pressure (LVEDP)>18 mmHg at catheterization for dyspnea

4. EF $\geq$ 50% on a clinically indicated echocardiogram or ventriculogram ***within 12 months*** prior to consent, in the absence of a change in cardiovascular status.
5. Stable medical therapy for 30 days as defined by:
   a. No addition or removal of ACE, ARB, beta-blockers, or calcium channel blockers (CCBs)
   b. No change in dosage of ACE, ARBs, beta-blockers or CCBs of more than 100%
6. Meet screening criteria
   a. VO_2 peak ≤ 60% normal value (see section 11: Screening Procedures) *with respiratory exchange ratio (RER) ≥ 1.0.*
   b. One of the following:
      i. NT-proBNP ≥ 400 pg/ml or BNP ≥ 200 pg/ml
      ii. NT-proBNP < 400 or BNP < 200 with mean pulmonary capillary wedge pressure (PCWP) > 20 mmHg at rest or > 25 mmHg with exercise.measured in proximity (within 2 weeks before or after) to the NT-proBNP or BNP level

8.3 Exclusion Criteria

1. Have a neuromuscular, orthopedic or other non-cardiac condition that prevents patient from exercise testing on a bicycle ergometer or from walking in a hallway
2. Non-cardiac condition limiting life expectancy to less than one year, per physician judgment
3. Current or anticipated future need for nitrate therapy
4. Valve disease (> mild aortic or mitral stenosis; > moderate aortic or mitral regurgitation)
5. Hypertrophic cardiomyopathy
6. Infiltrative or inflammatory myocardial disease (amyloid, sarcoid)
7. Pericardial disease
8. Primary pulmonary arteriopathy
9. Have experienced a myocardial infarction or unstable angina, or have undergone percutaneous transluminal coronary angiography (PTCA) or coronary artery bypass grafting (CABG) within ***60 days*** prior to consent, or requires either PTCA or CABG at the time of consent
10. Other clinically important causes of dyspnea such as morbid obesity or significant lung disease defined by clinical judgment or use of steroids or oxygen for lung disease
11. Systolic blood pressure < 110 mmHg or > 180 mm Hg
12. Diastolic blood pressure < 40 mmHg or > 100 mmHg
13. Resting heart rate (HR) > 100 bpm
14. A history of reduced ejection fraction (EF<50%)
15. Implanted metallic device which will interfere with MRI examination (in patients without atrial fibrillation)
16. Severe renal dysfunction (estimated GFR < 20 ml/min/1.73m^2 by modified MDRD equation)
    GFR (mL/min/1.73 m^2) = 175 x $(S_{cr})^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female) x (1.210 if African American) (conventional units)
17. Women of child bearing potential who do not have a negative pregnancy test at study entry and who are not using effective contraception
18. Hemoglobin <10 g/dL
19. Patients taking alpha antagonists or cytochrome P450 3A4 inhibitors (ketoconazole, itraconazole, erythromycin, saquinavir, cimetidine or serum protease inhibitors for HIV).
20. Patients with retinitis pigmentosa, previous diagnosis of nonischemic optic neuropathy, untreated proliferative retinopathy or unexplained visual disturbance
21. Patients with sickle cell anemia, multiple myeloma, leukemia or penile deformities placing them at risk for priapism (angulation, cavernosal fibrosis or Peyronie's disease)
22. Patients with severe liver disease (AST > 3x normal, alkaline phosphatase or bilirubin > 2x normal)
23. Consistent with ACC/AHA guidelines, persons with dyspnea and risk factors for coronary artery disease, should have had a stress test and those patients with a clinically indicated stress test demonstrating significant ischemia within a year prior to enrollment would be excluded

24. Listed for cardiac transplantation

Up to 58 patients with chronic atrial fibrillation will be enrolled in the study Once 58 patients with chronic atrial fibrillation have been enrolled, the DCC and DSMB will review the characteristics (atrial fibrillation versus sinus rhythm) of all patients enrolled and will increase this limit if needed to insure adequate enrollment to address the primary endpoint.

9. TREATMENT INTERVENTIONS

9.1 Intervention

The therapeutic intervention is double blinded therapy with placebo or the PDE-5 inhibitor sildenafil (as Revatio®). Study drug therapy will be given for 24 weeks starting with 20 mg by mouth three times a day (tid) for 12 weeks. If that dose is well tolerated, the dose will be increased to 60 mg tid at week 12 for the remaining 12 weeks of the study. Patients unable to tolerate the 60 mg tid will be maintained on the 20 mg tid dose. The active and placebo study medication will appear identical to preserve the double blind design of the study. If patients develop side effects thought potentially related to study drug (including but not limited to headache, flushing, dizziness, orthostatic lightheadedness, dyspepsia, nasal congestion, diarrhea, rash, visual disturbance), the dose will be adjusted to the previously tolerated dose. As recommended for patients with HF, patients will be encouraged to weigh daily and record weights. If patients have experienced enhanced responsiveness to their diuretic dosage (decreased weight *and* symptoms of over-diuresis such as lightheadedness), consideration can be given to reducing the diuretic dose rather than the study drug.

9.2 Control Group

The control group in this study will include patients randomized to placebo medication. Study drug (sildenafil or placebo) will be given in a double blind fashion and all patients will undergo identical study procedures including dose titration.

9.3 Concomitant Therapies

Current guidelines for management of DHF stress control of hypertension, rate control for patients with atrial fibrillation, avoidance of tachycardia in patients in normal sinus rhythm, maintenance of sinus rhythm, use of diuretics and revascularization if diastolic dysfunction is thought related to ischemia[44]. These recommendations are based on consensus opinion as there have been few clinical trials in DHF and no therapy has been proven to improve clinical outcomes (see section 4.3). Thus, treatment with agents to control blood pressure and maintain volume status is expected but no specific concomitant therapies are specified.

Study exclusion criteria outline drugs which can not be used due to concern over drug interaction (see exclusion criteria, section 8.3). These include nitrate preparations as well as alpha antagonists or cytochrome P450 3A4 inhibitors or serum protease inhibitors for HIV. If the patient develops clear indication for nitrates (angina which can not be managed with alternative antianginals), the patient will need to be withdrawn from the study. If the need for nitrates is emergent or urgent, the treatment assignment in the patient should be obtained emergently from the DCC so that patients treated with placebo could start treatment with nitrates immediately. If the patient was on active therapy, the exact time after the last dose of sildenafil when nitrates can be safely administered is not well defined. Although plasma levels of sildenafil at 24 hours post dose are much lower than at peak concentration, it is unknown whether nitrates can be safely co-administered at this time point. Thus, it is recommended that nitrates should not be administered to any patient previously on active study drug prior to 5 days after the last dose of sildenafil. Should there be an urgent need for nitrate administration, they should be administered in a monitored inpatient setting.

In patients with a history of volume retention or hypertension, standard therapy will specify a low sodium diet but no specific instructions regarding diet will be provided as part of the study. Daily aerobic

exercise is recommended for patients with HF as tolerated but no specific recommendations for activity will be provided as part of this study.

9.4 Dose Justification (also see Appendix 3; Section 22.3)

Most studies suggesting benefit of sildenafil in humans with SHF have utilized a single dose to assess acute effects. The early studies of chronic therapy in humans with PH were small and ill suited to assess dose response. Thus, the justification for the dosing chosen in the RELAX study is based on the recently published larger trial in pulmonary arteriopathies unrelated to HF which explored dose responsiveness [68] and the seminal animal (mouse) study [51].

In the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) study [68], oral doses of 20, 40 and 80 mg tid were compared to placebo in patients with symptomatic (NYHA class II-III, mean age 57 years) PH. No dose response was evident for the primary endpoint (6 minute walk distance) *or for tolerability*. However, the data presented suggest the presence of a dose response with decreases in heart rate, pulmonary artery pressure, and pulmonary vascular resistance appearing most dramatic with the highest dose.

In the seminal mouse study showing anti-hypertrophic and anti-fibrotic effects and effects to preserve LV systolic and diastolic function, the sildenafil dose was 100 mg/kg/day [51]. This dose was postulated to be equivalent to approximately 1 mg/kg/day in man due to a "near 100-fold higher rate of metabolism of sildenafil in the mouse". In a study of pharmacokinetics of sildenafil in mouse and man, oral bioavailability in mice (17%) is approximately 50% that observed in humans (38%), the volume of distribution is similar (1.0 l/kg in mouse versus 1.2 l/kg in man) but metabolism is faster with an elimination half life of 1.3 hours vs 3.7 hours in mouse vs man[83] . The maximal concentration at equivalent oral dose (1 mg/kg) was 30 ng/ml in mouse and 212 ng/ml in humans. The time to peak concentration was 0.5 hours in mouse and 1.2 hours in man. Thus, these data suggest that clearance is faster and that to achieve a similar peak or steady state concentration, the equivalent dose in humans would be significantly less than used in the mouse, although not clearly 100% less. Assuming an average body size of 70-90 kg, the planned dose range is 0.66- 2.57 mg/kg/day. Thus, the planned dose range is proportional to that used in the mouse where dramatic effects on myocardial structure and function were observed.

It is acknowledged that the absence of a relationship between the dose of sildenafil and the change in 6 minute walk distance in the SUPER trial may suggest that up-titration to a dose beyond 20 mg tid in RELAX is unnecessary. Recent studies suggest that binding of cGMP and PKG mediated phosphorylation of PDE-5 enhance binding (of cGMP). Binding of sildenafil produces similar conformational changes as seen with binding of cGMP (promoting further sildenafil binding) and further enhances binding by increasing intracellular cGMP and thus PKG mediated phosphorylation of PDE-5. This further increases sildenafil binding. Based on these observations regarding the biology of PDE-5 and sildenafil, one could hypothesize that the duration of sildenafil binding to PDE-5 is longer than would be predicted from it's pharmacokinetics and that dose (or plasma levels) do not correlate linearly with enzyme binding and biological effect[84]. These remain intriguing and important but as yet unproven hypotheses. However, there are only limited data regarding the dose response of pulmonary or peripheral vascular, myocardial, renal, neurohumoral or clinical outcome effects of sildenafil in pulmonary hypertension or systolic HF (reviewed in detail in Appendix 3). Some of these data support the importance of a dose up-titration strategy while other data do not. Unfortunately, there are no data concerning the dose response in the unique syndrome of DHF where the pathophysiology and dose responsiveness may differ from erectile dysfunction, pulmonary hypertension and systolic heart failure. Thus, acknowledging the size and the phase II nature of this trial in DHF, a dose which will maximize efficacy is chosen and some exploratory dose response data will be obtained. Specifically, in an exploratory analysis we will compare exercise capacity in sildenafil treated patients after 12 weeks at 20 tid to that observed after 12 weeks on 60 tid. Further, all studies will be performed at near peak sildenafil levels (45-120 minutes after last dose) and

levels will be checked at the time of the exercise study at 12 and 24 weeks. These data will provide further information regarding the relationship between dose and plasma levels and therapeutic response.

9.5 Side Effects

The known side effects of sildenafil include the following:
Prevalence > 10%:
Central nervous system: headache
Prevalence 1% to 10%:
Cardiovascular: flushing
Central nervous system: dizziness
Dermatologic: rash
Genitourinary: urinary tract infection
Ophthalmic: abnormal vision (color changes, blurred or increased sensitivity to light)
Respiratory: nasal congestion
Prevalence < 2% (limited to important or life-threatening):
Allergic reaction, angina pectoris, anorgasmia, asthma, AV block, cardiac arrest, cardiomyopathy, cataract, cerebral thrombosis, colitis, dyspnea, edema, exfoliative dermatitis, eye hemorrhage, gout, heart failure, hyperglycemia, hypotension, migraine, myocardial ischemia, neuralgia, photosensitivity, postural hypotension, priapism, rectal hemorrhage, seizure, shock, syncope, vertigo, tinnitus, transient or permanent unilateral or bilateral hearing loss.

In regards to potential toxicity, side effect prevalence was not dose dependent in the SUPER trial. Most adverse events were mild to moderate for all treatment groups and were not dose related as outlined above. Over 12 weeks, only two serious adverse events, postural hypotension and left ventricular dysfunction, were considered to be related to sildenafil. In contrast, in a large meta-analyses of 27 erectile dysfunction trials in 6659 men, studies which examined dose responsiveness did demonstrate a trend towards a dose response in the prevalence of side effects[85]. However, the rate of discontinuation for side effects was lower in the active vs placebo arms. Side effects were not serious and the incidence of chest pain, myocardial infarction or death was not increased in premarketing or postmarketing studies. In this metaanalysis, there was no apparent increase in side effects according to baseline characteristics (age, diabetes, etc).

According to previous studies (summarized in package insert for sildenafil), healthy elderly volunteers (65 years or over) had a reduced clearance of sildenafil, with free plasma concentrations approximately 40% greater than those seen in healthy younger volunteers (18-45 years). Sildenafil is eliminated predominantly by hepatic metabolism and is converted to an active metabolite with biological properties (PDE-5 inhibition) similar to the parent, sildenafil. Sildenafil is cleared predominantly by the CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal isoenzymes. The major circulating metabolite results from N-desmethylation of sildenafil, and is itself further metabolized. This metabolite has a PDE selectivity profile similar to sildenafil and an *in vitro* potency for PDE5 approximately 50% of the parent drug. Plasma concentrations of this metabolite are approximately 40% of those seen for sildenafil, so that the metabolite accounts for about 20% of sildenafil's pharmacologic effects. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered oral dose) and to a lesser extent in the urine (approximately 13% of the administered oral dose). In volunteers with mild (creatinine clearance = 50-80 mL/min) and moderate (creatinine clearance = 30-49 mL/min) renal impairment, the pharmacokinetics of a single oral dose of sildenafil (50 mg) were not altered. In volunteers with severe (creatinine clearance =<30 mL/min) renal impairment, sildenafil clearance was reduced, resulting in approximately doubling of AUC and C_{max} compared to age-matched volunteers with no renal impairment. However, while these studies suggest reduced clearance of sildenafil in patients with renal dysfunction, a study in patients with end stage renal disease on hemodialysis showed that pharmacokinetics were similar to normal subjects both before and after hemodialysis[86] .

Therefore, we initiate therapy with 20 mg tid recognizing the probable advanced age of study participants, up-titrate therapy at 12 weeks in the absence of side-effects, down titrate if increasing the dosage results in side effects but use a goal dose consistent with that providing the maximal hemodynamic effect in humans with PH and equivalent to that producing the observed myocardial effect in the mouse. Selection of this strategy provides the opportunity to maximize efficacy while minimizing side effects in this population.

At the study visits where the first dose of study drug (20 mg) is administered or the higher dose (60mg) is administered, systemic blood pressure measurement will be obtained in sitting and standing position one hour after administration of study drug. The dose of study drug will be stopped (therapy initiation visit) or reduced (therapy up-titration visit) in any patient in whom the SBP (seated or standing) measured 1 hour after the study drug during the drug initiation/titration visit is < 100 mm Hg or DBP < 40 mm Hg. Patients with chest pain, palpitations, diaphoresis will have a 12-lead ECG and a clinical evaluation as soon as possible. Study drug will be stopped in patients with acute coronary syndromes, AV block, or life-threatening arrhythmias.

At all study visits (in person or phone follow up), monitoring for toxicity will include questioning and encouragement to call the investigator about headache, flushing, dizziness, orthostatic lightheadedness, dyspepsia, nasal congestion, diarrhea, rash, visual disturbance, chest pain, palpitations, worsening dyspnea, peripheral edema, diaphoresis, tinnitus or hearing loss. On every study visit, investigators will obtain a medical history and perform a targeted cardiovascular physical examination. After each dose initiation or dose escalation, study coordinators will contact the patient by phone at 1 and 2 days after the dosing visit to monitor for adverse effects.

9.5.1 Potential for enhanced diuretic responsiveness:

Patients will be encouraged to weigh themselves daily and patients will undergo measurement of weight at each study visit on the same scale. In patients complaining of lightheadedness, dizziness or other symptoms of low blood pressure or with decreases in blood pressure on measurement, the study staff will determine if evidence of increased diuretic responsiveness is present (decrease in weight on same diuretic dose and without increased signs of volume overload on examination). If evidence of enhanced diuretic responsiveness is present, consideration will be given to reducing diuretic dose before adjusting study drug dosage.

9.6 Summary of the Risks and Benefits

9.6.1 Potential benefits:

This study involves administration of an agent (sildenafil) with potential beneficial effects in DHF. Thus, if patients receive active study drug (sildenafil) rather than placebo, they could potentially experience clinical benefit.

9.6.2 Potential risks associated with diagnostic studies completed as part of this protocol:

Blood drawing with a total of 120 mls over the 6 month study period with a maximum of 40 ml per week. Potential risks of blood drawing include bleeding at the puncture site, bruising and pain. These risks occur in a very small portion of the population. Exercise stress tests will be performed by trained professionals in a highly monitored setting. These studies are routinely obtained in clinical practice to assess clinical status and are considered safe. Patients with > Canadian class II angina or recent (< 60 days) myocardial infarction are excluded from this protocol. A resting heart rate (HR) of > 120 bpm, systolic BP > 180 or diastolic BP > 100 are relative contraindications for stress testing and patients with these parameters at screening will not be enrolled until standard medications have been adjusted to control BP and heart rate. Cardiac MRI and Echo Doppler are non-invasive, use no radiation and other than mild chest discomfort or claustrophobia are not associated with adverse affects.

9.6.3 Potential risks associated with the active study drug (sildenafil):

The concomitant use of potent cytochrome P450 3A4 inhibitors (e.g., erythromycin, ketoconazole, itraconazole) as well as the nonspecific CYP inhibitor, cimetidine, is associated with increased plasma levels of sildenafil. Patients taking these drugs are excluded from this protocol and patients enrolled in the study will be cautioned not to commence therapy with these agents. Patients enrolled in the trial will receive a card outlining potential drug interactions and be instructed to present this card if new medications are recommended during the trial. This wallet card will also contain the DCC Clinical Helpline number for emergent unblinding and for reporting of side effects. Patients will also be given a medic alert necklace indicating the patient may be taking sildenafil. Clearance is also reduced in patients > 65 and in those with severe renal or severe hepatic dysfunction where an initial dose of 20 mg is recommended. Patients with significant liver disease (AST>3x normal or alkaline phosphatase or bilirubin > 2x normal) are excluded from this protocol. Side effects reported to occur in ≥ 2% of patients in phase II/III clinical trials of Sildenafil have included headache (16%), flushing (10%), dyspepsia (7%), nasal congestion (4%), urinary tract infection (3%), abnormal vision (3%), and diarrhea, dizziness and rash (2% each). Transient visual changes can occur with sildenafil due to effects on PDE-6 in the eye. Sildenafil is quite specific for PDE-5 but some cross-reactivity can occur at higher doses and produce transient color distortion, halo-vision, or blurred vision. Ocular effects from PDE-5 inhibition include conjunctival hyperemia. However, extensive testing in PH patients and in erectile dysfunction patients treated with sildenafil has not revealed any permanent effects on visual acuity, visual fields, color sensitivity or fundoscopic examination associated with chronic daily or intermittent sildenafil dosing, even at doses of 50-100 mg (data on file, Pfizer).

In post marketing experience, non-arteritic anterior ischemic optic neuropathy (NION), a cause of decreased vision including permanent loss of vision, has been reported rarely post-marketing in temporal association with the use of PDE-5 inhibitors, including sildenafil. Most, but not all, of these patients had underlying anatomic or vascular risk factors for developing NION, including age over 50, diabetes, hypertension, coronary artery disease, hyperlipidemia and smoking. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors, to the patient's underlying vascular risk factors or anatomical defects, to a combination of these factors, or to other factors. Among 479,489 veterans who had received a prescription for sildenafil, a diagnosis of NION or possible NION was present (at some point) in 670 (0.140%). Use of drug and temporal relation of drug use and NION was not assessed. The RR for NION or possible NION for those with an “ever” prescription of sildenafil was 1.10 (95% CI: 1.02-1.20). Thus, the *absolute* increase in risk would be from 0.127 % to 0.140% or 0.013%. This study was unable to assess true incidence or the relationship of sildenafil use and NION and is significantly confounded by the similar risk profile for NION and erectile dysfunction[87]. The most common dose prescribed in this study was 100 mg (>99% of prescriptions). In two studies which examined the *incidence* of NION (irrespective of use of PDE-5 inhibitors), the annual incidence rate in the general population was 10.2 per 100,000 (95% CI 6.5-15.6) in one study[88] and 2.77 per 100,000 in the other[89]. Thus the chance of a patient on placebo developing NION during the 6 month study period would be 0.00005%. Clinical, epidemiological and post-marketing surveillance studies do not provide support for an association between sildenafil use and NION (Data on file, Pfizer).

Following the publication of a report in the Journal of Laryngology and Otology (April 2007), FDA conducted a search of its adverse event reporting system for cases of hearing loss in patients taking PDE5 inhibitors. Sudden hearing loss was defined as new hearing loss occurring over a period of 3 days or less following the last dose, both with and without tinnitus and dizziness. A total of 29 cases were identified in post-marketing surveillance of all forms of PDE5 inhibitors on the market (sildenafil, vardenafil or tadalafil). The incidence of new hearing loss in temporal association with PDE5 inhibitor use in clinical trials was also reviewed. **Sildenafil as Viagra®:** A total of 5 (0.02%) sildenafil treated patients, of the approximately 25,000 sildenafil-treated patients from all clinical studies combined, experienced sudden hearing loss. Fifteen of the 29 post-marketing cases occurred in patients taking sildenafil as Viagra. In 5 of the 15 cases, sudden hearing loss occurred after the first dose. Nine of the 15 cases were unilateral, one was bilateral and 5 did not specify. Sudden hearing loss was temporary (lasting 24 hrs to 2 weeks) in 4 of the 15 cases. In 8 cases, the sudden hearing loss was ongoing, and in 3 cases it was not reported if the sudden hearing loss was temporary or ongoing. **Sildenafil as Revatio® in patients with pulmonary hypertension**: A total of 5 (0.8%) sildenafil-treated patients among the approx-

imately 660 patients enrolled in all clinical studies reported hearing loss/impairment, including sudden hearing loss. Four of these cases occurred in an open-label extension study. Sildenafil therapy was continued in all 5 cases. In 2 cases, the sudden hearing loss resolved (2 months in one case, 1 day in the other). In 3 cases, the hearing impairment was still present at the end of the study. Four of the 29 post-marketing cases occurred in patients taking sildenafil as Revatio. The time to onset of sudden hearing loss ranged from less than 3 weeks to 11 months after beginning Revatio therapy. All 4 cases involved unilateral hearing loss and were ongoing. Revatio therapy was continued for three of the reported cases and discontinued in 1 case.

The FDA labeling revision advises patients using PDE5 inhibitors for erectile dysfunction to *discontinue* the medication and contact their physician if they have hearing changes (ringing in ears or hearing loss). The FDA advises patients using PDE5 inhibitors for pulmonary hypertension to *continue* the medication and contact their physician immediately if they have hearing changes (ringing in ears or hearing loss).

In the RELAX trial, a simple bedside assessment of hearing will be collected during the baseline physical examination. If patients note tinnitus or hearing loss, they will be advised to discontinue the study drug and contact the study staff immediately.

Use of sildenafil in patients with coronary disease and/or HF has been examined. Sildenafil (40 mg intravenously) administered to patients with stable ischemic heart disease not taking nitrates resulted in a 10% decrease in systolic BP. In 105 men with a mean (SD) age of 66 (9) years who had erectile dysfunction and known or highly suspected CAD with mean ejection fraction 56% (7%) (range, 39%-68%), sildenafil (50 or 100 mg) reduced blood pressure from 135 (19) mm Hg to 128 (17) mm Hg prior to stress testing. Sildenafil had no effect on symptoms, exercise duration, or presence or extent of exercise-induced ischemia, as assessed by exercise echocardiography. Sildenafil has been administered to patients with severe pulmonary hypertension (primarily class III symptoms) at doses from 75 mg bid to 200 mg bid[90]. Lepore JJ et al[69] studied 11 patients with left ventricular systolic dysfunction due to coronary artery disease or idiopathic dilated cardiomyopathy and secondary pulmonary hypertension. Oral sildenafil (50 mg), inhaled NO (80 ppm), and the combination of sildenafil and inhaled NO were administered during right-heart and micromanometer left-heart catheterization. Administration of sildenafil alone or in combination with inhaled NO improved pulmonary and systemic vascular resistance but did not change systemic arterial pressure or indexes of myocardial systolic or diastolic function. Katz et al [91] studied 63 patients with systolic HF (age 30-79; median EF =33%) taking standard HF therapy who were treated with sildenafil (50-100 mg; up to 40 doses in 14 weeks) for erectile dysfunction and reported no adverse events. Alaeddini J et al[92] studied 14 patients with HF and pulmonary hypertension treated with sildenafil 25 mg (n = 8) or 50 mg (n = 6) every 8 hours for </=3 doses with invasive serial hemodynamic measurements. There was no significant decrease in systolic (108 ± 19 vs 104 ± 21 mm Hg) or diastolic (62 ± 10 vs 59 ± 11 mm Hg) blood pressure after drug administration (p = NS for systolic and diastolic blood pressure). No patient experienced any side effect related to the administration of sildenafil. No adverse alteration in renal function was noted, and there was no development of a new supraventricular or ventricular tachyarrhythmia. Webster LJ et al[93] studied 35 patients with class II-III HF treated with sildenafil for erectile dysfunction with ambulatory blood pressure monitoring. Sildenafil caused a mean +/- SEM asymptomatic decrease in blood pressure of 6 +/- 3 mm Hg, and no patient experienced symptomatic hypotension or other significant adverse effects. Bocchi FA et al[70] studied 23 HF patients with acute administration and chronic therapy for erectile dysfunction of sildenafil (50mg orally prn) and found that sildenafil was well tolerated and improved exercise capacity. These studies provide assurance that the administration of sildenafil at the planned dose is safe in patients with HF. In DHF, there is less concern regarding hypotension as average blood pressure after treatment is ≈ 140 mmHg [11] and while patients are older, the meta-analysis of Fink et al did not report significant increases in side effect profile according to subgroups such as age, diabetes, hypertension[85].

10. RECRUITMENT PROCEDURES

10.1 Common Recruitment Procedures

While a large number of clinical and epidemiology studies have established that 30-50% of patients with HF have normal EF, patients with DHF are often not cared for by cardiologists, particularly at academic institutions. Close collaboration with non-cardiologist care providers at each site and cardiologists from the community will be important to insure adequate enrollment of patients with DHF. Some important recruitment strategies to be considered include:

1. Presentations or meetings with non-cardiologist and cardiologist practices within each RCC to educate care providers regarding DHF, the lack of proven therapies and the RELAX trial. *A slide set for these presentations will be generated and made available to participating sites.*
2. Screening of HF hospitalization records. Each site should have some method of identifying patients recently hospitalized with a discharge diagnosis of HF (DRG-127). Administrative data bases for most hospitals will have this data and as assessment of ejection fraction is considered a marker of quality of care for patients with new onset HF, most patients will have an assessment of EF. Study coordinators can use this list to screen for those patients with normal EF.
3. Many echo laboratories record a referral diagnosis and this is an additional method to screen for DHF patients. Study coordinators may negotiate access to the site echocardiographic laboratory records/data base and look for patients referred for evaluation of dyspnea or heart failure. A quick review of these echo reports looking for patients with normal EF and evidence of diastolic dysfunction (particularly the presence of left atrial enlargement) may be helpful in identifying patients.
4. Meetings with pulmonologists caring for patients with pulmonary hypertension. Patients may be identified by referring physicians as having an elevated RV systolic pressure obtained echocardiographically. These patients then undergo right heart catheterization and are found to have an elevated LV filling pressure and a picture consistent with DHF with secondary pulmonary hypertension. This represents a potential group of patients for study under this protocol.
5. Distribution of and posting of materials advertising the study to patient care areas to enhance referral again, including non-cardiac care floors/practices.

10.2 Informed Consent Procedures

10.2.1 Informed Consent

Patients will typically be recruited in the outpatient setting but may be initially identified as a potential candidate during a hospitalization for DHF. After a potential patient is identified, the study coordinator and/or site PI will review the inclusion and exclusion criteria. If the patient meets these criteria, the study coordinator and/or PI will explain the study, review the consent form, answer questions and if the patient is willing to participate, the consent form will be signed. The patient will then undergo the screening tests (safety labs and cardiopulmonary stress test if not available clinically within 3 months of enrollment in the absence of changes in clinical status). If the patient meets criteria for participation based on the screening labs and metabolic stress test, the patient will complete the baseline studies, be randomized and begin study drug.

10.2.2 Confidentiality and HIPAA Requirements

Patients enrolled in the study will be identified by a study number. Within each participating site, patients will also be identified by their site specific registration number and their name but these patient identifiers will not be released outside of the regional HFN site and any publications will exclude any kind of patient identifiers that could be correlated with the specific patient. Plasma samples, MRI, echocardiographic, radiographic, electrocardiographic, exercise, clinical and quality of life data will be collected specifically for the research protocol and identifying information removed before submission to the DCC or the Core Laboratories. Existing clinical and demographic data will be collected from the patient's record for the purposes of the research protocol but all data will be de-identified before submission to the DCC or Core Laboratories.

11. SCREENING PROCEDURES

Patients will be referred to the study or will be identified using the recruitment strategies outlined in section 10. The patient's clinical records will be reviewed to determine if the patient meets the entry criteria. Additional information may be collected from face to face or phone interview with the patient and/or the patient's physician. If the patient meets entry criteria and is willing to participate, informed consent is obtained.

The patient will have labs drawn (if not available within 90 days of consent) to determine eligibility (complete blood count, creatinine, bilirubin, alkaline phosphatase and AST), a NT-proBNP (or BNP) level and undergo a metabolic stress test (if not available within 90 days of enrollment). If the patient meets the second tier entry criteria (no prohibitive abnormalities in CBC, creatinine, or liver function tests, and peak VO_2 ≤ 60% normal value with respiratory exchange ratio (RER) ≥1.0), the patient will be randomized.

The values for peak VO_2 in normal persons and the values reflecting below the average are:

Males 20-29 years old: 43±7.2; entry criteria < 28.2 ml/kg/min
Females 20-29 years old: 36±6.9; entry criteria < 21.6 ml/kg/min
Males 30-39 years old: 42±7.0; entry criteria < 25.2 ml/kg/min
Females 30-39 years old: 34±6.2; entry criteria < 20.4 ml/kg/min
Males 40-49 years old: 40±7.2; entry criteria < 24 ml/kg/min
Females 40-49 years old: 32±6.2; entry criteria < 19.2 ml/kg/min
Males 50-59 years old: 36±7.1; entry criteria < 22 ml/kg/min
Females 50-59 years old: 29±5.4 entry criteria < 17.4 ml/kg/min
Males 60-69 years old: 33±7.3; entry criteria <19.8 ml/kg/min
Females 60-69 years old: 27±4.7: entry criteria < 16.2 ml/kg/min
Males 70-79 years old: 29±7.3: entry criteria <17.4 ml/kg/min
Females 70-79 years old: 27±5.8: entry criteria <16.2 ml/kg/min

These values are based on published normal values [94].

Both the screening study labs and the metabolic stress test may be performed and interpreted on site. If obtained solely as a screening test, the screening CPXT must be performed according to the RELAX CPXT protocol and will be interpreted locally but will be submitted to the CORE laboratory as the baseline study if patients are enrolled. The patient will then undergo baseline studies. After baseline studies have been completed, the patient will be randomized and the first dose of medication given. The screening study labs and metabolic stress test should be performed within two weeks of consent. The baseline studies, randomization and dosing of study drug should be performed within two weeks of the screening studies. Exceptions to these time limits will be permitted in special circumstances after review with the DCC staff.

11.1 Telephone Screening

If direct referral or recruitment measures as outlined in section 10 identify a patient who may be eligible for participation, the study coordinator may contact the patient to obtain further information needed for eligibility and to explain the study to ascertain the level of interest. Phone scripts for these types of patient contact will be generated.

11.2 Prescreen

Potential patients may be identified through recruitment strategies as outlined in section 10. Data pertinent to the inclusion and exclusion criteria will be obtained from the patient's medical record, face to face or phone contact of the patient and/or the referring care provider.

11.3 Screening

The screening procedures include:

1. Safety labs (if not available from clinical record within 90 days of consent) including complete blood count, creatinine, bilirubin, alkaline phosphatase and AST.
2. An NT-proBNP level (or BNP level if NT-proBNP not available at enrolling site)

3. Cardiopulmonary exercise test (if not available from clinical record within 90 days of consent) to determine if they are able to exercise and have a peak VO_2 in the range necessary to participate in the study. This study will be performed with the RELAX CPXT protocol and will be interpreted locally but will be submitted to the CORE laboratory as the baseline study if patients are enrolled.
4. Consistent with recommendations for the management of patients with diabetes, patients with diabetes should undergo yearly eye examinations. If patients have not had their yearly examination (visual acuity and fundoscopic examination) within 3 months of enrollment or if the results of this study are not available, an eye examination should be performed prior to enrollment in the study.

12. BASELINE EVALUATIONS AND RANDOMIZATION

12.1 Evaluations Performed During the Baseline Period

The following studies will be performed during the baseline period prior to randomization and starting study drug. Please see section 13 for a detailed description of these procedures as well as the schedule of evaluations as outlined in Appendix 22.1.

1. Limited Doppler echocardiography
2. Cardiac Magnetic Resonance Imaging (MRI) (Patients in sinus rhythm only at enrolling sites with MRI access)
3. Phlebotomy for Biomarkers
4. Metabolic stress test (Screening study may be submitted to the CORE CPXT laboratory as the baseline study if patient meets entry criteria)
5. Six minute walk test
6. Minnesota Living with Heart Failure Questionnaire
7. Creatinine
8. Electrocardiogram
9. Clinical history
10. Physical examination which will include simple bedside hearing assessment (ability to hear examiner's fingers rubbed together in front of each ear).

12.2 Randomization Procedures

After the patient has been consented and has completed the screening studies, patients meeting meeting the entry criteria based on the screening studies will undergo baseline studies and then be randomized. Randomization will be performed via a web based system (WebEZ) Randomization will be stratified by site and by the presence or absence of atrial fibrillation.

12.3 Blinding of Study Personnel

All enrolled patients will be assigned a study number and study drug (placebo or sildenafil) will have an identical appearance and will be allocated according to a predetermined randomization scheme. The DCC will allocate study drug and the study staff will not have access to information regarding whether the patient is on active or placebo therapy. As sildenafil may commonly have characteristic benign (enhanced erectile function) or rarely adverse (priapism) side effects in male patients, study subjects will be encouraged at consent and enrollment not to discuss any non-adverse changes in sexual function with the study staff. However, all patients will be assessed for adverse events at the serial study visits as outlined above. If a patient has experienced an adverse effect likely or potentially attributable to the study drug, the decision to discontinue the drug will be made without unblinding the patient or the study staff unless deemed urgent to care of the patient. In this case, the DCC will supply the information and the patient will be removed from the study.

12.4 Unblinding Procedures

The DCC Medical Monitor will be available to the attending physician to help consider the need for unblinding on a case by case basis. Unblinding will be permitted ONLY for the reason of subject safety. Specifically, the blind should be broken only for serious, unexpected and drug-related AEs or when required by local regulatory authorities, when the knowledge of treatment assignment is needed for subject safety. The site investigator must notify the DCC prior to the unblinding of any subject. The site investigator should direct the attending physician to contact the DCC Medical Monitor who will verify that a patient safety issue requires unblinding and will carry out the unblinding procedure. In an emergency, if the site investigator is not immediately available, the attending physician should contact the DCC Medical Monitor directly. Every effort should be made to maintain the blind for the site investigator and study coordinator. In the event of a blind breaking, only the treatment assignment for the subject in question will be revealed.

13. FOLLOW-UP EVALUATIONS

13.1 Evaluations Performed During the Follow-up Period

The following studies will be performed during the follow up period after starting study drug. Please see section 14 for a detailed description of these procedures section 22 (Appendix 22.1) for the schedule of evaluations.

Week -4 to 0: Consent, Screening studies and Baseline studies
Week 0: Randomization and initial dosing visit (Study Coordinator) (Phone follow up x 2 days)
Week 1: Phone contact to assess study drug tolerance (Study Coordinator)
Week 3: Study visit and evaluations (Study Coordinator)

1. Creatinine
2. History
3. Limited physical examination including bedside hearing assessment

Week 8: Phone contact to assess study drug tolerance (Study Coordinator)
Week 12: Study visit and evaluations and dose escalation (Study coordinator and PI)

1. History
2. Physical examination including bedside hearing assessment
3. Metabolic stress test with sildenafil level immediately prior to the study.
4. Six minute walk test
5. Minnesota Living with Heart Failure Questionnaire
6. Creatinine
7. Dose escalation (60 mg tid) (Phone follow up x 2 days)

Week 13: Phone contact to assess study drug tolerance (Study Coordinator)
Week 16: Phone contact to assess study drug tolerance (Study Coordinator)
Week 20: Phone contact to assess study drug tolerance (Study Coordinator)
Week 24: Final study visit and evaluations (Study coordinator and PI)

1. Limited Doppler echocardiography
2. Cardiac Magnetic Resonance Imaging (MRI) (Patients in sinus rhythm only)
3. Phlebotomy for Biomarkers
4. Metabolic stress test with sildenafil level immediately prior to the study.
5. Six minute walk test
6. Minnesota Living with Heart Failure Questionnaire
7. Creatinine
8. Clinical history
9. Physical examination including bedside hearing assessment

13.2 Off-Schedule Evaluations

Patient's returning for an AE will have an evaluation by the study coordinator and PI and an adverse event form will be filled out. Further evaluation for clinical care of the patient will be performed appropri-

ate to the care of the patient and the nature of the AE event. No other evaluations specific to the study will be performed.

13.3 Evaluations for Drop-Outs and Withdrawals

If a subject drops out of the study for any reason, the subject will be encouraged to complete as many of the study procedures as they will agree to as outlined in section 13.1 above.

14. OUTCOME DETERMINATIONS

14.1 Primary Outcome: Maximal oxygen uptake (peak VO_2) at cardiopulmonary exercise testing (CPXT).

For baseline or screening CPXT, the CPXT may be done at any time in the evaluation process. For studies on therapy (12 and 24 weeks), study patients should take their study medication on site approximately 1 hr (45-120 minutes) prior to blood draw for study labs. CPXT should be performed immediately after the blood draw. All screening CPXT should be performed using the same protocol as is used for baseline and follow up CPXT in RELAX and may be used as the baseline CPXT.

Cardiopulmonary exercise testing (CPXT) will be performed using the best exercise modality, determined by the CPXT lab and the patient. The chosen exercise modality should be used for all subsequent CPXT tests. Please see the Cardiopulmonary Exercise Testing Manual of Operating Procedures for details regarding the methods to be used during the CPXT.

A number of other exercise variables will be recorded to allow further post hoc exploratory analyses including time to ventilatory anaerobic threshold, VO2 max at ventilatory anaerobic threshold, and oxygen uptake efficiency slope[95] .

14.2 Secondary and Tertiary Outcomes

14.2.1 Composite Clinical Score

A composite clinical score in RELAX will be based on a ranking of all participants. All participants will be ranked sequentially with ranking stratified in one of three tiers based on:

1) Death (lowest tier)

The ranking within this tier is based on time to death from randomization date. The person with the shortest time from randomization to death is given the lowest rank within the tier.

2) Hospitalizations due to cardiovascular (CV) or renal causes (middle tier)

For patients alive, the ranking within this tier is based on time to hospitalization from randomization date. The person with the first cardiovascular or renal cause hospitalization will be given the lowest rank within the tier. Cardiovascular causes defined as initial primary cause for hospitalization as HF, acute coronary syndrome, cerebrovascular accident, peripheral vascular disease, arrhythmia or syncope. Renal causes defined as initial primary cause for hospitalization for worsening renal function, renal failure or hyperkalemia.

3) Change in MLWHFQ from baseline (highest tier)

For patients without an event meeting the first two criteria, the person with the least favorable change in MLWHFQ is given the lowest rank within this tier. Patients with an equal change in the MLWHFQ will receive an equal ranking.

The use of three tiers within the ranking reflects the greater adverse impact of death or CV hospitalization on clinical status without an arbitrary assignment as to the relative value of these events in relation to changes in quality of life.

14.2.2 Minnesota Living with Heart Failure Questionnaire:

The MLWHFQ is a self-administered, disease-specific measure of health related quality of life (QOL) that assesses patients' perceptions of the influence of HF on physical, socioeconomic and psychological aspects of life[96-98]. Patients respond to 21 items using a six-point response scale (0-5). The total summary score can range from 0-105 with a lower score reflecting better HF related QOL. Two subscale scores reflect physical (8 items) and emotional (5 items) impairment. This instrument has been extensively validated and widely used to assess treatment effect on clinical status in multiple trials of therapeutic interventions in HF.

14.2.3 Six minute hall walk

The six minute walk test is widely used to assess submaximal exercise performance in clinical trials conducted in patients with HF or pulmonary disease. Use of the six minute walk test as an outcome measure in randomized, blinded intervention trials of chronic HF has been comprehensively reviewed[99] and its use in elderly patients with HF has been specifically reviewed[100].

Six minute walks will be performed by registered RN or LPN study coordinators or trained exercise technicians using a standardized approach using a 25 meter course in an enclosed corridor with a chair placed at each end of the course. Patients will be allowed to use any mobility aids they traditionally use Patients will be instructed to walk from end to end at their own pace while attempting to cover as much ground as possible in the six minutes using a standardized script.

"The purpose of this test is to find out how far you can walk in six minutes. You will start from this point and walk back and forth between the two markers I showed you. You will go back and forth as many times as you can in the six-minute period. If you need to, you may stop and rest. Just remain where you are until you can go again. However, the most important thing about the test is that you cover as much ground as you possibly can during the six minutes. I will tell you the time, and I will let you know when the six minutes are up. When I say 'stop', please stand right where you are."

During the walk, the following words of encouragement will be provided at 30-second intervals "…you're doing well"…"keep up the good work"…"good job"…"you're doing fine".

The patient will be notified when 2, 4, and 6 minutes (stop) have elapsed and what the remaining time is. Patients will be allowed to slow or stop and rest during the walk but will be asked to resume walking as soon as they feel able. After six minutes, the distance walked will be measured to the nearest meter. Vital signs (heart rate and blood pressure) will be obtained before and immediately after the test in the standing position. Patients will indicate symptoms limiting ability to walk during the test (dyspnea, fatigue, chest pain, leg or joint pain, instability, other, none).

14.2.4 Cardiac MRI for assessment of LV and vascular structure and function

Patients in atrial fibrillation will not undergo baseline or follow-up MRI. Patients will undergo cardiac MRI for measurement of LV end diastolic volume, LV end systolic volume, LV mass, ascending aortic wall thickness and ascending aortic maximal and minimal cross sectional area (CSA). Systolic (SBP) and diastolic (DBP) blood pressure and heart rate will be assessed at the time of aortic imaging using a non-ferromagnestic sphygmomanometer. LV volumes and LV mass will be calculated according to Simpson's rule on traced endocardial and epicardial short axis LV images. Aortic wall thickness will be measured along the wall of the aorta adjacent to the superior vena cava in a region free of atherosclerotic plaque. Derived indices of ventricular and aortic function will include:Ejection fraction (%) = [(LV end diastolic – end systolic volume)/LV end diastolic volume] x 100%; Stroke volume (ml) = (LV end diastolic – end systolic volume); Aortic distensibility (10^{-3} mmHg) = (aortic CSA_{max} – aortic CSA_{min})/(aortic CSA_{min} x (SBP-DBP)); Effective arterial elastance (Ea, mmHg/ml) = (SBP x 0.9)/stroke volume; systemic vascular resistance (dyne*sec/cm^5)= 80 x [MAP/(stroke volume x heart rate)]

EKG gated MRI images will be obtained using a phased-array cardiac surface coil placed on the chest. After initial localization scans, short axis cine images 8 mm thick with a 2 mm gap will be obtained perpendicular to the long axis of the ventricle scanning from base to apex using a steady state free pre-

cession fast gradient echo sequence (fiesta) with the following parameters: TE min full, flip angle 45°, bandwidth 125 kHz, matrix 256x192-224, field of view 34-40 cm, 20 cardiac phases, 12 views per segment, and number of acquisitions 0.5-1.0. Each short axis slice typically requires 10-15 seconds to acquire depending on the heart rate, and parameters (matrix, views per segment, and number of acquisitions) will be adjusted on an individual basis according to heart rate and breath hold capacity. Using a mid ventricular short axis cine image, long axis views with the same parameters will be prescribed perpendicular to the plane of the ventricular septum.

Cardiac cycle-dependent changes in the aortic lumen will be assessed as previously described [32,50] with interleaved, velocity encoded, phase-contrast, gradient echo images acquired perpendicular to the course of the proximal ascending thoracic aorta approximately 4 cm above the aortic valve. Scans will have slices 6 mm thick with a 256 x 256 matrix, a 26 cm field of view, a 40° flip angle, an 11 ms repetition time, a 3.5 ms echo time and a through-plane velocity encoding of 150 cm/s.

To assess aortic wall thickness, double inversion recovery fast spin echo images will be acquired in the same slice position as that used to assess aortic distensibility. These scans will have an echo train length of 32, a repetition time of 2 x the R-R interval, a 650 ms inversion time, a 42 ms echo time, a 30 cm field of view and a 256 x 256 matrix [32,50].

Images will be transferred to a windows workstation and an appropriate software analysis package will be used to analyze images at a core MRI laboratory. The average of two measurements will be used for each parameter.

14.2.5 Doppler echocardiography

A limited 2 D, M-mode and Doppler echocardiogram will be performed in each subject in the left lateral (parasternal long axis view and right ventricular (RV) inflow view) and the left lateral decubitus (apical 4 chamber and long axis views) positions using an appropriate echocardiographic instrument equipped with a multifrequency transducer as well as the Doppler tissue imaging (DTI) program. Blood pressure and heart rate will be recorded after the patient has acclimated to the imaging environment. In the parasternal long axis view, the diameter of left ventricular outflow tract (LVOT) will be measured just below the aortic valve. In the RV inflow view, guided by color flow imaging, continuous wave Doppler imaging will be used to measure the peak tricuspid regurgitant velocity. In the apical long axis view, pulsed wave Doppler echocardiography will be used to measure the LVOT velocity profile. In the apical 4-chamber view, the transmitral inflow velocity profile obtained from pulsed wave Doppler imaging at the tips of the mitral valve will be obtained. Doppler tissue imaging will be used to measure the septal and lateral mitral annular velocity in diastole. Guided by color flow imaging, continuous wave Doppler imaging will be used to measure the peak tricuspid regurgitant velocity. Measurements and derived values will be obtained in triplicate and measurements made at a core echocardiographic laboratory. Measurements and derived values include:

Stroke volume = [3.14 x (LVOT diameter/2)^2] x time velocity integral of the pulsed wave LVOT velocity profile

Cardiac output = SV * heart rate

E velocity (cm/s) from the transmitral inflow velocity profile measured at leaflet tips.

E' velocity (cm/sec) (septal and lateral) from the diastolic mitral annular velocities measured with DTI.

The E/E' ratio (septal and lateral) will be calculated for each subject.

Operant Ed will be calculated as (E/E')/stroke volume as previously reported [27].

Peak pulmonary systolic pressure = (4*(Peak TR velocity in m/sec)^2 + 10) using the maximal TR velocity consistently measured (not following extrasystole)

Standardized image acquisition and measurement algorithms along with training materials will be provided to each participating center to enhance standardization of Doppler echocardiographic measurements.

14.2.6 Biomarker assays

All biomarker assays will be performed at a Core biomarker lab. cGMP and Aldosterone will be measured by RIA as previously described[101]. N-terminal pro BNP will be measured by the Roche assay as

previously described[102]. Plasma Amino-terminal propeptide of type III procollagen (PIIINP) levels have been shown to correlate with the extent of cardiac fibrosis in systolic HF and hypertensive heart disease and to reflect extracellular matrix metabolism, decreasing with therapies which reduce the degree of cardiac fibrosis and indices of LV diastolic dysfunction[103-110]. Plasma PIIINP will be determined using a radioimmunoassay with a commercially available kit (Orion Diagnostica, Espoo, Finland). The intra-assay and interassay variations for determining PIIINP are 5.3% and 7.7%, respectively. The sensitivity (lower detection limit) is 0.2 µg/L. Endothelin (marker of neurohumoral activation), troponin (marker of myocardial damage), uric acid (marker of oxidative stress) and cystatin C (marker of renal dysfunction) will also be measured.

14.2.7 Sildenafil levels

Peak sildenafil levels (45-120 minutes post dosing) will be determined at the 12 and 24 week visits and will be timed to coincide or immediately precede CPXT.

15. METHODS TO PROMOTE ADHERENCE TO THE INTERVENTIONS

Adherence to study drug: Patients will be instructed to bring all used and remaining bottles of study drug to each study visit. The percent compliance will be calculated at each study visit.

Adherence to study procedures: Adherence to study procedures will be enhanced by the following factors:

1. At recruitment and consent visits, the entire study will be carefully explained to the patient and figures used to indicate what procedures and visits will be required of the patient. The potential participant will be asked to carefully consider their ability to participate in the study and specifically their willingness to perform a total of four cardiopulmonary exercise tests.
2. When feasible, use of a clinically indicated screening CPXT will familiarize the patient with the procedure and better enable the patient to determine whether they are able to perform subsequent baseline, 12 and 24 week CPXT. Further, patients with non-cardiac dyspnea due to frailty, motivational factors, deconditioning and pulmonary disease will be identified and excluded (patients must meet RER≥1.0) since these patients are less likely to perform subsequent CPXT.
3. Designation of VO_2 as the primary endpoint emphasizes its importance to both the investigator and the patient and will enhance compliance.
4. Adherence to study drug and procedures will be promoted via the study visits and monthly phone visits when not seen in person.
5. There is currently no therapy proven to affect outcomes in DHF. This factor generally enhances compliance with an RCT of novel therapies.
6. Performance of RELAX within the limited number of sites in the HFN under the direct supervision of the principal investigators involved in the HFN steering committee should enhance compliance with the baseline and follow up studies.
7. Data completeness at each site will be carefully monitored by the DCC and those sites not providing complete follow up data will be contacted and strategies to enhance compliance identified.
8. In general, event rates in RCT are lower than those observed in observational studies of HF and this was observed in DHF even in a large RCT restricted to elderly persons (PEP-CHF)[47]. Power calculations indicate adequate power even considering death rates at 6 months similar to those observed in community based observational studies.

16. QUALITY CONTROL ACTIVITIES

16.1 Training Sessions and Certification Procedures

A Manual of Operating Procedures (MOP) details all study procedures. It contains specific instructions describing how the study is conducted, what procedures are performed, in what order, by whom and under what circumstances. A study coordinator training session will be held to provide appropriate instruction regarding the RELAX study.

17. PARTICIPANT SAFETY AND ADVERSE EVENTS

17.1 Institutional Review Boards

Before initiating this study, the protocol, site-specific informed consent forms, HIPAA forms, recruitment materials, and other relevant information will be reviewed by a properly constituted IRB at each participating clinical site. A copy of the signed and dated IRB approval at each clinical site will be retrieved prior to site activation and archived at the DCC. Any amendments to the protocol, other than simple administrative and typographical changes, must be approved by each IRB before they are implemented. The sites will seek annual renewals of their IRB approvals in accordance with local procedures.

17.2 Adverse Events

During a clinical trial, the reporting of adverse experience information can lead to important changes in the way a new treatment is developed, as well as provide integral safety data.

17.2.1 Definition of an Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical-investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this study drug. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered to be related to the medicinal product. Diseases, signs, symptoms, or laboratory abnormalities already existing at enrollment are not considered AEs unless they worsen (i.e., increase in intensity or frequency). Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Surgical procedures planned prior to randomization and the conditions leading to these measures are not AEs.

The relation between an adverse event and the study drug will be determined by the Investigator on the basis of his or her clinical judgment and the following definitions:

Reasonable Possibility – There is a reasonable possibility that the adverse event was caused by the study drug. The temporal relationship of the adverse event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide sufficient explanation for the observed event.

Not a reasonable possibility – There is not a reasonable possibility that the adverse event may have been caused by the study drug. The temporal relationship of the adverse event to trial drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide sufficient explanation for the observed event.

Drug-related means that there is a reasonable possibility that the adverse event may have been caused by the test agent.

An **unexpected** adverse event is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., U.S. package insert).

The intensity of the adverse event will be defined by the following criteria:

Mild: The adverse event is noticeable to the patient but does not interfere with routine activity

Moderate: The adverse event is discomforting and interferes with routine activity.

Severe: The adverse event significantly limits the patient's ability to perform routine activities despite symptomatic therapy.

Life -Threatening: The patient is at immediate risk of death.

17.2.2 Adverse events Anticipated in This Study

The following table separates the definite or probable study drug-related conditions, illnesses or adverse events from the pre-existing or concurrent illnesses seen in the study patient population.

Pre-existing, Predisposed or Concurrent Conditions, Illnesses or Adverse Events	Study Drug Related Conditions, Illnesses or Adverse Events
▪ Sudden death ▪ Arrhythmias ▪ Acute coronary syndrome ▪ Cerebrovascular accident ▪ Pulmonary embolism ▪ Hospitalizations for HF ▪ IV diuretic administration ▪ Deep vein thrombosis ▪ Dizziness ▪ Lightheadedness ▪ Syncope ▪ Worsening renal function	▪ Headache ▪ Flushing ▪ Dizziness, Lightheadedness ▪ Syncope ▪ Dyspepsia ▪ Worsening renal function ▪ Visual disturbance ▪ Priapism ▪ Tinnitus or hearing loss

17.3 Adverse Event Collection

For the RELAX trial, all AEs (serious and non-serious) will be recorded from start of study treatment through final study visit on the AE case report form.

17.4 Serious Adverse Events

A serious adverse event (SAE) is any untoward event that:

- Is fatal
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization, with the following exceptions:
    - Preplanned (prior to the study) hospital admissions unless the hospitalization is prolonged
    - Planned admissions (as part of a study, e.g., routine biopsies)

- Hospitalizations of less than 24 hours duration
- Hospitalization for elective procedure
- Emergency room visits

- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect.
- Important medical events that may not result in death, be life-threatening, or require inpatient hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening means that the patient or subject was, in the view of the investigator, at immediate risk of death from the AE as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.

Persistent or significant disability/incapacity means that the event resulted in permanent or significant and substantial disruption of the subject's ability to carry out normal life functions.

Associated with the use of the drug means that there is a reasonable possibility that the experience may have been caused by the drug.

17.4.1 Potential SAEs Anticipated in This Study

Serious adverse events anticipated based on the proposed study population and on use of the study drug in previous large randomized clinical trials for erectile dysfunction or pulmonary hypertension include:

1. Death
2. Worsening HF as defined by the need for escalation of HF therapy as an outpatient
3. Unplanned treatment for HF in an Emergency Department without hospital admission
4. Unplanned hospitalization for HF
5. Syncope
6. Visual disturbance
7. Priapism
8. Cerebrovascular accident
9. Acute coronary syndrome
10. Hearing loss

17.5 Procedures for Expedited Reporting of SAE

For the RELAX trial, all SAEs occurring from study drug initiation through final study visit require expedited reporting. The investigator must complete and submit a Pfizer IIR SAE form to DCRI Safety Surveillance within 24 hours of knowledge of the event.

DCRI Safety Surveillance
Telephone: 1-866-668-7799
Fax: 1-866-668-7138

The investigator must complete and submit a follow-up Pfizer IIR SAE form when important follow-up information (diagnosis, outcome, results of specific investigations, etc.) becomes available after submission of the initial form. Follow-up forms should be submitted according to the same process used for reporting the initial event as described above (i.e., within 24 hours of knowledge). All reportable events will be followed until resolution, stabilization, or 30 days after the last patient completes the final study visit, whichever occurs first. The investigators will be responsible for reporting AEs to their local institutional review boards (IRBs) in accordance with local guidelines.

DCRI Safety Surveillance will perform a clinical review of the forms to verify that all sections are complete, legible and consistent and will query the site for incomplete information, data clarification and/or

follow-up information. A narrative summary will be written for each SAE and SAE data will be entered into the Clintrial database.

DCRI Safety Surveillance will forward all SAE forms, regardless of relatedness, to Pfizer within 1 business day of receipt along with a copy of any queries sent to the site.

17.5.1 Regulatory Reporting

For any SAE that is assessed by the site investigator as both related to study drug (reasonable possibilty) and as unexpected per package labeling, site investigators are required to complete and submit the voluntary 3500 MedWatch form online at https://www.accessdata.fda.gov/scripts/medwatch/.
A copy of this MedWatch form should be sent to DCRI Safety Surveillance. Investigators are responsible for promptly reporting these events to their reviewing IRBs.

18. STATISTICAL CONSIDERATIONS

18.1 Sample Size and Power Considerations

18.1.1 Primary endpoint: Change in peak VO_2 (ΔVO_2)

No previous study has characterized the standard deviation of the ΔVO_2 in a study population similar to that planned here where DHF patients are studied and strict entry criteria and a screening cardiopulmonary exercise test (CPXT) are used to minimize enrollment of patients with non-cardiac dyspnea and select for patients who can perform near maximal exercise (RER≥1.0). Patients who are unwilling or unable to perform at this level of exercise will not be eligible for enrollment. This requirement will reduce variability in both the primary endpoint (Δ peak VO_2) and the secondary endpoint measuring submaximal exercise (Δ6MW). Recognizing the lack of data relevant to the planned study population, we have based power calculations on the SD for the ΔVO_2 observed in randomized clinical trials (RCT) in SHF where ΔVO_2 was a primary (SD=2.7 ml/kg/min)[111] or secondary (SD≈2.7 ml/kg/min) [112,113] endpoint and on limited RCT data in DHF[50] (SD=1.3 ml/kg/min; Dalane Kitzman, personal communication). A range of withdrawal due to death at 6 months was also estimated based on a large RCT in DHF (<2%)[47] and community based observational studies in DHF (13-16%)[7,11].

The relationship between power, effect size (mean difference in ΔVO_2) and SD of the ΔVO_2 using a two sample t-test and a two-sided alpha of 0.05 is shown in the table assuming 85% and 90% power. This analysis indicates that a sample size of 158 patients with complete data will have 85% power to detect a difference of 1.2 ml/kg/min in ΔVO_2 with a SD of ΔVO_2 =2.5 ml/kg/min. To account for up to 25% missing data, the projected sample size is approximately 215 patients.

Power	85% Power			90% Power		
	TE = 1.0 ml/kg/min	TE = 1.2 ml/kg/min	TE =1.5 ml/kg/min	TE = 1.0 ml/kg/min	TE = 1.2 ml/kg/min	TE =1.5 ml/kg/min
SD=2.0	146	102	66	172	120	78
SD=2.5	228	158	102	266	186	120
SD=3.0	326	228	146	382	266	172
TE=treatment effect (difference in Δ in peak VO2); SD = standard deviation of Δ in peak VO2						

In patients with SHF, a single dose of sildenafil resulted in statistically significant increases in peak VO_2 in three small, single center studies (n≤24 patients) and the magnitude of this effect was similar to or exceeded that detectable in the current study[64,70,114]. Data from two recent studies of chronic PDE-5 inhibition in SHF showed significantly greater increases in peak VO2 in sildenafil vs placebo treated patients with effect sizes of 1.7 and 2.0 ml/kg/mn[115,116]. As outlined in the Preliminary Studies

(Section 5.0), we hypothesize that we will observe a greater effect in DHF than SHF and with chronic vs acute therapy.

The RELAX trial is designed to test the hypothesis that chronic PDE-5 inhibition improves exercise capacity and clinical status in DHF *and* to explore the mechanisms responsible for such an effect. If treatment benefits for exercise performance and key pathophysiological mechanisms are demonstrated, a larger clinical outcome based trial may be warranted.

18.1.2 Secondary Endpoints: Composite clinical score

A composite clinical score in RELAX will be based on a ranking of all participants. All participants will be ranked sequentially with ranking stratified in one of three tiers based on:

1) Death (lowest tier)

The ranking within this tier is based on time to death from randomization date. The person with the shortest time from randomization to death is given the lowest rank within the tier.

2) Hospitalizations due to cardiovascular (CV) or renal causes (middle tier)

For patients alive, the ranking within this tier is based on time to hospitalization from randomization date. The person with the first cardiovascular or renal cause hospitalization will be given the lowest rank within the tier. Cardiovascular causes defined as initial primary cause for hospitalization as HF, acute coronary syndrome, cerebrovascular accident, peripheral vascular disease, arrhythmia or syncope. Renal causes defined as initial primary cause for hospitalization for worsening renal function, renal failure or hyperkalemia.

3) Change in MLWHFQ from baseline (highest tier)

For patients without an event meeting the first two criteria, the person with the least favorable change in MLWHFQ is given the lowest rank within this tier. Patients with an equal change in the MLWHFQ will receive an equal ranking.

The use of three tiers within the ranking reflects the greater adverse impact of death or CV hospitalization on clinical status without an arbitrary assignment as to the relative value of these events in relation to changes in quality of life.

As was the case with the primary endpoint, we have no data relevant to the composite clinical endpoint in this population. However, Smith et al [7] described functional status changes over 6 months in a population of 200 heart failure patients with preserved ejection fraction. In this population, 13% of subjects died, 27% declined in functional status, 50% maintained functional status and 10% improved. With 190 patients with complete data and an assumption of a proportional odds model for the multinomial response (Agresti)[117] we would have 87% power to detect a proportional odds shift of 3.0 and 73% power to detect a proportional odds shift of 2.5[118]. We would likely have more power with the rank-ordered data. Assuming approximately 10% missing data and an analysis sample size of 190 patients, we have 80% power to detect a statistically significant difference if $P(X<Y) = 0.65$ where $P(X<Y)$ is the probability that a randomly selected patient in the sildenafil group has a better outcome rank than a randomly selected control group patient.

18.1.3 Secondary Endpoint: 6 Minute Walk Distance

No previous study has characterized the standard deviation of the Δ6MW in a study population similar to that planned here where DHF patients are studied and strict entry criteria and a screening CPXT are used to minimize enrollment of patients with non-cardiac dyspnea and select for patients who can perform near maximal exercise (RER≥1.0). Recognizing the lack of data relevant to the planned study population, we have based power calculations on the SD for the Δ6MW observed in the PEP-CHF trial in DHF[47] (n≈650). In this trial, the SD for the Δ6MW was ≈70m (John Cleland, personal communication, 2/28/2007) which was similar to the SD used for sample size estimates in the SUPER trial of sildenafil in PH[68]. Further, this was similar to the SD for Δ6MW observed in a small (SD=80m, n=34) RCT of chronic sildenafil therapy in SHF (Marc Semigran, personal communication). As above, a range of withdrawal due to death at 6 months was also estimated based on a large RCT in DHF (<2%)[47] and community based observational studies in DHF (13-16%)[7,11].

With the assumed common standard deviation of 70m, an analysis sample size of 190 patients (allowing for 10% missing data) will have 90% power to detect a difference in Δ6MW of 33m. In the SUPER trial of PDE-5 inhibition in PH, the placebo corrected mean difference in Δ6MW between treatment groups was approximately 45m[68]. In RELAX, we hypothesize that improvements in exercise capacity will be mediated not only by effects on pulmonary vascular tone, but also by effects on the systemic vasculature, LV and kidney. Thus, the potential for greater benefit in DHF than in PH exists.

18.1.4 Secondary Endpoint: MRI assessment of LV mass

Given the very high reproducibility of LV mass assessment by MRI, MRI of the entire RELAX cohort will not be needed to provide excellent power to detect clinically relevant changes in LV mass. A single center study assessed the reproducibility of measurements of LV mass by MRI and reported that the mean difference between repeat MRI measurement of LV mass was -1.1 ± 4.2 g (LV mass index -0.6 ± 2.1 g/m^2) in 20 normal persons and -2.4 ± 8.4 g (LV mass index -1.3 ± 4.0 g/m^2) in 20 patients with LVH[119]. These inter-study reproducibility values were similar to several other small studies and markedly better than inter-study reproducibility of echocardiography. The "4-E study" [120] was a multicenter study using MRI assessment of LV mass and a core MRI laboratory to compare the effect of different anti-hypertensive therapies on LV mass in patients with hypertension. Power calculations for the 4-E study assumed a standard deviation of 24g in the change in LV mass from baseline (3 x the SD reported in the Grothues study).

Based on these previous studies, enrollment of 132 patients in the "Sinus Rhythm" arm of RELAX would provide 85% power to detect a difference in the change in LV mass of 14.1g between groups with a conservative assumption of 20% missing data and a standard deviation of 24g (see table below).

MRI assessment of LV mass (n=132)				**Assuming 20% missing data (available data for n=106)**			
SD	8g	16g	24g	SD	8g	16g	24g
Difference in change in LV mass detectable (g)				**Difference in change in LV mass detectable (g)**			
85% power	4.2	8.4	12.6	85% power	4.7	9.4	14.1
90% power	4.6	9.1	13.7	90% power	5.1	10.2	15.3

Other tertiary endpoints: Allowing for 10% missing data, our projected sample size of 215 subjects would provide 85% power to detect an effect size of 0.44 for any of the tertiary endpoints.

18.2 Analysis Policies

A statistical analysis plan will be completed before the data are analyzed in an unblinded fashion. Due to clinical interest in departures from both sides of the null hypothesis, all test statistics will be two-sided. The trial results will be reported according to guidelines specified in the CONSORT statement[121,122]. A flow diagram describing screening, recruitment, randomization, dropout, and vital status will be included in the primary manuscript. Adverse events and efficacy data will be presented by the two treatment groups. Adherence, dropout, and lost-to-follow-up will be carefully examined across the two treatment groups. Analyses of safety will be based on data from all randomized patients who received at least 1 dose of study drug. All primary analyses will be based on intent-to-treat (ITT) principles using all randomized participants.

18.2.1 Controlling Type-I Error

The primary endpoint of change in peak VO2 from baseline to 24 weeks will be evaluated at the two-sided 0.05 level. A conservative approach will be taken with the multiple analyses on key clinical subgroups. For subgroup analyses, a significance level of 0.001 will be required for statistical significance. Thus, subgroup analyses will be considered exploratory unless the p-value from an interaction test is smaller than 0.001.

18.2.2 Interim Analyses for Efficacy

There will be no interim analysis for efficacy due to the short duration and small size of the trial.

18.3 Randomization

A permuted block randomization scheme will be created with varying block sizes stratified by the presence or absence of atrial fibrillation. Enrollment of patients with atrial fibrillation is planned to be limited to 58 patients (30% of study population). Once 58 patients with chronic atrial fibrillation have been enrolled, the DCC and DSMB will review the characteristics (atrial fibrillation versus sinus rhythm) of all patients enrolled and will increase this limit if needed to insure adequate enrollment to address the primary endpoint. Once a patient has completed the screening and baseline period and evaluation for inclusion/exclusion criteria, the randomization process will begin. Patients will be randomized to receive one of the 2 treatment regimes with equal probability (1:1), via a Web based system (WebEZ). On the day of randomization, after the patient has successfully met all inclusion and exclusion criteria, the investigator or designee will log into the on-line WebEZ randomization system to obtain the assigned kit randomization numbers for the patient. The WebEZ system will also be used for re-supply of the site.

18.4 Statistical Comparisons of Baseline Factors

For continuous variables, baseline factors will be compared across groups using mean (standard deviation) and median (25th and 75th percentiles) summary measures. For categorical variables, baseline factors will be summarized using counts and percentages with statistical comparisons based on the Pearson's chi-square statistic. Given the size of this proof of concept trial, the potential for inequitable distributions of baseline characteristics between the treatment groups exists. We have pre-specified subgroup analysis for those variables most likely to impact response to PDE-5 inhibition. Randomization will be stratified by site and by rhythm (presence of atrial fibrillation), primarily to insure that there are equal numbers of sildenafil and placebo treated patients in the group without atrial fibrillation who will undergo MRI. If significant differences in baseline characteristics exist, an analysis adjusted for these characteristics will be performed.

18.5 Specification of the Primary Analyses

A general linear model with the change in peak VO2 measured at 24 weeks compared to the baseline peak VO_2 as the response variable and predictor variables including a treatment indicator and the baseline measure of peak VO2 will be used in the primary analysis. The treatment effect will be determined by the point estimate and 95% confidence interval for the treatment indicator.

18.6 Specification of the Secondary Analyses

A Wilcoxon test will be used to calculate the P(X<Y) and the p-value for the clinical composite score endpoint. Multivariate rank-ordered data will be analyzed according to the worst-rank approach of Lachin[123]. A mixed model repeated measures (MMRM) analysis will be used to compare differences in the slope of peak VO2 and 6MW distance across the two treatment groups[124]. Contrast estimates of differences in slopes of treatment by time (along with confidence intervals) will be used to estimate the treatment effect. The validity of this model in terms of meeting modeling assumptions will be assessed via standard modeling diagnostics and goodness-of-fit measures. Based on the MMRM framework, missing peak VO2 and 6MW distance data will not be imputed. Sensitivity analyses will be conducted using an inverse probability weighted method to account for missing data[125].

18.7 Subgroup Analyses

Pre-specified subgroup analysis will include comparison of the effect of therapy in patients with:

1. LV mass index above and below the median at entry into the study.
2. Estimated PASP above and below the median at entry into the study

3. Dose of sildenafil achieved
4. Presence of atrial fibrillation
5. Differences in background therapy

19. DATA MANAGEMENT PROCEDURES

19.1 Hardware and Software Configuration

19.1.1 Hardware and Database Software

Data will be stored in an Oracle database system. Oracle has advantages of processing efficiency and smooth linkage with other software systems. The application and database will be hosted on Solaris Unix servers at the DCC. Clintrial will be used for data entry.

19.1.2 Statistical Software

SAS will be used as the principal application for the management of analysis data files and statistical computations. S-Plus will be used to provide supplementary functions as needed.

19.1.3 Access Control and Confidentiality Procedures

Access to databases will be controlled centrally by the DCC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage.

19.1.4 Security

Database and Web servers will be secured by a firewall and through controlled physical access. Oracle has many security features to ensure that any staff member accessing the database has the proper authority to perform the functions he or she requests of the system. Within the secondary SAS databases, Unix group-access control maintains similar security. The Sun workstation login is secured by extensive user-password facilities under Unix.

19.1.5 Back-up Procedures

Database back-up will be performed automatically every day, and standard DCC policies and procedures will be applied to dictate tape rotation and retention practices.

19.1.6 Virus Protection

All disk drives that provide network services, and all user computers, will be protected using virus-scanning software. Standard DCC policies will be applied to update these protection systems periodically through the study.

19.1.7 Sources of Data

Data will be captured and forwarded to the DCC from the sites and the adjudication committees. First, basic clinical information, e.g., demographic information, will be recorded on paper case report forms (CRFs) and forwarded via parcel-delivery service to the DCC for data entry.

19.2 Data Management Activities

In general, the following data management procedures will be applied:

- Paper CRFs will be designed specifically for the needs of this study. The CRF will be partitioned into "booklets" according to the type of data captured (e.g., screening, clinical data, etc.). Identification information will identify key fields, e.g., the participant's ID number, initials, and date of birth as well as the date of the evaluation.

- The CRF will be printed on 3-part NCR paper. At regular intervals, the different parts of the CRF will be separated. One part will remain at the clinical sites while the others will be forwarded to the DCC using a parcel-delivery system.
- Personnel at clinical sites will record the data mandated by the protocol on the CRFs. They will be abstracted from the participant's medical charts and other source documents. All CRFs will be completed according to the current Good Clinical Practice (GCP) guidelines. Training on completing the CRFs will be included in the training session described in the HFN Manual of Procedures.
- A database will be created on the DCRI computer network specifically for this study. As described above, the database will be managed with Oracle using Clintrial.
- For every record type, the data dictionary will identify key fields (e.g., the participant's ID number and the type and date of evaluation); the field type (e.g., numeric, character, checklist, or date), and ranges for impossible and improbable values.
- All CRFs will be entered into the study database. Double data-entry by 2 different operators will be performed to ensure a high level of confidence in the data entered.

A series of computerized validation checks will be performed at the DCC. "Queries" will be generated, and data clarification forms (DCFs) for problems and exceptions uncovered will be forwarded to the clinical sites for investigation and resolution. Corrections will be made on the data clarification form (DCF) using current GCP standards and forwarded to the DCC. If corrections are needed to the CRF form prior to the initial submission to the DCC, a single line will be drawn through the original entry such that it is still visible. The correct value will be written close to the field and the correction initialed and dated by the HFN staff member making the change.

19.3 Data Management and Quality Control Procedures

Four levels of database quality control will be performed. The first level is the double data-entry process as described above. The second level consists of programmatic consistency checks and/or range checks. The third level of database quality is a record or panel level of control. Programs will be written to identify suspected duplicate and blank or missing records and records not double-entered within and across database tables. An independent auditing group will perform the fourth level of database quality control. These internal data quality and process compliance audits are routinely conducted on internal ongoing studies to document the frequency of random errors and identify systematic deviations so that they can be corrected. Other periodic quality control checks will document the frequency of random entry errors and identify systematic and process errors.

In general, the following issues will be addressed:

- Data completeness: Completion by the clinical centers of all evaluations mandated by the protocol are checked.
- Procedural errors: Errors in performing study procedures, e.g., taking the blood samples.

Remedial action will be taken as appropriate; otherwise, the protocol and Manual of Procedures may be revised as appropriate. Training and recertification will be made available to redress deficiencies and misunderstandings.

19.4 Reports and Summaries

A variety of standard progress reports will be prepared during the course of a trial and include:

- Data Status/Exception Reports: lag in entering CRFs into the database, missing visits, missing pages, listing of outstanding queries, and summary of totals of outstanding queries
- Quality Control Reports: duplicates, missing from table, blanks
- Data Surveillance Reports: query frequencies, perfect data
- Protocol Deviation Reports: numbers of ineligible participants enrolled in the study

Reports will be prepared for the periodic meetings of the Steering Group. Some reports, such as the Data Exception report, may be generated more frequently as required.

19.5 Biological Sample Management

This study will utilize a biomarkers core laboratory designated by the NHLBI and the DCC. Plasma specimens at baseline and 24 weeks will be processed according to the procedures provided by the core laboratory and sent to the core laboratory on dry ice. Planned analyses include:

- Sildenafil levels
- Aldosterone
- cGMP
- N – terminal pro BNP (NT - proBNP)
- Neurohormonal activation (Endothelin-1)
- Renal function (Cystatin C)
- Myocardial necrosis (Troponin T)
- Measures of collagen turnover/fibrosis (pro-collagen III NTP)
- Uric acid

20. STUDY ADMINISTRATION

20.1 Cooperative Agreement Mechanism

The administrative and funding mechanism used to undertake this project is a “Cooperative Agreement” (U01), which is an assistance mechanism. Under the cooperative agreement, the NHLBI assists, supports, and/or stimulates the study and is substantially involved with investigators in conducting the study by facilitating performance of the effort in a “partner” role. The NHLBI Project Scientist serves on the Steering Group, and he or another NHLBI scientist may serve on other project committees, when appropriate. At the same time, however, NHLBI does not assume a dominant role, direction, or prime responsibility for this research program.

As described below, governance of the project is conducted through a steering group. Principal investigators have lead responsibilities in all aspects of their trials and the project, including any modification of trial designs, conduct of the trials, quality control, data analysis and interpretation, preparation of publications, and collaboration with other investigators, unless otherwise provided for by the Steering Group.

Principal investigators retain custody of and have primary rights to their center-specific and collaborative data, subject to government rights-of-access consistent with current Health & Human Services (HHS), Public Health Service (PHS), and NIH policies. The protocols and governance policies call for the continual submission of data centrally to the DCC for the collaborative database. At a minimum, the database will contain the key variables selected by the Steering Group for standardization across all clinical centers; the submittal of copies of the collaborative datasets to each principal investigator upon completion of the project; procedures for data analysis, reporting and publication; and procedures to protect and ensure the privacy of medical and genetic data and records of individuals. The NHLBI Project Scientist, on behalf of the NHLBI, will have the same access, privileges, and responsibilities regarding the collaborative data as the other members of the Steering Group.

Principal investigators are also encouraged to publish and to publicly release and disseminate results, data, and other products of the project, concordant with the project protocols and governance, and the approved plan for making data and materials available to the scientific community and to the NHLBI. However, during or within 3 years beyond the end date of the project period of NHLBI support, unpublished data, unpublished results, data sets not previously released, or other study materials or products are to be made available to any third party only with the approval of the Steering Group.

Upon completion of the project, principal investigators are expected to put their intervention materials and procedure manuals into the public domain and/or make them available to other investigators, according to the approved plan for making data and materials available to the scientific community and

the NHLBI, for the conduct of research at no charge other than the costs of reproduction and distribution.

The NHLBI reserves the right to terminate or curtail the project (or an individual award) in the event of (a) failure to develop or implement mutually agreeable collaborative measurement, participant eligibility, and data management sections of the protocols; (b) substantial shortfall in patient recruitment, follow-up, data reporting, quality control, or other major breech of protocol; (c) substantive changes in the agreed-upon protocols with which NHLBI cannot concur, (d) reaching a major project outcome substantially before schedule with persuasive statistical significance, or (e) human subject ethical issues that may dictate a premature termination.

Any disagreement that may arise in scientific/programmatic matters (within the scope of the award) between award recipients and the NHLBI may be brought to arbitration. An arbitration panel will be composed of 3 members—one selected by the Steering Group (with the NHLBI member not voting) or by the individual principal investigator in the event of an individual disagreement; a second member selected by NHLBI, and the third member selected by the other 2 members. This special arbitration procedure in no way affects the principal investigator's right to appeal an adverse action that is otherwise appealable in accordance with the PHS regulations at 42 CFR part 50, Subpart D, and HHS regulation at 45 CFR part 16 or the rights of NHLBI under applicable statutes, regulations, and terms of the award.

20.2 Steering Committee

The Steering Group is the main governing body of the project. It is composed of the principal investigators of the clinical centers, the principal investigator of the DCC, the Heart Failure Network Chair, and the NHLBI Project Scientist. The clinical centers, the Data Coordinating Center, the Network Chair, and the NHLBI each have 1 vote on the Steering Group. All decisions are determined by majority vote.

All major scientific decisions are determined by the Steering Group. It assumes overall responsibility for the design and conduct of the trial. It appoints (and disbands) committees and subcommittees as the need arises; designs, approves, and implements the study protocols; oversees the development of the Manual of Procedures; monitors patient recruitment and treatment delivery; evaluates data collection and management; oversees quality assurance procedures; and implements changes and enhancements to the study as required. It also has primary responsibility for facilitating the conduct of the trials and reporting the project's results.

20.3 Data and Safety Monitoring Board

The NHLBI will establish a data and safety monitoring board in accordance with established policies (see http://www.nhlbi.nih.gov/funding/policies/dsmb_inst.htm) to ensure data quality and participant safety and to provide independent advice to the NHLBI regarding progress and the appropriateness of study continuation.

20.4 Monitoring

Monitoring activities will be performed at all sites in accordance with the DCRI standard operating procedures. Information regarding the types of visits will be outlined in the MOP.

20.5 Informed Consent Procedures

All HFN patients will provide written informed consent using procedures reviewed and approved by each clinical center's IRB. Informed consent will be undertaken by study personnel in person with the patient. The patient has the option of declining further participation in the study at that point. No further study procedures will be conducted until the signed documents have been provided to the HFN clinical site.

Sample informed consent documents are provided as a separate document but will be modified according to the specific needs of the IRB at each participating clinical site.

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22. APPENDICES

22.1 Appendix 1: Schedule of Evaluations * If a cardiopulmonary exercise test (CPXT) has already been done as part of the patients clinical care, only a baseline study is done. If a screening CPXT is done for the protocol and qualifies, that study can be used as the baseline study.

Procedure	Screen	Baseline	1 wk	3wk	8wk	12wk	13 wk	16wk	20wk	24wk
History and Physical w/ hearing assessment - PI	**x**	**x**		**x**		**x**				**x**
Study Coordinator visit	**x**	**x**		**x**		**x**				**x**
CBC, bilirubin, alkaline phosphatase, AST	**x**									
Clinical NT-proBNP (or BNP)	**x**									
Cardiopulmonary stress test*	**x**	**x- see ***				**x**				**x**
Creatinine	**x**	**x**		**x**		**x**				**x**
Electrocardiogram		**x**								
Phone contact			**x**		**x**		**x**	**x**	**x**	
Peak Sildenafil Level						**x**				**x**
Cardiac MRI		**x**								**x**
Minnesota Living HF Questionairre		**x**				**x**				**x**
6 minute hall walk		**x**				**x**				**x**
2 D and Doppler echo - limited		**x**								**x**
RELAX Biomarker panel		**x**								**x**

22.2 Appendix 2: Framingham Criteria for the Diagnosis of Heart Failure

All enrolling investigators are encouraged to review the documentation regarding the patient's diagnosis of HF considering the Framingham Criteria for the clinical diagnosis of HF.

Major criteria

Paroxysmal nocturnal dyspnea
Orthopnea
Elevated JVP
Pulmonary rales
Third heart sound
Cardiomegally on chest radiograph
Pulmonary edema on chest radiograph
Weight loss > 4.5 Kg during treatment for HF

Minor criteria

Peripheral edema
Night cough
Dyspnea on exertion
Hepatomegally
Pleural effusion
Heart rate > 120 bpm

* Diagnosis of HF requires 2 major or 1 major and 2 minor criteria present concurrently

22.3 Appendix 3: A Review of Previous Studies of the Pharmacodynamics and the Cardiovascular Dose-Response of Sildenafil for the Development of a Dosing Scheme for RELAX

Introduction: Sildenafil is currently approved in the US for acute, intermittent administration for the treatment of male impotence and for chronic administration for the treatment of pulmonary arterial hypertension (PAH). Sildenafil, and other type 5 phosphodiesterase (PDE5) inhibitors such as vardenafil and tadalafil, act to increase intracellular cGMP and potentiate the effect of nitric oxide (NO) and natriuretic peptide actions that are mediated by guanylate cyclase. The dosage of sildenafil recommended for these two indications differs, for acute intermittent administration, doses of 25-100 mg are recommended. For chronic administration in pulmonary hypertension, a dose of 20 mg tid is recommended. When considering a dosing protocol for sildenafil in RELAX, two issues arise: the pharmacodynamics of the drug and the slope of the dose-biological response curve.

1. Pharmacodynamics and kinetics:

Pharmacodynamic and pharmacokinetic data taken from pre-clinical development studies indicates that sildenafil is readily absorbed from the gastrointestinal tract, is primarily metabolized and excreted by the liver, and has a half-life of 3.2 hrs. While 92% of a single oral dose is absorbed (with peak levels achieved 1 h after ingestion), only 38% is bioavailable due to extensive first-pass metabolism by the liver. Published plasma levels of sildenafil measured after administration to humans are given in Table 1.

Table 1. Published data on plasma sildenafil levels following acute and chronic administration to humans.

Dose	Number of doses	Patients studied (N)	Route	[Sildenafil] (ng/ml)	Ref	Note
50 mg	1	Normal (1)	PO	265	2	1 h after dose
50 mg	1	Normal (3)	PO	610	3	Maximum level
25 mg	1	Normal (3)	IV	560	3	Maximum level
100 mg	1	Normal (?)	PO	450	1	1 h after dose
50 mg	1	SHF (13)	PO	237 ± 23	4	1 h after dose
40 mg tid	18	Normal at high altitude (6)	PO	254	5	1-2 h after dose
25 mg tid	Chronic	SHF (7)	PO	78 ± 23	6	Random level
50 mg tid	Chronic	SHF (4)	PO	88 ± 12	6	Random level
75 mg tid	Chronic	SHF (4)	PO	220 ± 84	6	Random level

SHF = systolic heart failure

Sildenafil is hepatically metabolized; in volunteers with mild (Ccre = 50-80 ml/min) and moderate (Ccre = 30-49 ml/min) renal impairment, the pharmacokinetics of a single oral dose of sildenafil (50 mg) are not altered. Plasma levels increase in patients with severe renal impairment (Ccre < 30 ml/min)(1).

The principle metabolite (UK 103320) of sildenafil circulates at about 40% of sildenafil concentration and has 50% of sildenafil's binding affinity to PDE5 (1). Thus UK 103320 contributes an additional 20% to PDE5 inhibition at a given level of circulating sildenafil, as it is not measured in the sildenafil plasma assay.

Summary: The peak plasma sildenafil concentration drawn –2 hours after administration of 40-50 mg is ~260 ng/ml (390 nM*). The plasma half-life after peak level is achieved is 3.2-4 hours. When administered every 8 hours, the trough is only 18% of the peak concentration. Thus, the peak level one to two hours after a dose during chronic administration is similar to that seen with single dose. That randomly drawn plasma levels intervals of sildenafil exhibit large variation is explained by the wide peak-to-trough ratio seen in tid dosing (6).

2. Relationship of Dose to Response

Several studies examining the relationship of sildenafil level to the biologic response have been performed. These include studies of vascular smooth muscle relaxation ex vivo, studies of animal models of pulmonary vasoconstriction, and clinical studies in patients with PAH and with left ventricular systolic dysfunction.

2A: Ex vivo studies of vascular tissue. Human coronary, internal mammary, and radial arteries were obtained from deceased organ donors, pre-constricted with the prostacyclin analogue U-46619, and exposed to sildenafil in modified Krebs solution (7). Rat aorta was harvested and preconstricted with phenylephrine prior to exposure to sildenafil (8). The concentration of sildenafil yielding half-maximal vasodilation and the range of concentrations yielding 10-90% vasodilation are given in Table 2.

Table 2.

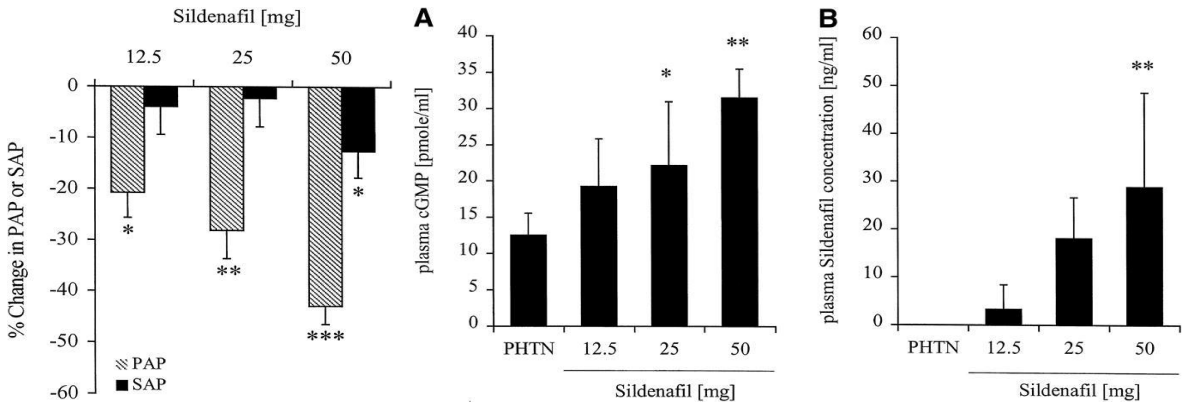
	[Sildenafil] yielding half maximal relaxation (nM)	Range of concentration-dependent response (nM)
Human (U46619) (ref 7)		
Coronary	50,000	100-100,000
Internal Mammary	5600	100-50,000
Radial	100	10-10,000
Rat (phenylephrine)(ref 8)		
Aorta	10	1-5,000

There is a direct correlation of ex vivo vasodilation caused by PDE5 inhibitors and drug concentration. The plasma concentration achieved clinically with sildenafil administration is at the low end of the concentration dependence of the ex vivo vasodilatory effect.

2B. Animal models of pulmonary hypertension, reperfusion injury, and ventricular remodeling.

2B1. Animal models of pulmonary hypertension show that the pulmonary vascular tone is especially sensitive to PDE5 inhibition (9). Pulmonary vasoconstriction was induced by U46619 in 5 previously instrumented, awake sheep (16-25 kg). Serial ascending doses (12.5, 25, 50 mg) of sildenafil were administered at 15-minute intervals orally. Figure 1 demonstrates the effect of dose on pulmonary and systemic arterial pressures, plasma cGMP and plasma sildenafil levels. Similar dose dependent effects of sildenafil were observed on cardiac output, pulmonary vascular resistance, and the ratio of pulmonary/systemic vascular resistance. These effects were abolished by infusion of the NO synthase inhibitor L-NAME. The sildenafil level 20 minutes after the administration of 50 mg of sildenafil was 37 $\pm$ 24 ng/ml.

*Figure 1. Effect of ascending doses of sildenafil in an ovine pharmacologic model of PAH. (*P<0.05, **P<0.01, ***P<0.001(ref 9))*



Based on their hemodynamic findings, the authors concluded that the pulmonary vasculature was more sensitive to sildenafil, and that significant systemic vasodilatory effects were meaningful only at the highest oral doses (~2 mg/kg).

2B2. Hypoxic neonatal piglets (10). Newborn piglets instrumented for hemodynamic monitoring and ventilated had hypoxemia induced by reducing the F_iO_2 to 0.15. After 30 minutes, the development of pulmonary hypertension occurred, with an increase in mean PA pressure by 20%. Systemic arterial pressure was unchanged. Infusion of sildenafil at doses ranging from 0-2.0 mg/kg/hr (n=6 animals/treatment arm, 3 treatment arms) was begun. After 90 minutes of infusion, there was a dose dependent reduction in mean PA pressure and pulmonary vascular resistance, with only a dose of 2 mg/kg/hr leading to a reduction in PA pressure to its normoxic baseline. There was no effect of sildenafil on systemic pressure. Plasma sildenafil levels were not determined.

2B3. Ischemic reperfusion injury of isolated rat hearts (11). Sildenafil may inhibit PDE5 present in cardiomyocytes (12), increasing myocardial cGMP. NO-donor compounds have been observed to decrease reperfusion injury after ischemic injury proportionate to an increase in myocardial cGMP. It has therefore been proposed that sildenafil may decrease cardiac reperfusion injury. In order to test this hypothesis, isolated, reperfused contracting rat hearts were treated with 10-200 nM sildenafil for 10 minutes prior to transient coronary ligation followed by reperfusion.

There was a dose dependent increase in myocardial cGMP in response to sildenafil treatment. This was not accompanied by a dose dependence of sildenafil on reduction of infarct size. Myocardial cAMP levels after ischemia and reperfusion were observed to be lower in hearts treated with 50 nM sildenafil than those of controls, but were unchanged relative to control with exposure to 200 nM sildenafil. cAMP levels were not measured in hearts treated with 10 or 20 nM sildenafil. This is consistent with the recent observations by Elrod et al., using murine “knock outs” of eNOS and iNOS, that the cardioprotective effects of sildenafil occur independent of nitric oxide synthesis and cGMP-mediated signaling (14).

The trend towards greater reperfusion injury in the rat hearts exposed to 200 nM sildenafil than in those treated with the lower dose and its association with greater myocardial [cAMP] levels has raised the possibility that high levels of cGMP may inhibit PDE3 hydrolysis of cAMP. However, recent studies by Takimoto et al. have demonstrated that exposure of cardiomyocytes to sildenafil concentrations between 0.1 and 1.0 μM decreases the contractile response to β-adrenergic stimulation (15), arguing against a functional significance of the augmentation of myocardial cAMP by exposure to higher doses of sildenafil.

2B4. Pressure overload hypertrophy (13). In order to induce myocardial hypertrophy, adult mice were subjected to thoracic aortic constriction (TAC) for a period of 1-9 weeks. In one set of experiments, sildenafil was fed to the mice at a dose of 100 mg/kg/day at the onset of TAC, and in another set, sildenafil was given at 7-10 days after surgery. In the first set of animals, the development of myocardial hypertrophy and fibrosis was blunted, and in the second set of animals, preexisting hypertrophy

and fibrosis were reversed. Contractile function was also preserved in the animals by sildenafil treatment despite the increased afterload. Sildenafil had no effect on sham-operated animals.

The dose of 100 mg/kg/day was chosen to approximate a dose of 1 mg/kg/day in humans, as there is a lower bioavailability of sildenafil in mice and a more rapid rate of metabolism. The mean free plasma concentration of sildenafil in the mice was 10.4 ± 2.3 nM. As the sildenafil was administered to the mice in their food, this is likely to be comparable to continuous administration for clinical studies, rather than what is observed with intermittent tid administration in chronic studies. Measurement of the "area under the curve" would be a better assessment of exposure to sildenafil in this model for comparison with chronic clinical administration.

Summary:
• Animal models have demonstrated a positive relationship between the dose of sildenafil administered and the magnitude of the increase in cGMP in plasma or tissue.
• There is a dose-response to the pulmonary vasodilator effect observed in animal models of pulmonary hypertension, with the magnitude of systemic vasodilatation being significantly less, if it occurs at all.
• The effect of sildenafil on myocardial reperfusion injury was not dose dependent, despite a positive relationship of dose with myocardial [cGMP].
• The plasma levels of sildenafil that prevented or reversed myocardial hypertrophy in TAC mice were lower than either peak or random levels observed in clinical studies, but may not be comparable with levels achieved by the intermittent dosing of drug as it is administered to humans.

2C.Clinical Studies of Pulmonary Arterial Hypertension and of Heart Failure

2C1. Acute effects of administration of sildenafil to PAH patients.

Assessment of pulmonary vasodilator response. The acute hemodynamic response to oral sildenafil was assessed 30 minutes after administration to 10 PAH patients. Three of the 10 patients had a "pulmonary vasodilator response" (defined as a > 20% reduction in PVR) to 50 mg sildenafil with one additional patient having a response after administration of 100 mg (16).

Treatment of postoperative PAH. Ten pediatric patients < 1 year of age with atrial or ventricular septal defects and PAH that persisted after cardiac surgery were treated with escalating doses of sildenafil. All patients were also receiving 20 ppm inhaled NO to avoid RV failure. As seen in Table 3, there was no relationship of PA pressure or any other hemodynamic variable to sildenafil dose. All patients were treated for a period of 7-10 days with 2.0 mg/kg sildenafil every 4 hours without the recurrence of pulmonary hypertension, or other adverse effects as the nitric oxide was weaned (17).

Table 3. Hemodynamic data at baseline and in response to ascending doses of sildenafil.

	Baseline (iNO 20 ppm)	**+Sildenafil 0.5 mg/kg**	**1.0 mg/kg**	**1.5 mg/kg**	**2.0 mg/kg**
MPAP (mmHg)	27 ± 2	23 ± 2*	22 ± 1*	22 ± 1*	22 ± 1*
MAP (mmHg)	62 ± 4	59 ± 4	60 ± 5	59 ± 4	60 ± 4
LAP (mmHg)	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 0
CVP (mmHg)	8 ± 1	9 ± 2	8 ± 1	8 ± 1	9 ± 1

LAP = left atrial pressure; CVP = central venous pressure. *P <0.05 vs. NO alone.

2C2. Effects of chronic administration of sildenafil to PAH patients. In a double-blind study, 278 patients with PAH (either idiopathic, or secondary to repaired congenital heart disease or connective tissue disease) were randomized to receive placebo or 20, 40, or 80 mg sildenafil tid for 12 weeks. Exercise capacity and right heart hemodynamics (Table 4) were measured at baseline and after 12 weeks

of blinded therapy (18). As seen in Table 4, there was a trend to a lower PA pressure, a higher CI, and a lower PVR in patients randomized to the 80 mg sildenafil treatment arm. Nonetheless, the magnitude of change in these hemodynamic parameters was the greatest of all the treatment arms.

Table 4. Mean change in hemodynamic variables in patients randomized to placebo or sildenafil.

	PA pressure (mmHg)		CI (l/min/m^2)		PVR (dyn/sec-cm^5)						
	Base		Δ at 12w		Base		Δ at 12w		Base		Δ at 12w

† P<0.05, * P<0.01 vs. baseline.

† P<0.05, * P<0.01 vs. baseline.

Exercise capacity, assessed as six-minute walk distance, improved similarly in patients randomized to the three different doses of sildenafil (Figure 3). In the more significantly impaired patients (baseline 6-minute walk distance of <325 m) a trend towards an improved exercise capacity with increasing sildenafil dose was observed (Figure 4). Thus the dose dependence of the hemodynamic effects of sildenafil may become relevant to exercise capacity in the most compromised patients. Of relevance to the RELAX study is the observation that the median 6-minute walk distance in the PEP-CHF (19) trial, a DHF patient population similar to the proposed RELAX study population, was 294 m.

Figure 3. Changes from baseline in six minute walk distance in PAH patients in the placebo and sildenafil treatment arms.

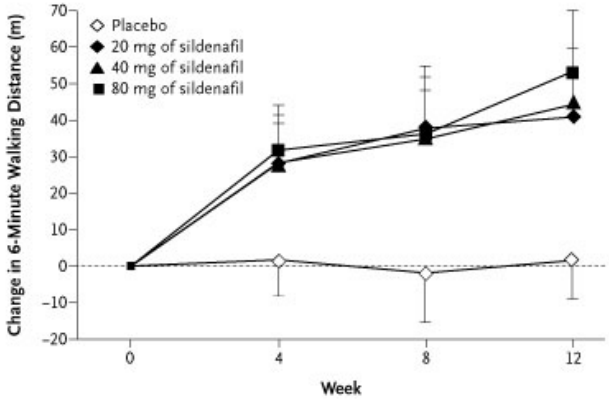
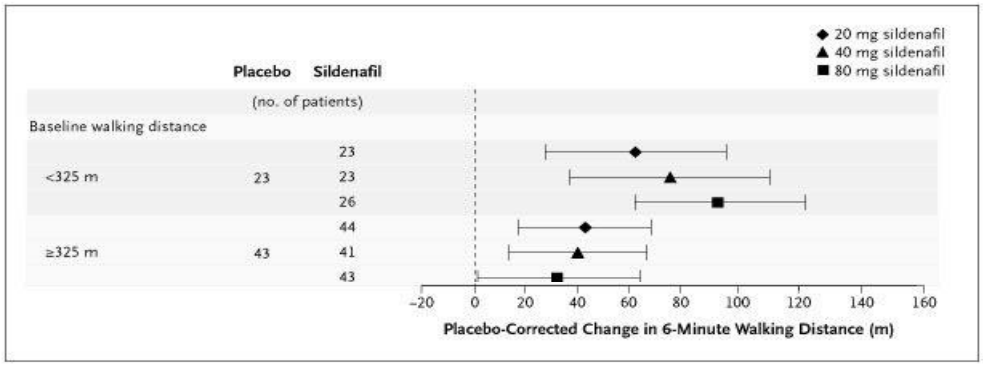


Figure 4. Effect of sildenafil dose on six-minute walk distance in patients stratified by baseline six-minute walk distance



2C3. Flow mediated vasodilation in chronic heart failure patients (20). Flow-mediated vasodilation is believed to occur in response to an increase in endothelial NO release in response to shear stress. The acute effect of sildenafil on flow-mediated vasodilation was assessed by sonographic imaging of the change in brachial artery diameter after arterial occlusion of 1, 3 and 5 minutes. Measurements were made one hour after the oral administration of placebo or 12.5, 25, or 50 mg of sildenafil to patients with NYHA Class II and III heart failure due to LV systolic dysfunction (n=12 in each treatment group). A change in arterial flow-mediated dilation was observed only after administration of 25 mg or 50 mg of sildenafil, not at the lower dose of 12. 5 mg (Figure 5).

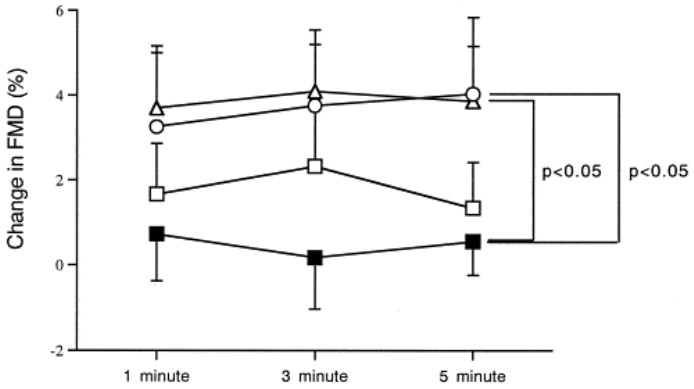


*Figure 5. Change in flow-mediated dilation (FMD) from pre treatment values after release of 1, 3, or 5 minutes of brachial artery occlusion in heart failure patients treated with 12.5 mg (□), 25 mg (**O**), or 50 mg (△) of sildenafil.*

2C4. Acute effects on exercise capacity and hemodynamics of administration of sildenafil to patients with heart failure due to LV systolic dysfunction (4). Rest and exercise right heart hemodynamics were measured before and one-hour after the oral administration of 50 mg of sildenafil to 13 patients with NYHA Class III heart failure secondary to LV systolic dysfunction. Peak VO_2 increased by 15±9% after sildenafil treatment only in those patients with moderate PA hypertension (mean PA pressure > 25 mmHg). The mean plasma sildenafil level was 237 ± 23 ng/ml and there was a trend to a greater increase in peak VO_2 in patients with sildenafil levels above the median when compared to those below the median.

2C5. Acute effects on exercise capacity, ventilatory efficiency, and endothelial function of administration of sildenafil to patients with heart failure due to LV systolic dysfunction (21). 15 HF patients (NYHA class II to III) with LV systolic dysfunction were randomly assigned to receive placebo or sildenafil (25 and 50 mg) according to a double-blind, crossover design. Diffusing lung capacity for carbon monoxide (DLCO) at rest and its normalization for alveolar volume (DLco/VA), as well as brachial artery flow-mediated hyperemic response were investigated before and 1 hour after drug administration. Cycle ergometry was performed to assess exercise capacity (peak VO2 and ventilatory efficiency (VE/VCO_2 slope) at baseline and after drug randomization. Table 5 shows that only administration of 50 mg showed improvements in pulmonary diffusion capacity, endothelial function, exercise capacity, and ventilatory efficiency.

*Table 5. Changes in pulmonary, endothelial, and exercise capacity after sildenafil administration (*p<0.05 vs. placebo).*

	Placebo	Sildenafil (25 mg)	Sildenafil (50 mg)

DLco/VA	4.2±0.8	4.4±0.7	4.9±0.8*
Brachial Hyperemic Flow (ml/min)	420±100	470±100	530±90*
Peak VO_2 (ml/min/kg)	16±4	17±3	19±4*
VE/VCO_2 slope	33±4	31±4	28±4*

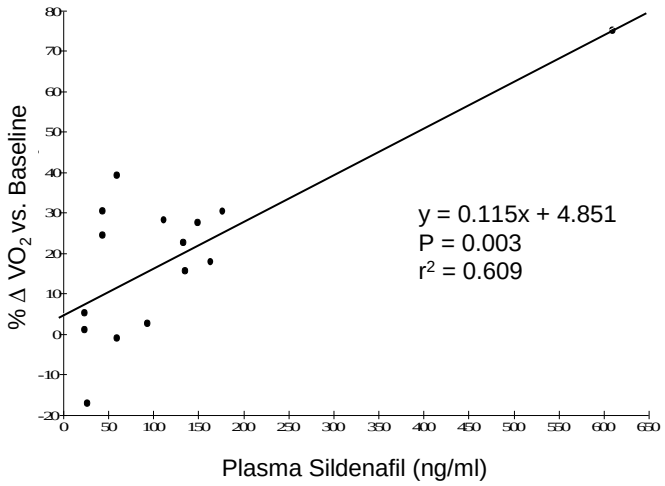
2C6. Effects of chronic administration of sildenafil to patients with heart failure due to LV systolic dysfunction (6). Based on the results of the beneficial acute effects of sildenafil in heart failure patients with pulmonary hypertension, 34 patients with chronic NYHA Class 2b and 3 heart failure due to LV systolic dysfunction and pulmonary hypertension (mean PA pressure > 25 mmHg) were randomized to receive either sildenafil or placebo for 12 weeks. Sildenafil was begun at a dose of 25 mg tid, with a weekly forced uptitration to a maximum of 75 mg tid, as tolerated by blood pressure and symptoms of lightheadedness. Table 1 shows that 27% of patients tolerated uptitration to 75 mg tid. Rest and exercise right heart hemodynamics, exercise capacity and first-pass radionuclide ventriculography was performed. In the study population overall, there was a 16 ± 6% increase in peak VO_2 that was associated with a reduction in PVR and an improvement in RV function. There was a trend towards a correlation between dose and the improvement in exercise capacity (Table 6)

Table 6. Change in exercise capacity by dose of sildenafil (6).

Sildenafil dose	Baseline peak VO_2	12 w peak VO_2	%Δ
25 mg tid (N=7)	10.8 ± 0.7	12.0 ± 0.9	12.0± 6.0
50 mg tid (N=4)	13.9 ± 1.5	16.4 ± 2.8	16.2± 7.8
75 mg tid (N=4)	12.7 ± 1.8	15.6 ± 1.4	27.4± 12.7

There was also a correlation between the improvement in exercise capacity and random plasma sildenafil levels drawn at the end of the placebo controlled period of study (Figure 6).

Figure 6. Relationship between plasma sildenafil levels measured at week 12 and change in exercise capacity from baseline in chronic heart failure patients randomized to sildenafil.



Summary:

• In a large, randomized trial of PAH patients that included randomization to three possible doses of chronic sildenafil therapy, an association between dose and hemodynamic changes was observed. A positive dose-response relationship for the clinical outcome of exercise capacity was seen in a posthoc analysis when patients were stratified by baseline exercise capacity. There was a trend to a dose-related increase in side effects related to vasodilatory effects of the drug.

• A positive relationship between plasma sildenafil level and clinical response was observed in three studies of patients with LV systolic dysfunction assessing the response to either acute or chronic sildenafil administration. In these clinical studies, higher doses of sildenafil were not statistically associated with greater adverse events than low doses, and the incidence of adverse events was low.

3. Conclusions:

Plasma sildenafil levels achieved in clinical studies of 20-80 mg tid are within the range of levels identified in preclinical and clinical studies as having a positive dose-response relationship with the cGMP dependent hemodynamic effects of this agent. This range of doses does appear to be safe to administer chronically to humans, and side effects unrelated to the vasodilatory effect of PDE5 inhibition do not appear to be dose related.

The optimum dose necessary to achieve beneficial myocardial effects of PDE5 inhibition is less clear. Analysis of changes in RV and LV volumes and mass in PAH and LV systolic dysfunction patients currently receiving chronic sildenafil therapy will help to better define the effect of sildenafil in these patient populations. At this time, it would be prudent for future studies, including the RELAX study of diastolic heart failure, to continue to explore a range of doses

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