1 SELECTION OF SUBJECTS

1.1 Screening phase

1.1.1 Inclusion criteria

1. Males or females, 12 years of age or older

2. Diagnosis of sickle cell disease (including, but not limited to SS, SC, SD, or Sβ°/+ thalassemia)

3. Provision of informed consent and, where applicable, assent

1.2 Main interventional trial

1.2.1 Inclusion criteria

1. Males or females, greater than or equal to 12 years of age and less than or equal to 70 years of age *(added upper age limit via protocol version 7.0)*

2. For female subjects, on a reliable method of birth control or not physically able to bear children

3. Electrophoretic documentation of sickle cell disease (including, but not limited to SS, SC, SD, or Sβ°/+ thalassemia)

4. At least mild pulmonary hypertension with TRV ≥ 2.7 m/s by echocardiogram

5. Six minute walk distance of 150–500 m

6. In the opinion of the investigator, ability to complete the protocol scheduled assessments during the 16 week, double-blind phase

7. Provision of informed consent and, where applicable, assent

8. Subjects with systemic hypertension must be on a stable antihypertensive regimen for greater than or equal to 90 days and a stable dose for greater than or equal to 30 days. *(Added via protocol version 7.0)*

1.2.2 Exclusion criteria

1. Current pregnancy or lactation

2. Any one of the following medical conditions:

- Stroke within the last six weeks
- New diagnosis of pulmonary embolism within the last three months
- History of retinal detachment or retinal hemorrhage in the last 6 months
- Non-arteritic anterior ischemic optic neuropathy (NAION) in one or both eyes
- History of sustained priapism requiring medical or surgical treatment, unless currently impotent or on transfusion program within the last two years
- Any unstable (chronic or acute) condition that in the opinion of the investigator will prevent completion of the study

3. Subjects taking nitrate-based vasodilators (including, but not limited to nicorandil [available in the UK only]), prostacyclin (inhaled, subcutaneous or intravenous) or endothelin antagonists. Subjects taking calcium channel

blockers will be allowed to participate provided they are on a stable dose for ≥ 3 months.

4. Left ventricular ejection fraction < 40% or clinically significant ischemic, valvular or constrictive heart disease: LVEF <40% or SF < 22%

5. Subjects who are in other research studies with investigational drugs, with the exception of hydroxyurea, unless the other trial has been approved by the walk-PHaSST Executive Committee for co-participation

6. Acute or chronic impairment (other than dyspnea), limiting the ability to comply with study requirements (in particular with 6MWT), e.g., angina pectoris, intermittent claudication, symptomatic hip osteonecrosis

7. Tonsillectomies for sleep apnea within 3 months prior to randomization

8. Active therapy for pulmonary hypertension, including prostacyclin analog, endothelin-1 antagonists, or PDE-5 inhibitor

9. Protease inhibitor therapy for the treatment of HIV

10. Subjects taking potent CYP3A4 inhibitor therapy (e.g., itraconazole, ritonavir, ketoconazole)

11. Subjects who are anticoagulated **and** have proliferative retinopathy (unless they have had ophthalmologist recommended intervention (e.g., phototherapy) or have been otherwise cleared by an ophthalmologist to participate in the study) *(Added via protocol version 6.0)*

12. Subjects with systolic blood pressure greater than or equal to 140 mmHg OR diastolic blood pressure greater than or equal to 90 mmHg. *(Added via protocol version 7.0)*

1.2.3 Randomization criteria

1. Second 6MW distance between 150–500 meters and within 15% of the previous 6MW

2. Second echocardiogram with a TRV ≥ 2.7 m/s

3. For subjects undergoing right heart catheterization, pulmonary capillary wedge pressure ≤ 24 mmHg

4. An ophthalmological exam sometime within the past year for patients not being anticoagulated. A completed baseline ophthalmological exam for patients being anticoagulated and who otherwise qualify (see Exclusion Criteria #11) *(Added via protocol version 6.0)*

2 RIGHT HEART CATHETERIZATION METHODS

Subjects enrolled in the trial with TRV ≥ 3.0 m/s (at least moderate PH) underwent RHC (using standard techniques and while the subject was in steady state) at baseline and after 16 weeks. Following premedication, RHC was performed under local anesthesia with a heparin-bonded thermodilution Swan-Ganz catheter inserted percutaneously into the right internal jugular or a femoral vein depending on anatomic landmarks. Catheter was positioned in the pulmonary artery with pressure monitoring. Catheter tip position was to be evaluated by fluoroscopy or chest radiography. Fluoroscopy could be used according

to standard clinical practice. A 20-gauge heparin-bonded cannula inserted percutaneously into a radial, brachial or femoral artery could be used for blood sampling and monitoring of systemic arterial pressure. Mean vascular pressure levels were determined by electronic integration of the pressure signals. Heart rate and vascular pressures were monitored continuously. Cardiac output was measured in triplicate by thermodilution or by the Fick method with measured oxygen consumption. Systemic and pulmonary vascular resistances were calculated using the mean cardiac index and mean vascular pressures.

In subjects undergoing right heart catheterization the acute hemodynamic effects of inhaled NO and oral sildenafil were assessed at rest at baseline and at the end of the study.

Table S1. Inhaled NO and oral sildenafil challenge at baseline

	Intervention	**Duration**	**Assessment**
Step 1	Baseline		Resting hemodynamics
Step 2	Inhaled NO 40 ppm	10 minutes	Resting hemodynamics[1]
Step 3	None	10 minutes	Resting hemodynamics
Step 4	Sildenafil 60 mg orally	30 minutes post oral dose	Resting hemodynamics
Step 5		60 minutes post oral dose	Resting hemodynamics

[1] Resting hemodynamics assessed at the end of the 10 minute period of inhaled NO, while the subject is still inhaling the NO.

Right heart catheterization was to be repeated at the end of the double-blind phase. This hemodynamic assessment was to follow the guidelines below:

- Subjects took their nighttime dose of study medication, but did not take their morning dose prior to right heart catheterization.
- Subjects brought study medication to visit.
- Study medication was administered with direct observation by study coordinator
- Right heart catheterization was performed approximately 4 hours after study medication is administered
- Baseline hemodynamic measurements were performed as outlined in Table S1 (steps 1–3).
- Blinded study medication was administered and hemodynamic measurements were performed at 30 and 60 minutes post dosing as outlined in steps 4 and 5 of Table S1.

If during right heart catheterization, it was not possible to obtain an adequate reading of the pulmonary capillary wedge pressure, a left heart catheterization was performed for the measurement of left ventricular end-diastolic pressure.

The pulmonary artery catheter is a triple lumen central venous catheter with two lumens devoted to the pressure transduction functions. The distal port transduces pressure from the pulmonary artery and from the left atrium, when in the “wedge” position. The central venous port transduces right atrial pressures. During catheter placement, right ventricular pressures are transduced. Pulmonary artery systolic and diastolic pressures, right atrial pressure, pulmonary capillary wedge pressure, systemic artery systolic and diastolic pressures, cardiac output, and calculated systemic and pulmonary vascular resistances were be obtained using this methodology.

Baseline and endpoint RHC procedures were to be conducted under similar conditions (e.g., level of sedation and oxygen). Endpoint RHC was to be conducted at trough levels of study drug: approximately 4 hours post-dose.

Figure S1. Right heart catheterization

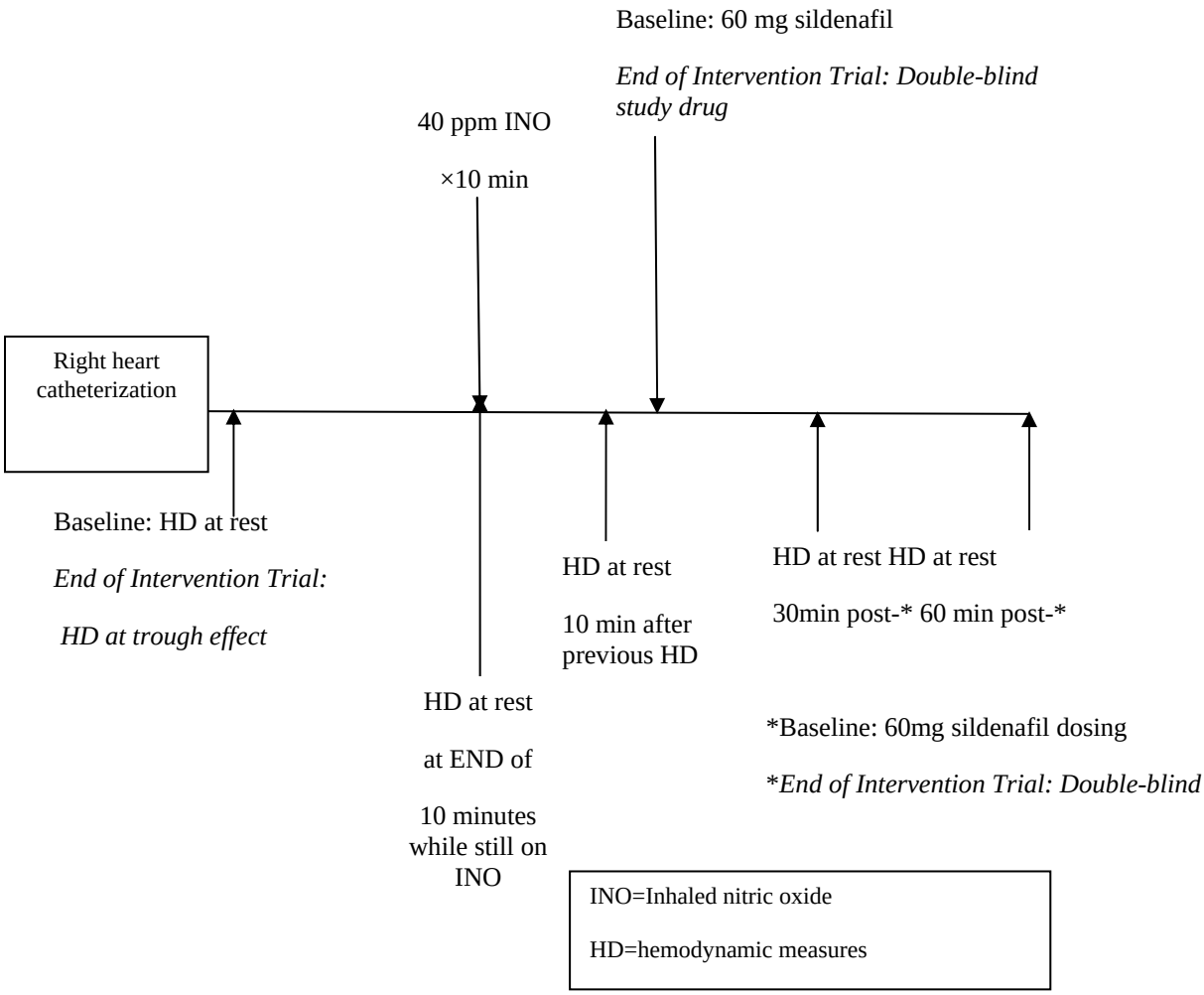

Table S2. Borg Dyspnea scoring
The Borg Dyspnea grade was obtained immediately following the six minute walk test using the visual analog scale below:

SCALE	SEVERITY	SCALE	SEVERITY
0	None at all	5	Severe
0.5	Very Very Slight (just noticeable)	6	
1	Very slight	7	Very Severe
2	Slight	8	
3	Moderate	9	Very Very Severe
4	Somewhat Severe	10	Maximum

Baseline imbalances in patient characteristics

There were also no treatment group differences in TRV at baseline (p=0.38). Because there were no baseline treatment group differences in these variables, we conclude that the treatment effect (or lack thereof) was not confounded by baseline differences in these variables.

Additionally, we included each of these parameters in separate mixed effects regression models to examine interaction effects on the 6MWD. That is, was the impact of sildenafil vs. placebo different based on the SCD parameter (e.g., on hydroxyurea (HU) vs not on HU)? Of this set of variables, only one raised the possibility of interaction with treatment (p=0.06). **Fig. S2** describes that interaction. Subjects on sildenafil who were not on HU during the study had a significant decrease in 6MWD as compared to a) all placebo subjects, regardless of HU use and compared to b) sildenafil subjects on HU. These data could be interpreted as the pain contributed a the lack of improvement in 6MWD for the sildenafil subjects although this is conjecture until confirmed or refuted by further

studies. Hydroxyurea could have also protected subjects from the pain that sildenafil might have otherwise caused, i.e., pain that may have led to the decrease in 6MWD in the sildenafil subjects not on HU.

Based on the key study finding that a greater number of sildenafil vs. placebo subjects experienced serious pain events, it might logically follow that the subset of sildenafil subjects on HU (n=21) should have been less likely to experience these serious pain events than those not on HU (n=16). However, this is not borne out by the data. In fact, for subjects on sildenafil, 48% of subjects also on HU experienced a serious pain event compared to 19% of subjects not on HU (Fisher's exact p=0.09). These data also do not conclusively refute the HU protection hypothesis because they are confounded by subjects who experienced regular and/or more severe sickle cell pain were also more likely to be prescribed hydroxyurea. Finally we do not have data on adherence to HU therapy during the study.

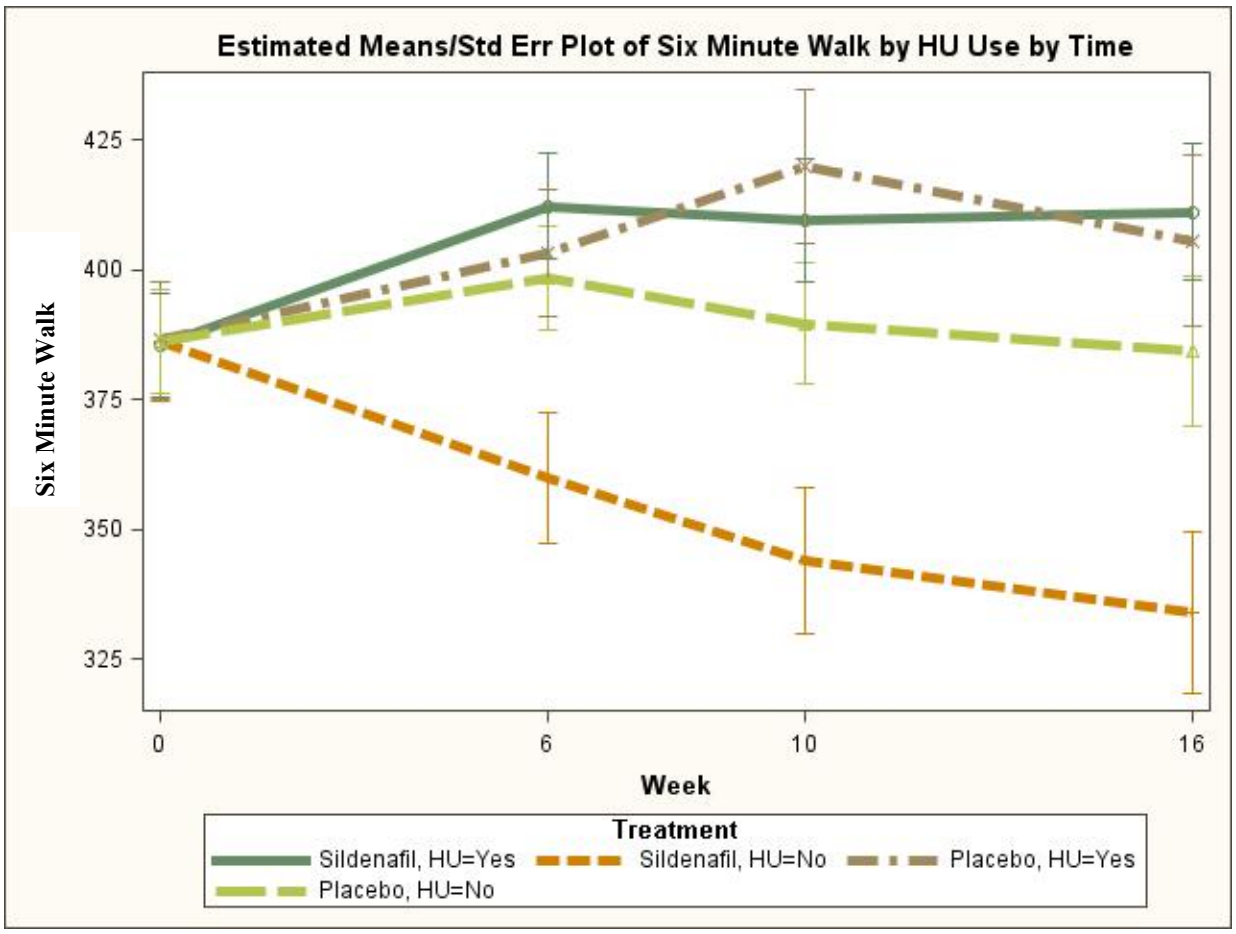

Figure S2. Interaction between hydroxyurea and sildenafil use and effect on 6MW
Subjects on sildenafil who were not on HU during the study had a significant decrease in 6MWD as compared to a) all placebo subjects, regardless of HU use and compared to b) sildenafil subjects on HU.

Table S3. Number and percentage of subjects experiencing treatment-emergent adverse events (frequencies ≥10% for body system or individual preferred term)

System Organ Class Preferred Term	Sildenafil (N = 37) n (%)	Placebo (N = 37) n (%)	P –value[1]
Any treatment-emergent AE	30 (81)	28 (76)	0.55
Congenital, familial, and genetic disorders (SCA with crisis)	18 (49)	13 (35)	0.23
Nervous System Disorders	14 (38)	4 (11)	0.004
Headache	13 (35)	4 (11)	0.01
Musculoskeletal and connective tissue disorders	5 (14)	6 (16)	0.75
Blood and lymphatic system disorders	4 (11)	6 (16)	0.49
Eye Disorders	5 (14)	4 (11)	0.73
Vision blurred	5 (14)	1 (3)	0.09
General disorders and administration site conditions	6 (16)	3 (8)	0.28
Infections and infestations	5 (14)	2 (5)	0.24

Note: System organ class and preferred term were based on MedDRA Version 10.1.
Note: If a subject experienced more than one episode of an adverse event, the subject was counted once for that preferred term. If a subject had more than one adverse event in a system organ class, the subject was counted once for that system organ class.
[1]P-value corresponds to a Cochran-Mantel-Haenszel chi-square test comparing treatment groups, controlling for TRV stratum. NC = not calculated due to lack of data.

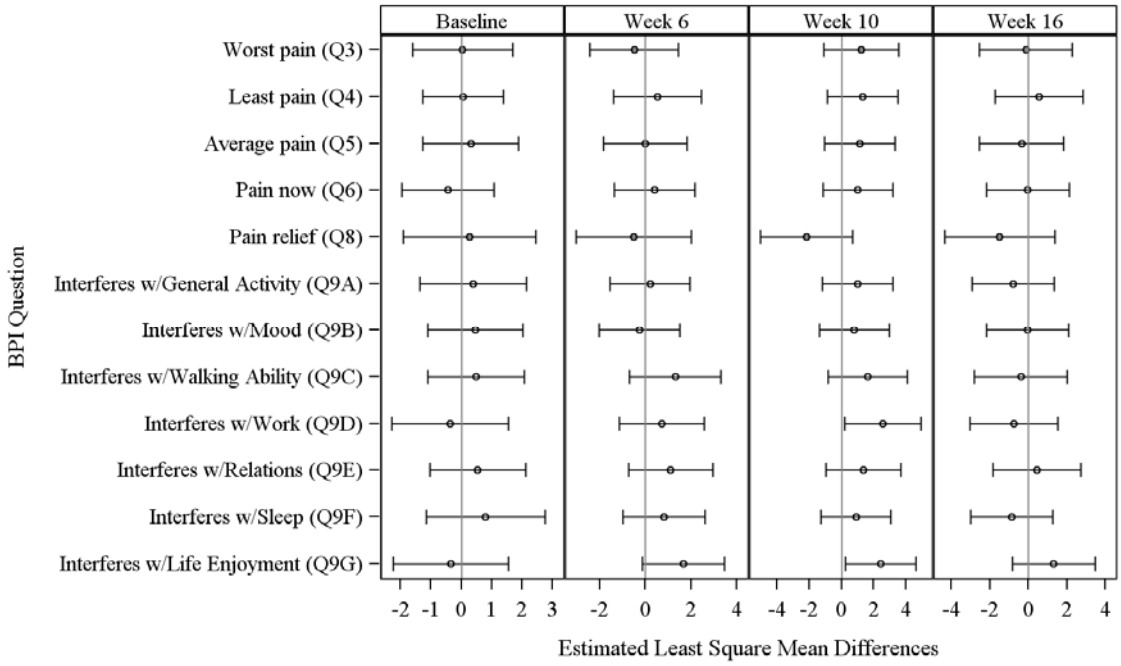

Figure S3. Least square means differences (Sildenafil – Placebo) and confidence intervals are based on a linear mixed effects model with treatment, baseline value, and time as fixed effects, and subject as a random effect

Estimated differences to the right of the zero reference line indicate greater pain/discomfort in the Sildenafil group. Global test of no treatment difference across all questions and post-baseline timepoints: p=0.62. Global test of no treatment difference by time point: Week 6 (p=0.63), Week 10 (p=0.04), Week 16 (p=0.99).