Supplemental Methods

Study design.

Subjects with a thalassemia syndrome (alpha, beta or E-beta thalassemia), confirmed by hemoglobin electrophoresis or by molecular diagnosis, aged ≥ 7 years old with Doppler-defined risk for PH (TRV>2.5 m/s) were eligible. Exclusion criteria included patients without a measurable TRV by local site cardiologists' readings, pregnancy, hepatic or renal insufficiency, history of retinal hemorrhage/detachment, cardiac disease with adjustment of cardiac medications 60 days prior to enrollment, symptomatic coronary artery disease in the last year, a new diagnosis of pulmonary embolism within 90 days of participation, or current/prior treatment with known PH therapies. Patients were characterized as TM if they required > 8 transfusions/year and had a history of chronic transfusion dependence at the time they were enrolled into the Thalassemia Longitudinal Cohort (TLC), a project which collects longitudinal (annual) clinical and laboratory data from 428 patients at 16 centers. Patients requiring < 8 transfusions/year with no history of long-term chronic transfusion at the time of enrollment into the TLC were classified as TI.

Study Dose Modification.

A sildenafil dose adjustment plan was anticipated for symptomatic or dose-limiting headache or facial/skin edema if the symptoms were severe enough as determined by either the participant or their physician to warrant holding the drug. If dose reduction was required during a participant's initial sildenafil dose, sildenafil was held for one week and re-challenged if symptoms had resolved. If symptoms recurred upon re-challenge, sildenafil was discontinued. If dose reduction was required after dose escalation, then sildenafil was held for one day and

restarted at the previously tolerated dose for one week. If symptoms recurred at the lower dose, sildenafil was discontinued. If the lower dose was tolerated for one week, sildenafil dose was again increased to 100 mg TID. If symptoms recurred upon second increase, sildenafil was held for one day and the dose decreased to previously tolerated dose for the duration of the trial.

Safety and Efficacy.

Safety assessments included adverse event (AE) and serious adverse event (SAE) reporting, clinical laboratory assessments, physical examination and vital signs. Safety and efficacy assessments were conducted at baseline, and weeks 2, 4, 8, and 12.

The 6-Minute Walk Test.

The 6MWT was performed in accordance with the guidelines established by the American Thoracic Society (1).

Pulmonary Function Testing.

Spirometry was performed via standard practices (2) at each local site in a pulmonary function laboratory. Variables were reported as raw measures and as percent-predicted based on age, gender, race, and height, gas exchanging capacity of the lungs by single-breath diffusion capacity for carbon monoxide (D_LCO). The D_LCO was subsequently corrected for hemoglobin concentration.

Laboratory studies.

Routine laboratory tests (complete blood count, serum chemistry profile and ferritin) were performed in the local laboratories of the participating institutions. Plasma and erythrocyte

arginine concentration was analyzed as previously described (3). Biomarkers of hemolysis, coagulation, inflammation and adhesion were measured through standard methods by the Frans Kuypers laboratory at Children's Hospital & Research Center Oakland (rbclab.com) and through LabCorp – Esoterix Clinical Trials (Cranford, NJ; www.labcorp.com).

Statistical Analysis.

This pilot study had 80% power at level α=0.05 to detect a 60 m change in the 6MWT among N=10 participants, assuming a 60 m standard deviation for 12-week change (4, 5). Average 6 minute walk distance is unknown for thalassemia and has not previously reported. Since previous data was not available to generate a sample size and anticipate the standard deviation prior to conducting the study, we based the power calculation on a similar population of patients with a hemolytic anemia and higher than normal risk of PH (4).

Descriptive statistics were reported as the number and percent, or the mean and standard deviation/standard error. Linear mixed models with participant-specific intercepts and slopes controlled for time effects were used to test the mean difference between baseline and week 12 measurements. Correlation analysis and repeated measure regression were used to test for associations between 6MWT, laboratory parameters and other covariates of interest. Log-transformation was used as needed to correct for skew in the data. All analyses were performed at the Data Coordinating Center (New England Research Institutes, Watertown, MA) with SAS statistical software (9.2, SAS Institute, Cary, NC) and R (2.11.1, The R Foundation for Statistical Computing, http://www.r-project.org/). P-values less than 0.05 were considered statistically significant.

1. Society AT. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111-7.
2. Laszlo G. Standardisation of lung function testing: helpful guidance from the ATS/ERS Task Force. Thorax. 2006;61(9):744-6.
3. Morris CR, Suh JH, Hagar W, Larkin S, Bland DA, Steinberg MH, et al. Erythrocyte Glutamine Depletion, Altered Redox Environment, and Pulmonary Hypertension in Sickle Cell Disease. Blood. 2008;140:104-12
4. Machado RF, Martyr S, Kato GJ, Barst RJ, Anthi A, Robinson MR, et al. Sildenafil therapy in patients with sickle cell disease and pulmonary hypertension. Br J Haematol. 2005;130(3):445-53.
5. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med. 2005;353(20):2148-57.

On-Line Supplement Table 1: Effect of Sildenafil on Clinical and Laboratory Variables at Baseline vs. week 12, Mean (SD) in Patients with Thalassemia Major (TM) Compared to Thalassemia Intermedia (TI)

Variables	Before Sildenafil		After Sildenafil		P-value[1]	P-value[2]	P-value[3]
	TM N=5*	TI N=5**	TM N=5[+]	TI N=4[++]			
6MWT and Dyspnea							
6MWT (m)	476.0 (137.0)	525.4 (44.1)	473.7 (147.2)	542.2 (72.8)	0.28	0.43	0.46
Borg Dyspnea Score before walk	1.6 (1.5)	1.0 (2.2)	0.7 (1.2)	0.2 (0.4)	0.23	0.37	0.40
Borg Dyspnea Score after walk	2.7 (1.3)	1.8 (1.9)	2.3 (1.5)	1.3 (1.2)	0.42	0.57	0.15
Blood Pressure and Pulse							
Systolic BP (mm Hg)	106.0 (8.5)	103.6 (10.6)	107.3 (9.7)	110.0 (16.5)	0.89	0.26	0.99
Diastolic BP (mm Hg)	64.4 (5.6)	62.2 (6.1)	67.5 (3.1)	62.8 (11.7)	0.13	0.89	0.52
Heart Rate (per minute)	79.4 (10.9)	83.4 (6.1)	84.0 (16.1)	88.6 (12.7)	0.74	0.25	0.70
Pulse Pressure (mm Hg)	41.6 (11.9)	41.4 (6.9)	39.8 (8.7)	47.2 (15.2)	0.65	0.37	0.65
Echo Parameters							
Local TRV (m/s)	3.4 (1.1)	3.0 (0.3)	-	-	-	-	-
Central TRV (m/s)	3.2 (1.0)	2.8 (0.2)	2.5 (0.7)	2.7 (0.4)	**0.03**	0.55	**0.02**
LVEF (%)	66.3 (2.6)	65.8 (2.1)	66.9 (1.7)	67.2 (1.3)	0.38	0.23	0.74
Right atrial size (cm2)	17.7 (4.3)	17.5 (3.0)	19.4 (5.4)	18.0 (2.6)	0.42	0.37	0.86
Left atrial volume (ml)	48.9 (21.7)	63.2 (9.0)	41.4 (30.7)	59.1 (6.9)	**0.001**	0.37	0.20
Left ventricular end systolic volume (ml)	22.8 (3.8)	38.6 (7.9)	18.1 (3.8)	35.7 (7.5)	**0.03**	0.11	**0.01**
Left ventricular end diastolic volume (ml)	67.9 (11.5)	112.5 (20.5)	54.7 (10.8)	108.6 (18.4)	0.07	0.14	**0.003**
Left ventricular mass (g)	122.8 (35.9)	139.9 (16.7)	109.4 (39.5)	162.8 (25.9)	0.20	**0.01**	**0.01**
LV septal wall thickness	0.9 (0.2)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.66	0.23	0.93
LV posterior wall thickness	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.39	0.91	0.61
LV mean wall thickness	0.9 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)	0.46	0.36	0.81
Cardiac Index (L/min/m^2)	2.2 (0.2)	3.6 (0.9)	1.8 (0.3)	3.8 (1.1)	0.11	0.41	**0.01**
Arterial compliance	1.5 (0.4)	1.0 (0.2)	1.8 (0.5)	1.1 (0.2)	0.18	0.44	**0.01**
Echo Parameters Indexed to BSA							
Right atrial size(cm^2/m^2)	11.1 (2.3)	10.2 (1.5)	11.8 (3.4)	10.4 (1.5)	0.41	0.39	0.60
Left atrial volume (ml/m2)	30.8 (13.5)	36.9 (6.6)	25.7 (18.5)	34.4 (5.3)	**0.001**	0.33	0.29
Left ventricular end systolic volume (ml/m2)	14.2 (1.6)	22.4 (4.1)	11.0 (1.9)	20.8 (4.7)	**0.02**	0.14	**0.01**
Left ventricular end diastolic volume (ml/m2)	42.6 (6.7)	65.4 (11.9)	33.2 (5.4)	63.3 (12.0)	0.054	0.21	**0.004**

Clinical Lab Analyses							
WBC (10^3/μL)	9.7 (3.8)	17.6 (3.5)	9.8 (6.0)	19.2 (7.0)	0.80	0.63	**0.02**
Platelet Count (10^3/μL)	376.0 (256.8)	659.6 (147.9)	371.0 (351.0)	647.8 (175.0)	0.62	0.67	0.14
Hemoglobin (g/dL)	10.8 (1.4)	10.0 (2.8)	11.2 (1.2)	9.3 (2.6)	0.63	**0.03**	0.40
Hematocrit (%)	32.7 (4.4)	31.2 (7.8)	33.9 (4.1)	29.9 (7.7)	0.59	**0.02**	0.57
Ferritin (ng/mL)	2174 (2469)	1568 (748.8)	2145 (1615)	1563 (852.7)	0.88	0.50	0.62
Bilirubin (mg/dL)	1.5 (0.9)	2.6 (0.9)	1.8 (0.8)	2.4 (1.0)	0.90	0.50	0.31
ALT (U/L)	22.4 (15.2)	51.2 (46.1)	20.5 (8.6)	38.2 (25.2)	0.76	0.64	0.48
AST (U/L)	63.0 (55.2)	49.5 (36.5)	18.7 (8.0)	48.4 (22.2)	-	0.85	0.12
Creatinine (mg/dL)	1.0 (0.4)	0.6 (0.2)	0.9 (0.3)	0.6 (0.2)	**0.04**	0.70	**0.03**
BNP (pg/ml) (normal:0-100 pg/ml)	68.9 (82.0)	38.9 (17.4)	81.9 (142.5)	29.8 (15.1)	0.57	0.29	0.84
Coagulation							
PAI-1 Activity (IU/mL)	8.2 (5.0)	8.4 (4.5)	6.0 (0.0)	8.7 (5.9)	0.39	0.96	0.21
PF12 MONO (pmol/L)	617.2 (960.3)	1159 (2038)	121.8 (48.3)	1362 (1990)	0.32	0.63	0.24
TAT COMPLX (ng/mL)	66.3 (141.3)	124.4 (265.9)	5.2 (2.5)	180.4 (263.4)	0.57	0.30	0.38
TF (Tissue Factor concentration (pg/ml))	310.0 (54.6)	329.5 (176.2)	244.2 (84.2)	335.1 (164.2)	0.16	0.51	0.56
Inflammation							
IFN-gamma (pg/mL)	30.9 (16.5)	81.4 (80.1)	22.8 (6.2)	21.5 (10.7)	0.49	0.10	0.47
IL-13 (pg/mL)	11.2 (3.7)	12.4 (2.8)	8.7 (5.8)	8.9 (3.5)	0.46	0.09	0.44
IL-1 beta (pg/mL)	9.9 (3.5)	23.5 (19.4)	7.5 (1.5)	9.3 (3.5)	0.38	0.16	0.16
IL-10 (pg/mL)	13.6 (3.3)	27.4 (20.1)	11.0 (2.7)	13.1 (2.1)	0.13	0.17	0.15
IL-12p70 (pg/mL)	14.0 (6.3)	35.6 (31.6)	13.4 (3.8)	11.2 (2.1)	0.99	0.15	0.43
IL-2 (pg/mL)	26.2 (10.4)	61.7 (50.2)	18.8 (8.5)	26.1 (8.0)	0.35	0.14	0.11
IL-4 (pg/mL)	16.0 (8.6)	37.1 (37.1)	10.3 (4.4)	13.1 (4.0)	0.15	0.24	0.37
IL-5 (pg/mL)	7.0 (1.9)	10.7 (6.2)	6.2 (0.7)	5.9 (0.9)	0.40	0.14	0.39
IL-6 (pg/mL)	12.5 (3.9)	23.3 (21.3)	10.6 (1.2)	25.5 (31.2)	0.44	0.91	0.19
IL-8 (pg/mL)	17.4 (4.2)	23.7 (11.7)	14.4 (1.7)	15.2 (2.9)	0.35	0.12	0.24
TNF-alpha (pg/mL)	17.4 (8.0)	47.2 (44.2)	14.4 (3.8)	13.3 (5.1)	0.62	0.12	0.41
INF-alpha	139.2 (260.5)	83.2 (127.6)	178.5 (272.6)	92.2 (131.1)	0.45	0.15	0.97
IL-18	525.4 (165.2)	804.0 (378.9)	631.7 (210.8)	557.3 (397.6)	0.38	0.37	0.58

Hemolysis/Arginine – Nitric Oxide Pathway							
LDH (IU/L)	167.4 (38.3)	242.0 (86.4)	176.3 (37.1)	335.4 (186.2)	0.40	0.47	0.10
Cell Free Hemoglobin (ug/ml)	190.2 (100.1)	174.3 (139.1)	163.0 (80.2)	90.5 (26.0)	0.52	0.20	0.14
Arginine (Plasma)	65.7 (18.2)	46.3 (28.1)	95.7 (36.9)	75.0 (34.1)	0.17	0.22	0.15
Arginine (erythrocyte)	4.4 (3.5)	5.1 (3.6)	8.4 (4.0)	7.5 (2.0)	**0.02**	0.19	0.47
Arginase concentration (ng/ml)	4.8 (2.8)	92.6 (60.2)	11.6 (9.2)	56.3 (56.0)	0.18	0.20	**0.001**
Arginase activity (U/L)	1.6 (1.5)	9.5 (4.9)	2.8 (2.1)	9.2 (8.5)	0.53	0.71	**0.004**
Plasma Arginine/Ornithine	2.8 (0.9)	1.1 (0.5)	2.4 (0.7)	3.1 (3.8)	0.19	0.26	0.65
Plasma Arginine/(Ornithine+Citrulline)	1.8 (0.5)	0.8 (0.4)	1.9 (0.7)	1.3 (1.1)	0.98	0.36	0.08
Serum NOx concentration (μM)	48.9 (22.6)	38.2 (14.9)	61.1 (32.0)	52.5 (34.3)	0.43	0.21	0.52
VEGF (pg/mL)	550.8 (519.0)	1514 (948.0)	591.5 (399.7)	1119 (665.9)	0.82	0.32	0.19
Adhesion							
L-selection concentration (ng/ml)	2820 (2142)	241.3 (395.6)	2664 (1732)	67.4 (24.0)	0.49	0.33	**<0.001**
PECAM (ng/ml)	181.1 (54.5)	221.1 (96.6)	228.6 (141.4)	335.2 (239.0)	0.61	0.26	0.51
sICAM-3 (ng/ml)	147.3 (78.6)	240.1 (40.7)	153.6 (50.2)	255.3 (20.5)	0.88	0.50	**0.02**
sE-SELECTIN (ng/ml)	120.4 (86.1)	218.5 (139.0)	130.6 (87.3)	248.4 (220.6)	0.61	0.48	0.34
sICAM-1 (ng/ml)	433.7 (45.0)	437.3 (236.3)	462.6 (119.0)	453.3 (275.2)	0.83	0.74	0.94
sP-SELECTIN (ng/ml)	267.3 (89.7)	374.3 (139.2)	422.9 (157.4)	455.7 (196.7)	0.06	0.36	0.39
sVCAM-1 (ng/ml)	1356 (1489)	811.5 (495.2)	1749 (1532)	1048 (880.6)	0.53	0.26	0.35

* N=2 for AST; N=4 for bilirubin

** N=2 for right atrial size; N=3 for 6MWT, Borg Dyspnea Score before walk, Borg Dyspnea Score after walk, left atrial volume, AST

[+] N=4 for bilirubin, AST, LDH, VEGF

[++] N=4 for bilirubin

[1] P-value to compare the change in variable over time (from baseline to week 12) among TM

[2] P-value to compare the change in variable over time (from baseline to week 12) among TI

[3] P-value to compare the difference in variable between TM and TI over time

Abbreviations: 6-minute walk test (6MWT); blood pressure (BP); echocardiography (Echo); tricuspid regurgitant jet velocity (TRV); left ventricular ejection fraction (LVEF); left ventricular (LV); body surface index (BSA); white blood cell count (WBC); alanine aminotransferase (ALT); aspartate aminotransferase (AST); brain natriuretic peptide(BNP); plasminogen activator inhibitor-1 (PAI-1); prothrombin fragment 1.2, monoclonal (PF1.2 MONO); thrombin-anti-thrombin complex (TAT); tissue factor (TF); interferon (IFN); interleukin (IL); tumor necrosis factor (TNF); lactate dehydrogenase (LDH); nitric oxide metabolites (NOx); vascular endothelial growth factor (VEGF); platelet endothelial cell adhesion molecule (PECAM); soluble intercellular adhesion molecule (sICAM); soluble vascular cell adhesion molecule (sVCAM).