Online-data Supplement:

Randomized Placebo-controlled Safety and Tolerability Trial of FK506 for Pulmonary Arterial Hypertension

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Content of online-data supplement:

Material and Methods
Statistics
Results
Figures, Tables and Legends
Transform Protocol
Consent form
Termination form
Placebo titration protocol

Material and Methods:

In addition to checking medication compliance at every visit by counting unused pills, we used MEMS monitors (Aardex). These monitors contained integrated micro-circuitry in the medication bottle lid that recorded the time and date of each opening. The data was downloaded by the research coordinator at the end of the trial and compared with the FK506 level variability. Possible non-compliance was defined as not opening the bottle or opening the bottle more than once on a calendar day.

Statistical Analysis:

Descriptive statistics such as medians, interquartile range, counts, and percentages were displayed for baseline patient characteristics. Drug levels, clinical, and serological biomarkers

were displayed graphically. For clinical and serological biomarkers (primary, secondary, and exploratory endpoints), we compared whether the change from baseline differed by (1) study arm (low, medium, high level FK506 and placebo) and (2) study drug (all FK506 levels) versus placebo in patients who completed the study per-protocol using a linear mixed-effect model with a random intercept for subject to account for within-subject correlation between baseline and 16 week measures.

Our methods follow the intent-to-treat (ITT) principle where possible. Because not all patients provided 16-week measures, we were unable to adhere completely to the ITT principle. To evaluate the sensitivity of our findings to incomplete follow-up of the clinical outcomes, we specified a worst-case scenario sensitivity analysis for all statistically significant findings in the linear models. In the worst-case scenario sensitivity analysis, study dropout subjects in the placebo arm were assigned the best-observed value and study dropout subjects in the active treatment arms had the worst observed value in their clinical measures.

Finally, for the exploratory biomarkers BMPR2 and Id1, the baseline and 16-week values were compared to healthy controls using the Mann-Whitney-Wilcoxon test. The 16-week comparisons both included and excluded the patients in the placebo arm. We also graphically displayed the change in BMPR2 and Id1 in clinical responders vs. non-responders and tested the change using the Mann-Whitney-Wilcoxon test. Clinical response was defined as achieving any of the following criteria: >40 m increase in 6MWD, decrease in NT-pro BNP of at least 200, increase in RV-FAC of at least 1.8%, or a decrease in RV-GLS of at least 2.5.

All analyses were performed in R, version 3.2. Statistical significance was specified a priori at p value of 0.05. All formal tests were intended to generate hypotheses for future studies.

Results:

Feasibility of targeting low-dose FK506

Following the initiation and dosing protocol, we found that on average seven blood draws were required to demonstrate a difference between the median trough levels in the placebo, low, medium and high level FK506 arms **(Figure 2A).** This time-point corresponded to approximately 50 days after initiation of FK506 (**Figure 2B**), slightly longer than the estimated 4 weeks we

anticipated being necessary to reach steady state. Given the known broad bioavailability range of oral dosing of FK506 with a mean of 27% (range 5-65%)[12,13] as well as the abundant drug-drug interactions of FK506 with drugs also metabolized by the cytochrome P450 system (CYP3A4 in specific), we correlated the daily FK506 doses with FK506 blood levels over the course of the trial, hypothesizing that there would be a large overlap between FK506 dose and blood levels. We found that even when FK506 was dosed to achieve sub-immunosuppressive levels there was a clear dose response that showed that higher FK506 doses resulted in higher FK506 blood levels with a relatively small IQR (**Figure 2C**). We presented FK506 doses reflective of at least 3 patients with median and IQR and single observations as a single bar. Our results suggest a favorable and narrow range of low-dose FK506 bioavailability in PAH.

Furthermore, using MEMS electronic compliance monitors we were able to correlate time and frequency of pill bottle opening with the number of necessary blood draws to correlate FK506 level outliers with inaccuracies in potential medication compliance (**Supplement Figure S3**). All patients except one patient who was in the low-level FK506 arm had excellent compliance of over 85%. There did not appear to be a relationship between compliance and the number of blood draws (Spearman Correlation 0.08). However, the data suggests that fewer blood draws were required to reach low and medium FK506 blood levels compared to high dose.

Online-data *Supplement*:

Figure legends:

Supplement Figure E1: Titration algorithm for FK506

Supplement Figure E2: Timeline and assessments during Transform study

RC= Routine Care, standard practice at enrolling center, S= Study related procedure at enrolling center.

Supplement Figure E3: No Correlation between compliance and Variability in FK50K levels

Proportion of compliant days of patients in each treatment group in relation to the number of required blood draws to reach the goal FK506 level, with placebo patients receiving a sham titration.

Black dotted line -------- representing days of 85% compliance, defined by opening drug bottle once a day as instructed.

Supplement Table E1: Safety and efficacy parameters in placebo and each FK506 treatment arm.

Table E1 lists baseline, 16 week, 18 week (when available), change from baseline to 16 week (delta bl-16w), change from baseline to 18 weeks (delta bl-18w), measurements for creatinine, WBC, Hgb, 6MWD, NT-proBNP, DLCO, Uric acid, RVFAC, TAPSE, RVGLS. Depicted are the median as well as the 25^{th} and 75^{th} percentile in the 4 treatment arms (placebo, low-level (<2 ng/ml), medium-level (2-3 ng/ml) and high-level (3-5 ng/ml) FK506.

Supplement Table E2: Comparison of primary, secondary and exploratory endpoints in the 4 treatment arms

Table 2 lists the p-values of a comparison of changes from baseline of clinical and serological biomarkers differed by (1) study arm (low, medium, high level FK506 and placebo) and (2) study drug (all FK506 levels) versus placebo in patients who completed the study per-protocol using a

linear mixed-effect model with a random intercept for subject to account for within-subject correlation between baseline and 16 week. To evaluate the sensitivity of our findings to incomplete follow-up of the clinical outcomes, we specified a worst-case scenario sensitivity analysis for all statistically significant findings in the linear models. In the worst-case scenario sensitivity analysis, study dropout subjects in the placebo arm were assigned the best-observed value and study dropout subjects in the active treatment arms had the worst observed value in their clinical measures. Worst-case scenario sensitivity analysis * p-value 0.470, ** p-value 0.044 .

Supplement Figure E4: Change in BMPR2 biomarkers

Change in Cofilin-1 (**A**), LIMK-1 (**B**), mir-21 (**C**), mir27a (**D**), Smurf-1 (**E**) RNA expression in peripheral blood mononuclear cells (PBMCs) and IL-6 plasma levels (**F**) with increasing FK506 treatment doses. RNA expression data presented as relative expression to controls and normalized to GAPDH ($\Delta\Delta$ CT relative quantification method). Data presented as median change between baseline and 16 week follow-up with interquartile ranges (IQR). The red line represents no change.

Supplement Figure E5: Change in clinical response in BMPR2 responders

The line plot shows the change in NT-proBNP (m) (**A**) and RVGLS (%) (**B**) in relation to a change in BMPR2 expression. 3 Groups were defined according to the change in BMPR2 expression in peripheral blood mononuclear cells (PBMCs) at 16-week follow-up compared to baseline: BMPR2 reduced (= reduced BMPR2 expression), slight increase in BMPR2 (increase in BMPR2 expression but < 1, the mean of healthy controls), large increased (increase in BMPR2 expression > 1).

Supplement Figure E6: Change in BMPR2 and Id1 in clinical responders

Median change in BMPR2 (**A**) and Id1 (**B**) in non-responders and clinical responders defined as 6MWD change > 40 m, AND/OR NT-proBNP negative change > 200 pg/ml, AND/OR RV-FAC > 1.8% AND/OR RVGLS negative change > 2.5% among the 17 patients receiving FK506 (excluding

the placebo treated patients). Data presented as mean change between baseline and 16 week follow-up with interquartile ranges. Wilcoxon-Mann-Whitney test (change in BMPR2: p=0.565 change in Id1 p=0.250.

Supplement Figure E7: Change in BMPR2 Expression in hereditary PAH patients.

Overall change in BMPR2 expression in peripheral blood mononuclear cells (PBMCs) from baseline (red icon) to 16 weeks follow-up (blue icon) in the placebo, low, medium and high level FK506 group compared to the BMPR2 expression of healthy volunteers (controls) at baseline. Larger icons represent the 4 patients with known BMPR2 mutations. RNA expression data presented as relative expression to controls and normalized to GAPDH ($\Delta\Delta$ CT relative quantification method).

Table E1	Placebo			Low dose			Medium dose			High dose		
	median	25th	75th	median	25th	75th	median	25th	75th	median	25th	75th
creatinine												
baseline	1.0	0.9	1.1	0.9	0.8	1.0	0.9	0.8	1.1	1.2	1.2	1.2
16 weeks	0.9	0.8	1.0	0.9	0.8	1.0	1.1	0.9	1.3	1.2	1	1.2
delta bl-16w	-0.1	-0.2	0	0	-0.1	0	0	-0.1	0.1	0	-0.2	0
WBC												
baseline	5.3	4.5	8.0	6.6	6.0	7.8	6.2	5.8	6.7	6.7	6.8	8.1
16 weeks	6.6	5.7	7.2	6.3	6.1	6.9	6.6	5.9	7.9	7.2	6.9	8.6
delta bl-16w	1.3	-0.3	2.8	0.4	-0.6	0.8	-0.1	-0.6	0.2	0.5	0.3	0.9
Hgb												
baseline	14.4	13.6	14.9	15.1	14.1	15.7	14.6	12.9	15	15.8	14.8	16.1
16 weeks	14.1	13.6	14.9	14.5	13.2	15.3	13	12.8	14.3	16.9	15.4	17.5
delta bl-16w	-0.12	-0.4	0	-0.6	-0.6	-0.3	0.6	0.4	0.7	0.8	0.6	0.9
6mwd												
baseline	507	464.8	577	550.5	448.5	572.2	541	475	579	512	494.8	524.8
16 weeks	546.5	507.5	588.5	533.5	421.2	582	552	530.5	555.5	521	472	564
18 weeks	508.3	406.5	578.9	486.2	323.5	563.1	556.3	531.6	585.2	533.4	509	548.6
delta bl - 16w	14.5	3.5	38.3	0	-20.3	3	41	-9.5	59	0	-20	9
delta bl - 18w	2.8	-1.7	4.0	-18.3	-42.3	2.6	38.9	17	60.1	12.4	11.9	17
NTpro BNP												
baseline	1.9	1.8	1.9	2.2	1.8	3	2	1.7	2.1	2.6	2.3	2.7
16 weeks	1.74	1.6	1.8	2.2	19	2.9	1.9	1.8	2.1	2.1	1.9	2.9
18 weeks	76.5	53.3	378.8	856	114.2	1782	47	38.5	57	360	173	696
delta bl - 16w	-22	-27.8	-10.3	-46.5	-122.5	13.78	40	-0.5	44.5	-63	-233	-11
delta bl - 18w	11	-18	103.8	112.5	9.8	218.3	-51	-60.5	-34	18	-14	45
DLCO												
baseline	93.5	75.5	104	90	78.3	104.8	88	88	96	86.5	73	91
16 weeks	89.5	77.3	106.2	87.5	76	107.2	83	82.5	89.5	77	76	84
delta bl - 16w	4.5	-0.75	11.3	-3.5	-11.3	2	-14	-35	-9.5	-2	-8	2
Uric acid												
baseline	4.75	4.38	5	6.3	5.6	6.5	5.3	4.8	6.2	5	4.7	5.6
16 weeks	4.6	4.2	5.4	6.1	5.3	6.6	5	5	5.5	5.1	4.9	5.1
delta bl -16w	0.1	-0.2	0.3	0.2	-0.2	0.5	-0.3	-0.5	0.1	0	-0.1	0.1
RVFAC												
baseline	25.48	21.1	27.55	23.64	22.04	26.22	29.55	24.28	30.46	22.88	21.92	27.89
16 weeks	25.62	21.69	28.56	24.69	22.06	26.58	30	27.2	31.66	26.2	21.21	28
delta bl- 16w	0.56	-0.44	0.9375	-0.205	-0.75	0.895	1.96	1.205	3.665	-0.71	-1.69	1.47
TAPSE												
baseline	1.7	1.45	2.325	1.8	1.55	2.05	2.05	1.825	2.25	1.7	1.6	2
16 weeks	1.7	1.588	2.225	2	1.85	2.075	2.1	1.95	2.325	1.9	1.5	2.25
delta bl - 16w	0.025	-0.0875	0.1	0.05	0	0.1	0.1	0.075	0.15	0	-0.1	0
RVGLS												
baseline	-17.21	-21.8	-14.56	-16	-20.46	-7.96	-19.05	-19.93	-18.01	-15.77	-19.79	-11.27
16 weeks	-19.38	-22.39	-15.97	-14.72	-20.56	-8.358	-19.47	-20.22	-18.92	-19	-19.44	-11.16
delta bl - 16w	-0.81	-1.6075	-0.2825	-0.195	-1.1725	1.42	-0.42	-0.915	-0.29	0.11	-0.42	0.35

Table E2

Endpoints		Comparing individual arms	Any treatment vs placebo
Primary			
	Creatinine	0.378	0.369
	WBC	0.655	0.235
	Hemoglobin	<0.001*	0.204
Secondary			
	6 MWD	0.475	0.245
	NT-proBNP	0.283	0.52
	DLCO	0.113	0.26
	Uric Acid	0.902	0.985
	RVFAC	0.349	0.528
	TAPSE	0.925	0.564
	RVGLS	0.92	0.513
Exploratory			
	BMPR2	0.666	0.52
	Id1	0.493	0.221
	Cofilin-1	0.349	0.473
	SMURF-1	0.527	0.154
	LIMK-1	0.971	0.718
	IL-6	0.343	0.585
	miR-21	0.010**	0.489
	miR-27	0.746	0.867

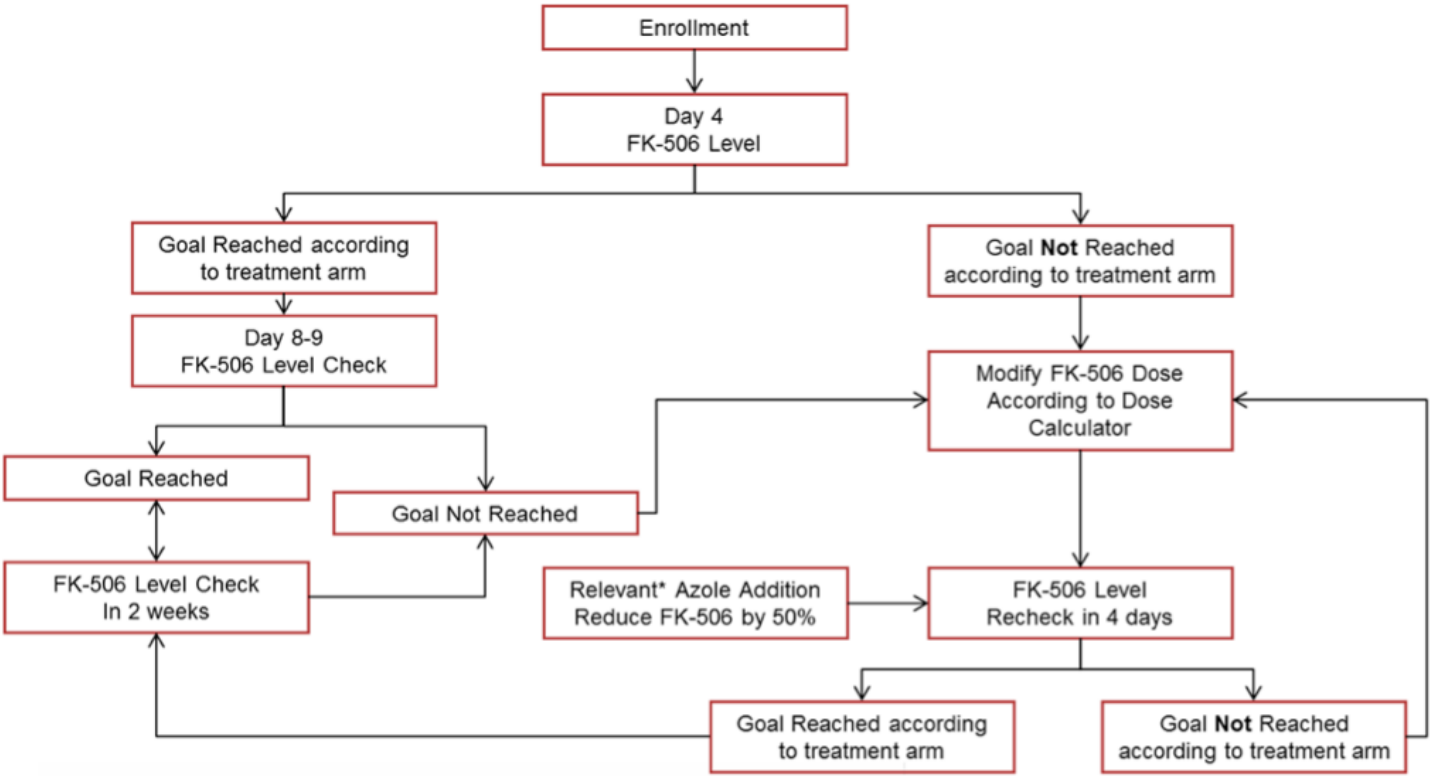
CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Title page
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Page 2
	2b	Specific objectives or hypotheses	Page 2
		Methods	
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 2-3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	Page 3
	4b	Settings and locations where the data were collected	Page 3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Page 3-4
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Page 4-5
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Page 6
Sample size	7a	How sample size was determined	Page 6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Page 6
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Page 3
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Page 3
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Page 3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Page 4

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Page 5-6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Supplement
		Results	
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Fig. 1, page 6
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 6
	14b	Why the trial ended or was stopped	Page 6
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Fig1, Page 6
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Tab 2, Figs 3,4,5, pp7-9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Fig 5, page 10
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Page 11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Page 12
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Page 11-12
Other information			
Registration	23	Registration number and name of trial registry	Page 1
Protocol	24	Where the full trial protocol can be accessed, if available	supplement
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Title page

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Figure E1



Tacrolimus dose modification formula:

$$\text{New Dose (mg)} = \frac{\text{Actual Dose (mg)} \times \text{Trough}_{\text{target}}\text{(ng/mL)}}{\text{Actual Trough from lab (ng/mL)}^{**}}$$

*Round to the nearest 0.5mg capsule size

** Tacrolimus levels reported by the core lab as <2ng/ml should be entered into the calculator as 1ng/ml.

Figure E2

TIME LINE: TRANsFoRM PAH STUDY - All Events Combined

	Screening	Baseline	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 16****	Week 18
Visit #	0	1				2		3			4	5
Day #	-30 - 0	0	7 ± 3	14 ± 3	21 ± 3	28 ± 3	42 ± 3	56 ± 3	70 ± 3	84 ± 7	112 ± 7	126 ± 14
Telephone Contact				S			S		S	S		
Informed Consent	S											
History and physical exam												
Medical history	RC	RC										
Symptom assessment	RC	RC		S		S	S	S	S	S	RC	S
Medications	RC	RC		S		S	S	S	S	S	RC	S
Vital Signs & Weight	RC	RC				S		S			RC	S
Physical Exam including NYHA functional class		RC				S		S			RC	S
General Testing												
Comprehensive Metabolic Panel, CBC		RC				S		S			RC	
Serum HCG (if applicable)		S***										
FK-506 Level (according to algorithm*), estimate			S	S	S	S	S	S	S	S	S	
Endpoint assessment												
6 minute walk distance		S				S		S			S	S
NT-pro-BNP		RC				S		S			RC	S
Echocardiograms		RC***									RC	
PFT with DLCO		RC***									S	
Serum/Plasma for biomarkers		S									S	
Uric Acid		S									S	
Study Procedures												
Dispense study drug		S				S		S				
Dose titration advice (according to drug calculator**)		S	S	S	S	S	S	S	S	S	S	
Medication Compliance				S		S	S	S	S	S	S	
Adverse Events				S		S	S	S	S	S	S	S
Randomization		S										
Study Termination												S

RC = Standard practice at enrolling centers
S = Study related procedure at enrolling centers

* see attached algorithm of FK-level frequency: Level check after 4 days for 2 consecutive times until steady state is reached, then every 2 weeks; days and total number of FK506 levels vary between patients

** see attached FK-dose calculator

*** If not done within 4 weeks of baseline

**** End of study drug

Figure E3

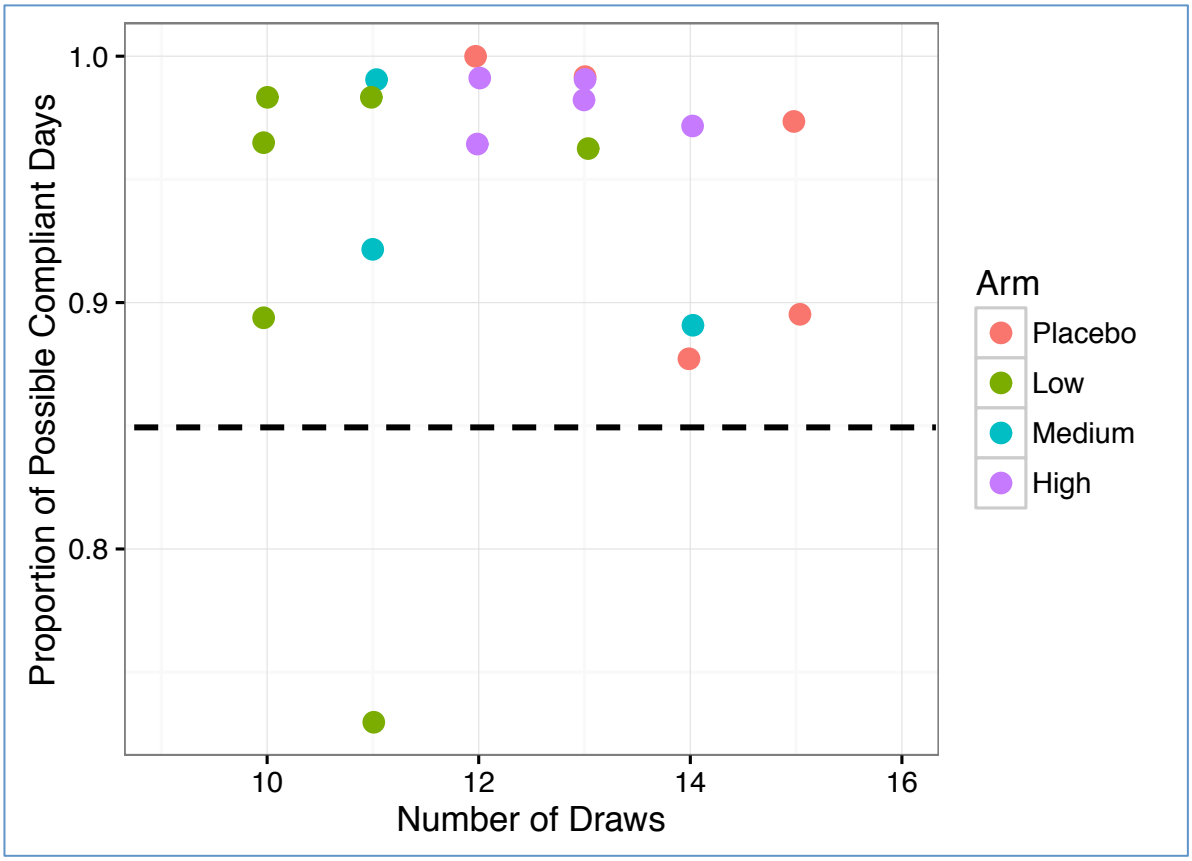

Proportion of Possible Compliant Days
1.0
0.9
0.8
10
12
14
16
Number of Draws
Arm
Placebo
Low
Medium
High

Figure E4

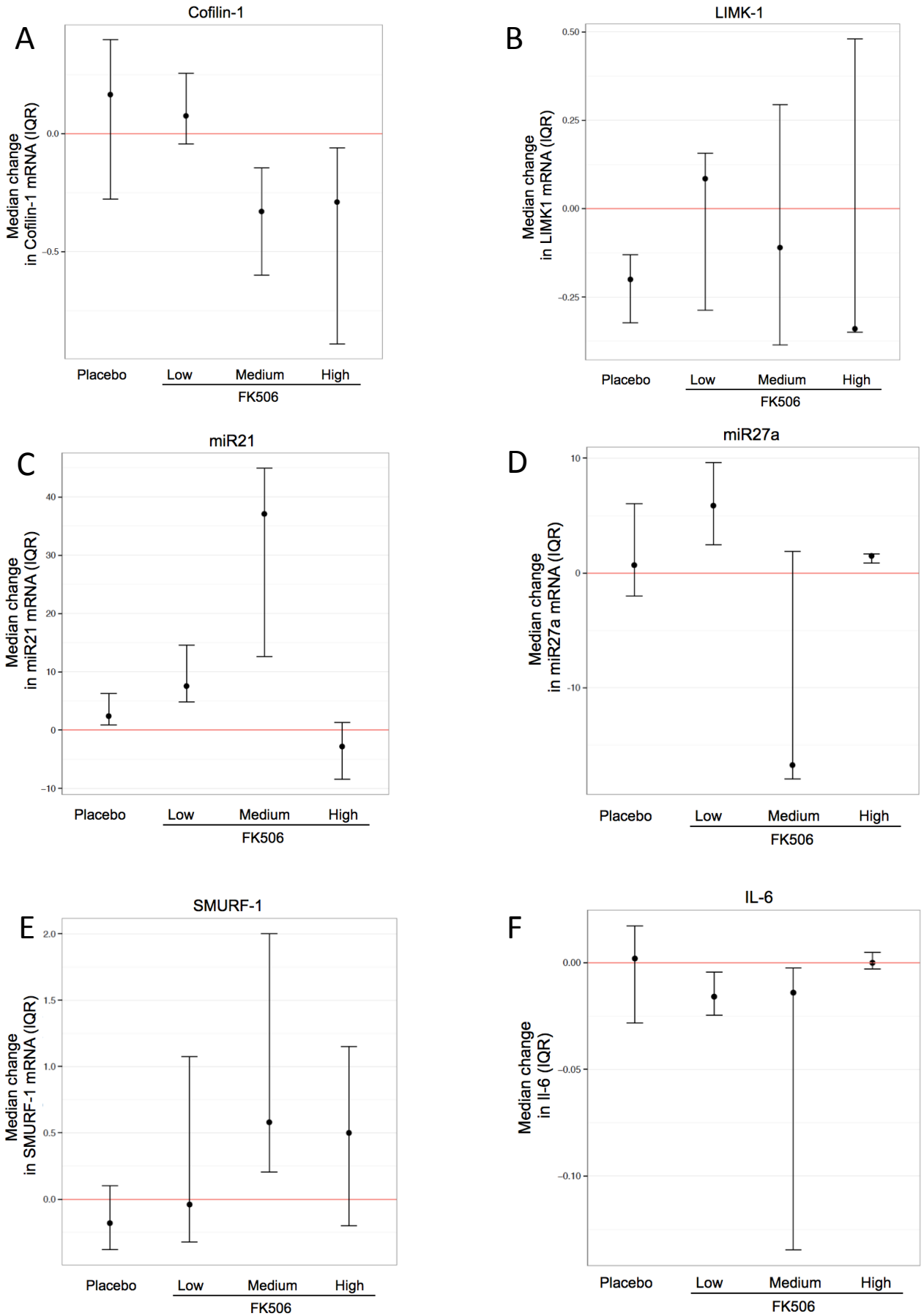

A
Cofilin-1
Median change in Cofilin-1 mRNA (IQR)
0.0
−0.5
Placebo
Low
Medium
High
FK506
B
LIMK-1
Median change in LIMK1 mRNA (IQR)
0.50
0.25
0.00
−0.25
Placebo
Low
Medium
High
FK506
C
miR21
Median change in miR21 mRNA (IQR)
40
30
20
10
0
−10
Placebo
Low
Medium
High
FK506
D
miR27a
Median change in miR27a mRNA (IQR)
10
0
−10
Placebo
Low
Medium
High
FK506
E
SMURF-1
Median change in SMURF-1 mRNA (IQR)
2.0
1.5
1.0
0.5
0.0
Placebo
Low
Medium
High
FK506
F
IL-6
Median change in Il-6 (IQR)
0.00
−0.05
−0.10
Placebo
Low
Medium
High
FK506

Figure E5

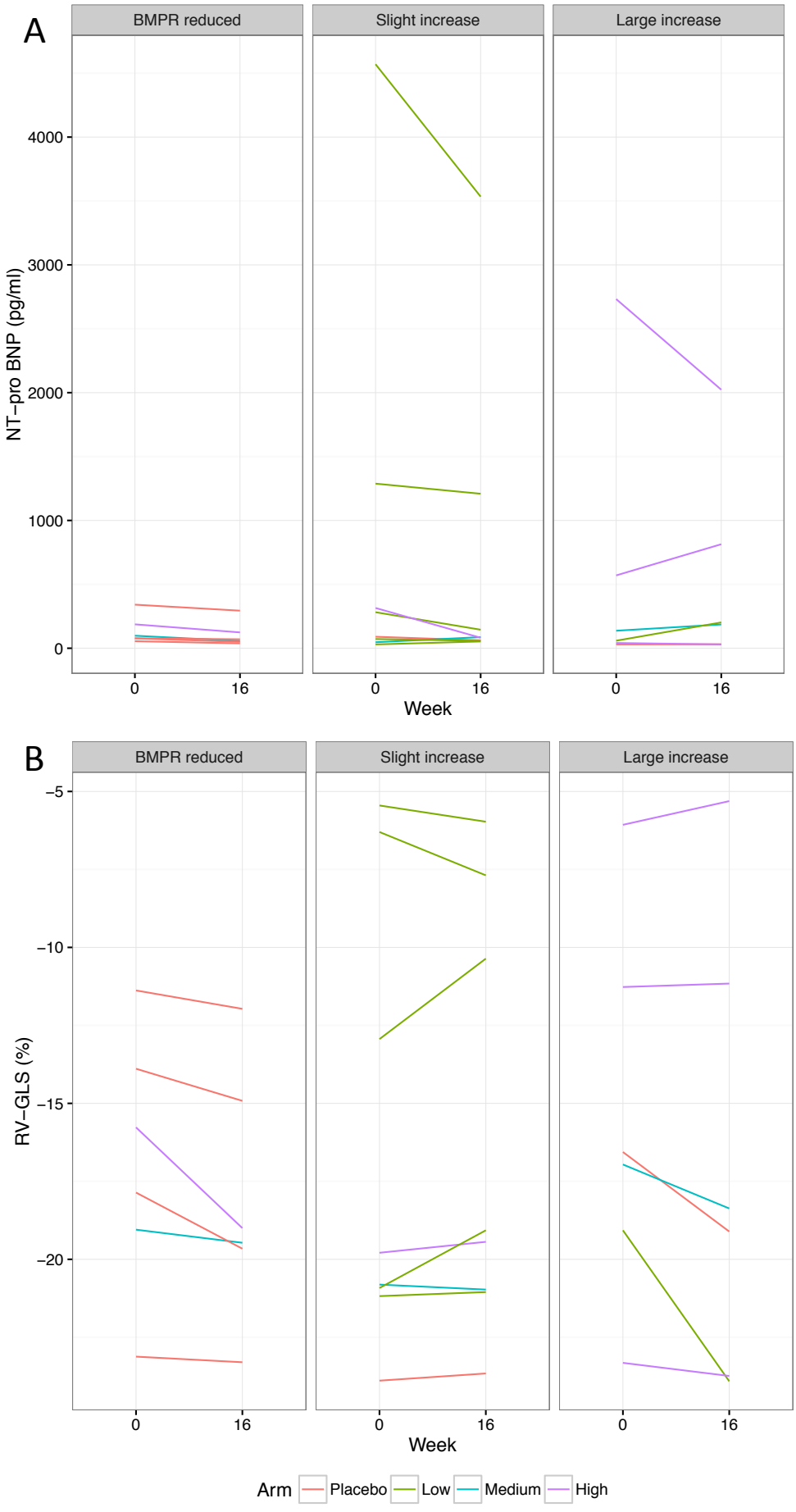

A
BMPR reduced
Slight increase
Large increase
NT−pro BNP (pg/ml)
4000
3000
2000
1000
0
0
16
Week
B
BMPR reduced
Slight increase
Large increase
RV−GLS (%)
−5
−10
−15
−20
0
16
Week
Arm
Placebo
Low
Medium
High

Figure E6

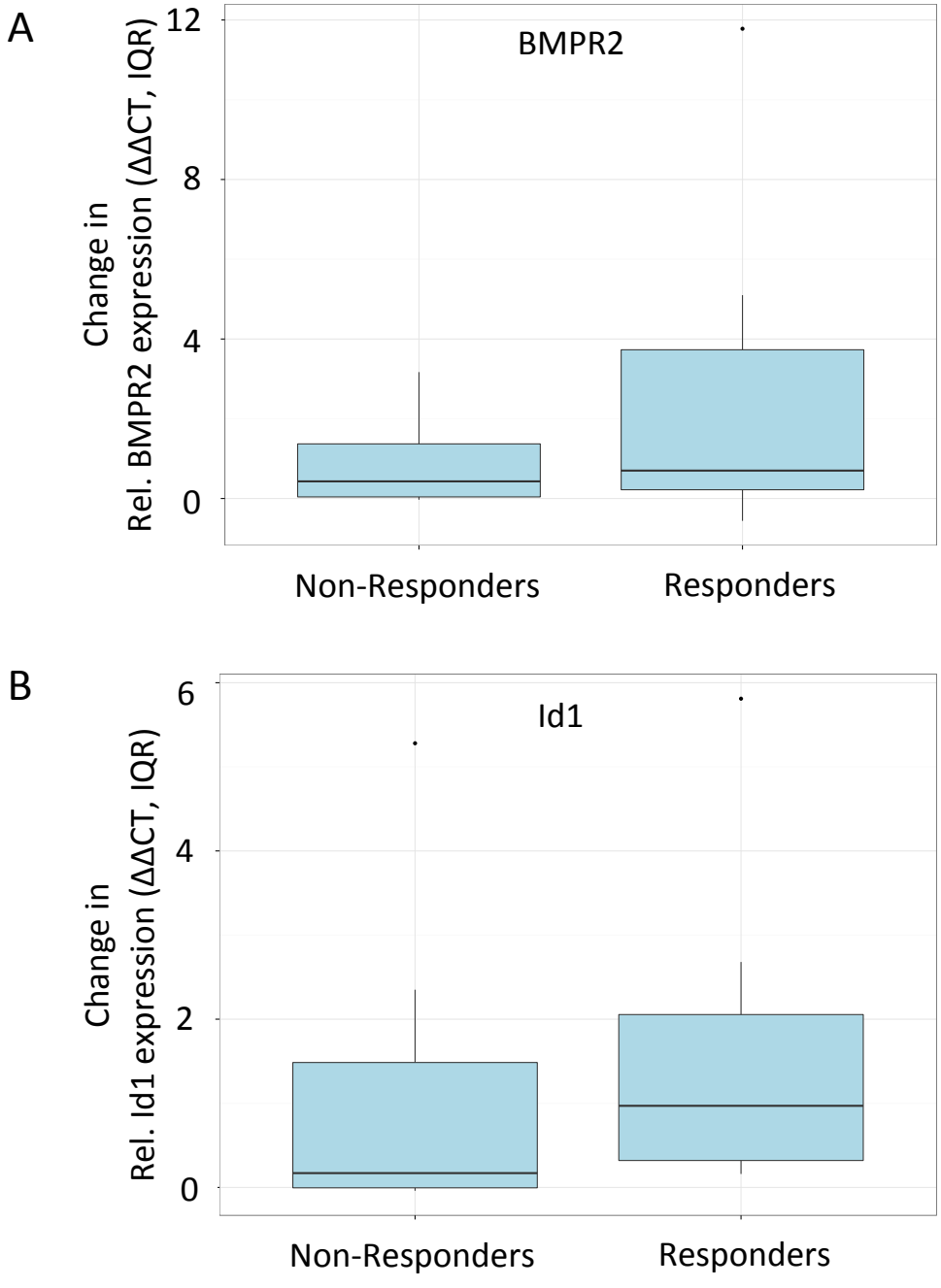
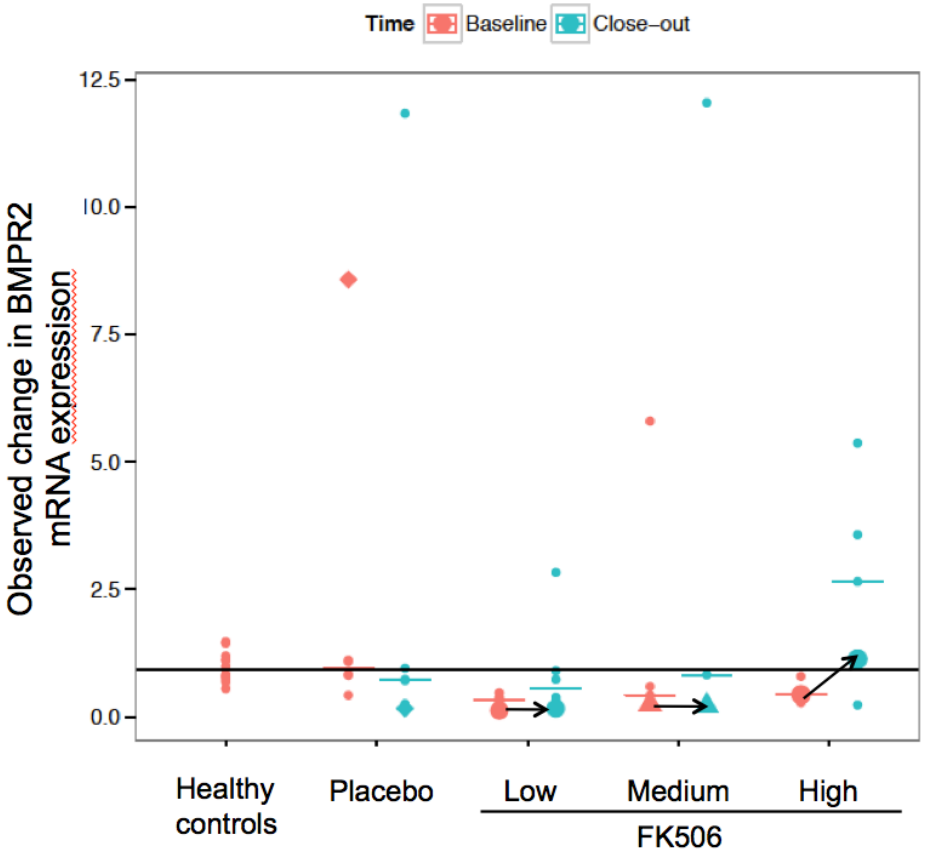

A
BMPR2
Change in
Rel. BMPR2 expression (ΔΔCT, IQR)
12
8
4
0
Non-Responders
Responders
B
Id1
Change in
Rel. Id1 expression (ΔΔCT, IQR)
6
4
2
0
Non-Responders
Responders

Figure E7


Time
Baseline
Close–out
Observed change in BMPR2 mRNA expression
12.5
10.0
7.5
5.0
2.5
0.0
Healthy controls
Placebo
Low
Medium
High
FK506

TrANsFoRM PAH:

Phase II Study of SafeTy, Tolerability, ANd Efficacy of FK-506 (TacRoliMus) in Pulmonary Arterial Hypertension

PROTOCOL
Version 9.0
02/21/2013

Stanford University School of Medicine

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PROTOCOL SUMMARY:

Objectives:

Primary objective of the study is to evaluate the safety of FK-506 (at 3 different blood levels) versus placebo in patients with pulmonary arterial hypertension (PAH)

Secondary objectives of the study are:

Assess the effect of placebo versus FK-506 (3 different doses) on time from randomization to clinical worsening (TTCW) at week 16. TTCW is a composite of multiple events as defined by the following set:

- All cause mortality
- Transplantation
- Atrial septostomy
- Need for escalation of therapies as deemed by site investigator
- Worsening of NYHA/WHO classification by at least 1 point.
- Hospitalization for right heart failure.

Assess the effect of placebo versus FK-506 (3 different doses) on change in 6MWD from baseline to 16 weeks.

Assess the effect of placebo versus FK-506 (3 different doses) on change in NT-pro BNP from baseline to 16 weeks.

Assess the effect of placebo versus FK-506 (3 different doses) on change in Uric Acid from baseline to 16 weeks.

Assess the effect of placebo versus FK-506 (3 different doses) on change in % predicated Hgb adjusted DLOC from baseline to 16 weeks.

Assess the effect of placebo versus FK-506 (3 different doses) on change in echocardiographic parameters such as RV size, RA size, and TAPSE from baseline to 16 weeks.

Study Design:

Randomized, placebo-controlled, double-blind study of 40 patients. Eligible patients will be randomly assigned to either placebo or FK-506 at 3 different blood level goals. The four arms of the study are as follows:

- Arm 1 - Placebo
- Arm 2 – FK-506 goal <2 ng/mL
- Arm 3 – FK-506 goal 2-3 ng/mL
- Arm 4 – FK-506 goal 3-5 ng/mL

Study Population:

Inclusion Criteria

1. Age ≥ 18 and < 70 years
2. Diagnosis of WHO Group I Pulmonary Arterial Hypertension (PAH) (Idiopathic (I)PAH, Heritable PAH (including Hereditary Hemorrhagic Telangiectasia), Associated (A)PAH (including collagen vascular disorders, drugs+toxins exposure, congenital heart disease, and portopulmonary disease).
3. Stable on active PAH treatment including any prostacycline or phosphodiesterase inhibitors and the endothelin antagonist Ambrisentan alone or in combination (stability defined as: <10% change in 6MWD, no change in NYHA class, no hospitalization or addition of PAH therapy for at least 3 months).
4. Previous Right Heart Catheterization that documented:
    a. Mean PAP ≥ 25 mmHg.
    b. Pulmonary capillary wedge pressure < 15 mmHg.
    c. Pulmonary Vascular Resistance ≥ 3.0 Wood units or 240 dynes/sec/cm5
5. All NYHA/WHO functional classes.
6. Willingness of female subjects to use birth control, or be post-menopausal, or status post hysterectomy.

Exclusion criteria:

1. WHO Group II – V Pulmonary Hypertension.
2. Current or prior experimental PAH treatments within the last 6 months (including but not limited to tyrosine kinase inhibitors, rho-kinase inhibitors, or cGMP modulators).
3. Current active treatment with the dual endothelin receptor antagonist bosentan.
4. TLC < 60% predicted; if TLC b/w 60 and 70% predicted, high resolution computed tomography must be available to exclude significant interstitial lung disease.
5. FEV1 / FVC < 70% predicted and FEV1 < 60% predicted
6. Significant left-sided heart disease (based on screening Echocardiogram):
    a. Significant aortic or mitral valve disease
    b. Diastolic dysfunction ≥ Grade II
    c. LV systolic function < 45%
    d. Pericardial constriction
    e. Restrictive cardiomyopathy
    f. Significant coronary disease with demonstrable ischemia.
7. Chronic renal insufficiency defined as an estimated creatinine clearance < 30 ml/min (by MDRD equation).
8. Current atrial arrhythmias not under optimal control.
9. Uncontrolled systemic hypertension: SBP > 160 mm or DBP > 100mm
10. Severe hypotension: SBP < 80 mmHg.
11. Pregnant or breast-feeding.
12. Psychiatric, addictive, or other disorder that compromises patient's ability to provide informed consent, to follow study protocol, and adhere to treatment instructions.
13. Active cyclosporine use.

14. Known allergy or hypersensitivity to FK-506.
15. Planned initiation of cardiac or pulmonary rehabilitation during period of study.
16. Human Immunodeficiency Virus infection.
17. Moderate to severe hepatic dysfunction with a Child Pugh score >10.
18. Hyperkalemia defined as Potassium > 5.1 mEq/L at screening .
19. Known active infection requiring antibiotic, antifungal, or antiviral therapies.
20. Co-morbid conditions that would impair a patient's exercise performance and ability to assess WHO functional class, including but not limited to chronic low-back pain or peripheral musculoskeletal problems.

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Chapter 1: Background

Background: A loss of function mutation in bone morphogenetic protein (BMP) receptor II (BMPRII) is present in >80% of familial and ~20% of sporadic idiopathic (I) PAH *(Machado et al. Hum Mutat 2006, 27:121-32)*. Even patients with IPAH without a BMPRII mutation or with other causes of PAH have reduced expression of BMPRII, reinforcing the importance of BMPRII signaling in the pathogenesis of PAH *(Humbert M et al. Eur Respir J 2002, 20:518-23).* Furthermore BMPR2 receptor gene therapy attenuates experimental hypoxic pulmonary hypertension in rats *(Reynolds et al. Am J Physiol Lung Cell Mol Physiol 2007*). Therefore increasing BMPRII signaling in patients with pulmonary arterial hypertension might prevent or reverse disease.

Detailed Methods of experimental data: We screened 3600 FDA approved drugs and bioactive compounds for their ability to activate BMP signaling, using a C2C12 mouse myoblast cell line stably transfected with a reporter plasmid expressing a **B**MP **r**esponse **e**lement (BRE) from the Id1 promoter fused to the luciferase-gene (BRE-luc). We then determined whether the best qHTS-BMPRII activator can induce Smad phosphorylation (phospho), Id1 expression and promote PAEC survival and tube formation using BMP4 as a positive control. Next we investigated whether the qHTS-BMPRII-activator would prevent PAH in mice with a conditional deletion in BMPRII in ECs (BMPRII-SCL-CreER^T) that develop exaggerated PAH after 3 weeks of hypoxia (10% O_2). In order to assess whether the BMPRII activator could also reverse PAH, we used 2 models of severe experimental PH in rats: 1. Monocrotaline induced pulmonary hypertension with development of severe medial hypertrophy of the pulmonary arteries 3 weeks after injection. 2. SUGEN (VEGF-Receptor blocker) and 3-week chronic hypoxia induced pulmonary hypertension with development of neointima formation in pulmonary arteries 8 weeks after initiation of the stimulus. Both groups were treated with FK-506 for 3 weeks via a sc osmotic pump (0.05mg/kg/d) after PAH and remodeling of the pulmonary arteries was established. The serum level of FK-506 in mice and rats was aimed to be 0.2ng/ml.

Results of experimental data: FK-506, an agent that can induce BMPRIA phosphorylation, was the main activator of Id1 expression. FK-506, at a dose of 15 ng/ml, the therapeutic serum level used to induce immunosuppression, and at a much lower dose of 0.2ng/ml increased Id1 protein expression 1h following stimulation, in a manner comparable to BMP4 (10 ng/ml) ($n=3$, $p<0.06$). This was preceded by phospho-Smad 1/5/8 at 15 min, similar to BMP4 ($n=3$, $p<0.001$). FK-506 induced p-Smad 1/5/8 and Id1 expression in PAECs harvested from six different IPAH patients at the time of transplant, including 3/6 patients that did not respond to BMP4. Both BMP4 and FK-506 improved survival of PAECs ($n=5$, $p<0.001$) and induced tube formation in an angiogenesis assay ($n=3$, $p<0.01$). A 3-week preventive treatment with FK-506 (0.05mg/kg/d) (serum levels 0.2ng/ml) in mice with a conditional deletion in BMPRII in ECs exposed to 3 weeks of hypoxia prevented the development of PAH and right ventricular hypertrophy (RVH); RV systolic pressure: 32±0.9 vs 21±2.3 mmHg, $p<0.001$; RVH: 36.2±2.5 vs 26.9±4.5, $p<0.01$, both $n=5$. To test whether FK-506 could also reverse PAH, we induced PAH in rats with monocrotaline (60mg once s.c) and began treatment with FK-506 3 weeks after injection, a time when PAH was established (RVSP 50.8 ± 2.7mmHg, $n=7$). The survival after a 3-week treatment with FK-506 did not differ in the FK-506 (57%) compared to the vehicle group (66%), yet of

those that that survived the PAH was significantly reduced compared to vehicle treated animals (RVSP 39.5 ± 4.7 vs 68.6 ± 4.2 mmHg, n=14).

Next we showed that the combined stimulus of SUGEN (20mg/kg s.c) and 3-weeks of chronic hypoxia induced PAH in rats when rats were returned to RT and left for another 5 weeks (RVSP 55.1±10.7 vs control 25.1±0.5mmHg, RVH 0.24±0.005 vs 0.44±0.07, n=4, $p<0.05$) but that a 3-week sc treatment of FK-506 at the time of established PAH could prevent progression and induce regression of PAH in FK-506 treated vs vehicle treated animals (RVSP 66.5±4.1mmHg vs 39.5±0.6 mmHg, RVH 0.49±0.07 vs0.34±0.02, n=4, $p<0.05$). Neointima formation in small pulmonary arteries (alveolar wall and alveolar duct vessels) per total vessel number decreased from 61.2±6.1% to 16.2±5.8% (n=4, $p<0.01$). At the low dose of FK-506 of 0.2 ng/ml we did not observe any effect on total or differential WBC count not did we find an immunosuppressive effect measured by decreased nuclear NFATc2.

Conclusion of experimental data: We have identified FK-506 (Tacrolimus) in a quantitative high throughput screen (qHTS) of FDA approved drugs and bioactive compounds as a drug that activates BMPRII signaling, restores normal function of pulmonary artery endothelial cells (PAECs), prevent and reverse experimental PAH in mice and rats. Our results have be presented in a mini-symposium at the American Thoracic Society in Denver May 2011, at the Aspen Lung conference 2012 and are currently pending publication. Given that Fk-506 is already FDA approved and is used for immunosuppression after solid organ transplantation, it would be an ideal candidate drug to use in patients with pulmonary arterial hypertension where BMPRII signaling is dysfunctional.

Chapter 2: Objectives & Specific Aims

Objectives

This is a Phase II, randomized, double-blind, placebo-controlled trial. The proposed research project involves one primary and two secondary specific aims. Specific aims 1 examines the safety and tolerability of FK506 in patients with PAH while specific aims 2 & 3 evaluate the effect of FK506 on clinical worsening (#2) and clinical markers such as exercise tolerance and disease biomarkers (#3). Patients in this protocol may be concurrently treated with other PAH therapies.

Specific Aims

Primary Aims:

1. To determine whether FK-506 is safe and tolerable at 3 different doses for 16 weeks in patients with PAH.

Secondary Aims:

2. To determine whether FK-506 affects time to clinical worsening as defined by all cause mortality, reduction in exercise tolerance, and hospitalization for right heart failure in patients with PAH.

3. To determine whether FK-506 affects clinical disease parameters such as six minute walk distance (6MWD), NT-pro BNP, Uric Acid, and percent predicted hemoglobin adjusted DLCO in patients with PAH.

Other aims include the demonstration of patient compliance and feasibility of routine blood testing for drug levels in PAH and to give preliminary insight into the dose necessary to plan for preliminary dose-finding and Phase III studies.

Chapter 3: Screening, Patient Selection, and Randomization

Inclusion criteria:

1. Age ≥ 18 and < 70 years
2. Diagnosis of WHO Group I Pulmonary Arterial Hypertension (PAH) (Idiopathic (I)PAH, Heritable PAH (including Hereditary Hemorrhagic Telangiectasia), Associated (A)PAH (including collagen vascular disorders, drugs+toxins exposure, congenital heart disease, and portopulmonary disease).
3. Stable on active PAH treatment including any prostacycline or phosphodiesterase inhibitors and the endothelin antagonist Ambrisentan alone or in combination (stability defined as: <10% change in 6MWD, no change in NYHA class, no hospitalization or addition of PAH therapy for at least 3 months).
4. Previous Right Heart Catheterization that documented:
    a. Mean PAP ≥ 25 mmHg.
    b. Pulmonary capillary wedge pressure < 15 mmHg.
    c. Pulmonary Vascular Resistance ≥ 3.0 Wood units or 240 dynes/sec/cm5
5. All NYHA/WHO functional classes.
6. Willingness of female subjects to use birth control, or be post-menopausal, or be status post hysterectomy.
    a. Post-menopausal state will be defined as follows:
    - Women above age 45 yrs: We diagnose menopause as 12 months of amenorrhea in the absence of other biological or physiological causes. A high serum FSH is **not** required to make the diagnosis.
    - Women below age 45 yrs: Diagnosis of menopause or premature ovarian failure will need to be made by a primary obstetrics and gynecology physician based on STRAW criteria.

Exclusion criteria:

1. WHO Group II – V Pulmonary Hypertension.
2. Current or prior experimental PAH treatments within the last 6 months (including but not limited to tyrosine kinase inhibitors, rho-kinase inhibitors, or cGMP modulators).
3. Current active treatment with the dual endothelin receptor antagonist bosentan.
4. TLC < 60% predicted; if TLC b/w 60 and 70% predicted, high resolution computed tomography must be available to exclude significant interstitial lung disease.
5. FEV1 / FVC < 70% predicted and FEV1 < 60% predicted
6. Significant left-sided heart disease (based on screening Echocardiogram):
    a. Significant aortic or mitral valve disease
    b. Diastolic dysfunction ≥ Grade II
    c. LV systolic function < 45%
    d. Pericardial constriction
    e. Restrictive cardiomyopathy
    f. Significant coronary disease with demonstrable ischemia.
7. Chronic renal insufficiency defined as an estimated creatinine clearance < 30 ml/min (by MDRD equation).
8. Current atrial arrhythmias not under optimal control.
9. Uncontrolled systemic hypertension: SBP > 160 mm or DBP > 100mm

10. Severe hypotension: SBP < 80 mmHg.
11. Pregnant or breast-feeding.
12. Psychiatric, addictive, or other disorder that compromises patient's ability to provide informed consent, follow study protocol, and adhere to treatment instructions.
13. Active cyclosporine use.
14. Known allergy or hypersensitivity to FK-506.
15. Planned initiation of cardiac or pulmonary rehabilitation during period of study.
16. Human Immunodeficiency Virus infection.
17. Moderate to severe hepatic dysfunction with a Child Pugh score >10.
18. Hyperkalemia defined as Potassium > 5.1 mEq/L at screening .
19. Known active infection requiring antibiotic, antifungal, or antiviral therapies.
20. Co-morbid conditions that would impair a patient's exercise performance and ability to assess WHO functional class, including but not limited to chronic low-back pain or peripheral musculoskeletal problems.

Clarification of selected eligibility issues

Hemodynamics
Hemodynamics from prior right and left heart catheterizations will be reviewed. If both a pulmonary capillary wedge pressure and left ventricular end-diastolic pressure (LVEDP) are measured, the LVEDP will be used for the inclusion criteria. These hemodynamic criteria need to be met within a single catheterization procedure at any time before study enrollment.

PAH diagnosis
The subtype of PAH will be determined using traditional guidelines. The diagnosis of collagen vascular disease will be based on American College of Rheumatology criteria, which incorporates clinical and laboratory results. The diagnosis of familial PAH will be made if a relative of the patient meets (or met) the criteria for PAH.
A diagnosis of congenital cardiac shunt will be made if the patient has (or had) a cardiac defect (including, but not limited to, atrial septal defect, ventricular septal defect, patent ductus arteriosus, etc.). Patients with repaired cardiac defects will be classified in this category.

Pulmonary function testing and imaging
Pulmonary function results from clinical testing within the year prior to screening may be used. DLCO will be performed ± 4 weeks from baseline visit. Computerized tomography results from the previous two years may be used if the total lung capacity is mildly reduced (60%< TLC% < 70%). The study may be high-resolution or non-high resolution to meet this requirement. A report of the computed tomographic scan will be sufficient to meet this criterion.

Limitations to six minute walk testing
As most of the patients in this protocol will have performed the 6MWT previously for clinical care, previous testing without musculoskeletal limitation will satisfy this criterion. Otherwise, the absence of notable limitations at screening (e.g., walking aids, reported difficulty walking) will satisfy this criterion.

Acceptable contraceptive precautions

Women of childbearing potential (premenopausal with a uterus) will be questioned about sexual activity and the use of effective contraception. For this protocol, acceptable contraception includes one or more of the following: condoms; diaphragm; oral or parenteral hormone therapy; bilateral tubal ligation, or sexual partner with a vasectomy.

Other concomitant medications

Drugs that might ***increase*** Tacrolimus levels (= CYP3A inhibitors that decrease Tacrolimus metabolism and increase it's bioavailability) are:

- Calcium channel blockers (diltiazem, nicardipine, nifedipine, verapamil)
- Antifungal agents (clotrimazole , fluconazole, itraconazole, ketoconazole, voriconazole)
- Macrolide antibiotics (clarithromycin, erythromycin, troleandomycin)
- Prokinetic agents (cisapride, metoclopramide)
- Others (bromocriptin, cimetidine, cyclosporine, cyclosporine, danazol, ethinyl estradiol, methylprednisone, lansoprazole, omeprazole, protease inhibitors, nefazodone, magnesium-aluminum hydroxide)

If patients are on these drugs at baseline, these don't represent a contraindication to be included in the study. We will monitor FK506 levels very frequently and therefore will adjust the FK506 dose. Should any of these drugs become necessary while patient is participating in the trial, we will treat the initiation of these medications the same as a dose change in FK506 and will assess the FK506 blood level after 4 days (x2) as described by the FK506 blood level algorithm.

Drugs that may ***decrease*** Tacrolimus levels:

- Anticonvulsants (carbamazepine, Phenobarbital, phenytoin)
- Herbal Medication (St. John's Wort)
- Antimicrobials (rifabutin, caspofungin, rifampin)
- Other drugs (sirolimus)

If patients are on these drugs at baseline, these don't represent a contraindication to be included in the study. We will monitor FK506 levels very frequently and therefore will adjust the FK506 dose. Should any of these drugs become necessary while patient is participating in the trial, we will treat the initiation of these medications the same as a dose change in FK506 and will assess the FK506 blood level after 4 days (x2) as described by the FK506 blood level algorithm. Antifungal agents which impact CYP3A and are known to increase FK506 levels will be noted and dose reduction / blood levels followed per algorithm included.

Other investigational agents

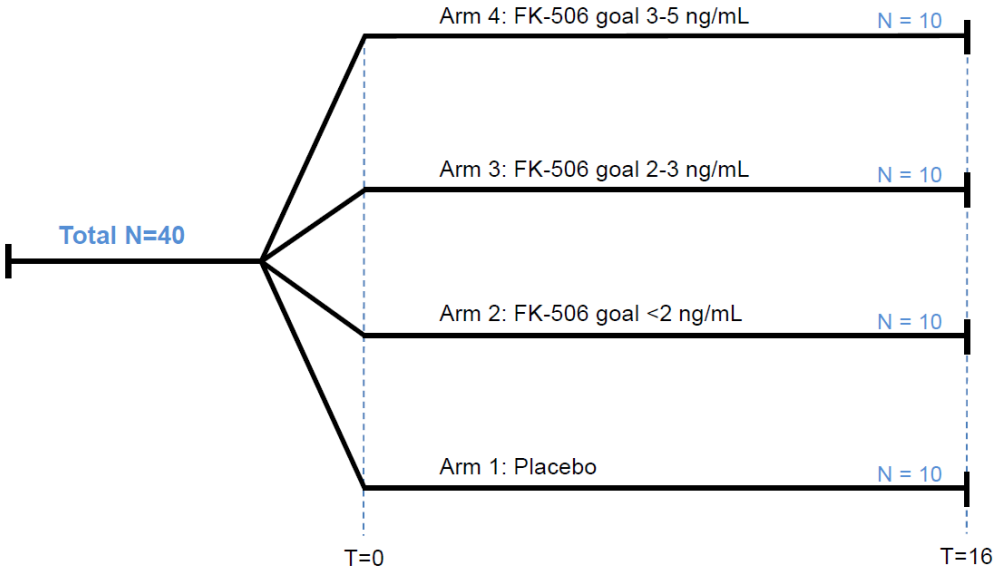
Patients who are currently using a non-FDA-approved drug for treatment of PAH will be excluded. This means that patients enrolled in clinical trials of non-FDA-approved drugs will be excluded from this study.

Randomization:

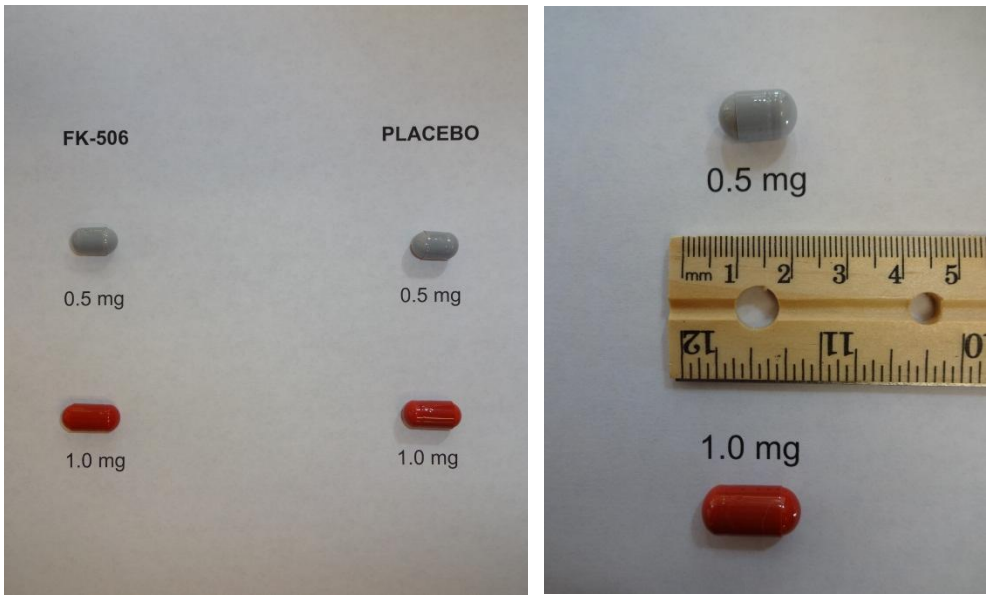
Subjects are randomized to placebo or FK506 at three different doses in a 1:1:1:1 ratio. Randomization will take place in blocks of 4.

Chapter 4: Treatment

At total of 40 study participants will be randomized to Placebo versus FK506 at three different blood levels (< 2 ng/ml, 2-3 ng/ml and 3-5 ng/ml) in a 1:1:1:1 fashion (see schematic below).



We will utilize FK506 (0.5 mg pill and 1.0 mg pill) and matching placebo (both supplied by UCSF pharmacy). To assure complete double-blinding, placebo and FK506 pills are encapsulated into similar shape and color capsules (as shown below).



Study medication
Labeling
Bottles will be fully labeled and packaged by the Research Pharmacy to meet state and FDA requirements.

Distribution
Each patient will receive one bottle of 1mg and one bottle of 0.5 mg pills.

Storage
Medication will be stored at controlled room temperature not to exceed 30°C (86°F), in a locked, secure area in the department of pharmacy at Stanford. Temperature in the drug storage area must be checked and recorded daily. These temperature readings must also be available for review by study monitor. Should the temperature exceed the above range, the monitor must be notified within one business day of discovery of the event.

Administration
The Research Coordinator or Investigator will instruct the subjects on how many tablets to take per day, depending on the instructions from the FK-adjustment committee. The study bottles will be labeled and color-coded (blue and yellow) to discriminate between the 0.5 mg and 1.0 mg tablets.

Medication Compliance
We will use MEMS monitors (below left) to measure patient's compliance. The MEMS monitors are drug packages with integral electronic micro circuitry designed to compile the dosing histories of ambulatory patients' prescribed medications. Each monitor consists of a conventional medicine bottle fitted with a special closure that records the time and date of each opening and closing of the container through integrated micro-circuitry. A Reader (below right) transfers the dosing history data from the MEMS monitor to a MS-Windows based computer. Patient will receive their medication and the medication compliance will be checked at each visit by downloading the information of the MEMS (on daily opening of the medication bottle) at each visit by the Research Coordinator.

Lost medication
If a patient loses one or both of the bottles of study medication, the local Research Coordinator should contact the Research Pharmacy. A replacement bottle with the appropriate amount of study medication will be shipped either to the patient's home by Federal Express or to the local Field Center for pick-up by the patient. If patient loses his/her medication more than twice, patient will be excluded from the study.

Medication resupply
At the 4 week and 8 week study visit, the subject will return the current bottles of the study medications and the Research Coordinator will dispense new bottles for the next study period while noting the dispensation on the Drug Accountability Form (See Section 9.2.2 Distribution).

Expired medication
The Research Coordinator will check the expiration date on the kit dispensed to a research subject to ensure that the expiration date is not within the study period for the patient. Well in advance of the expiration of these medications, the Research Pharmacy will resupply the study centers with fresh study medications.

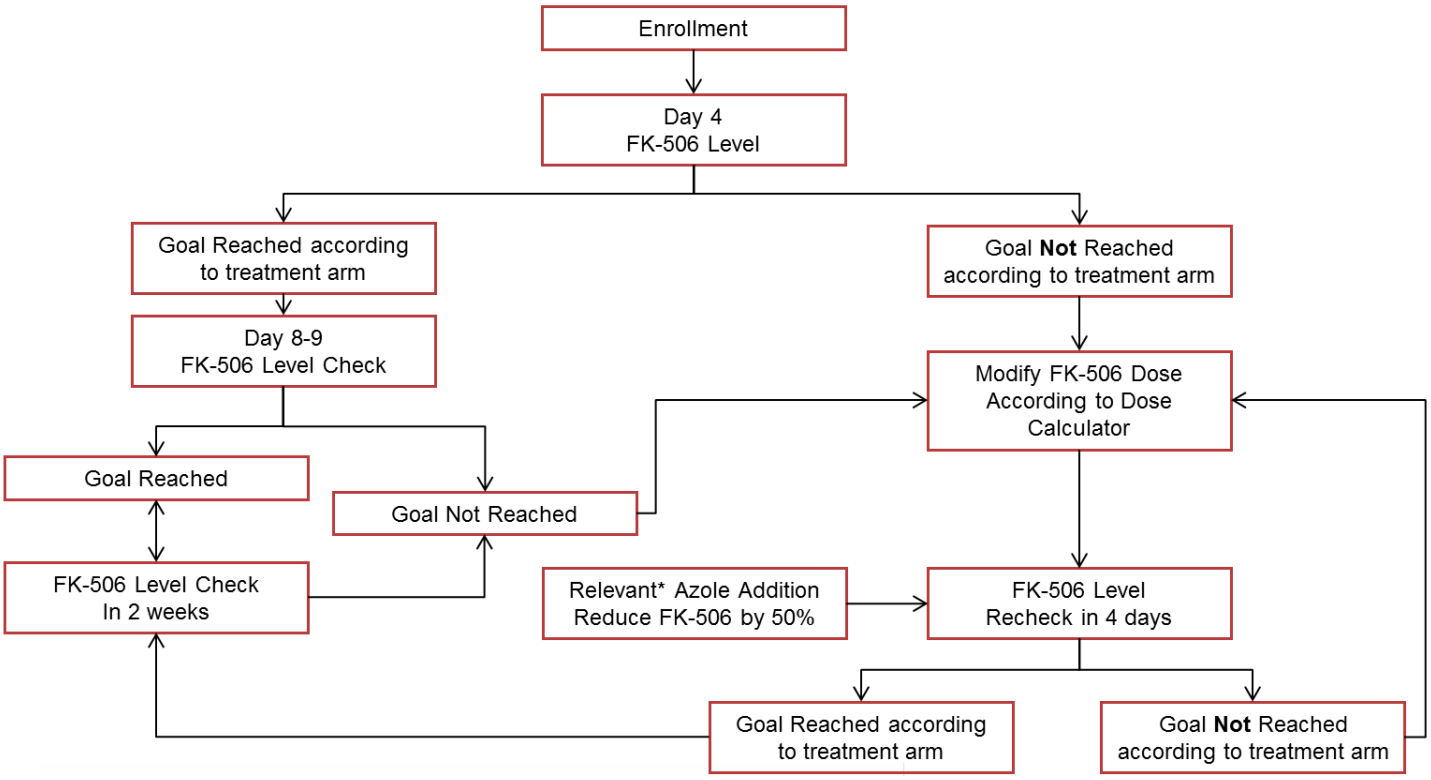
Investigational drug return
The Research Coordinator and investigators will dispense two bottles of drug product to study patients at each study visit during the treatment phase, one with Tacrolimus 1.0 mg and the other one with Tacrolimus 0.5 mg pills. Patients will be asked to return bottles at each clinic visit, compliance with be measured with the MEMS device. Pill bottles will therefore be collected by the Research Coordinator at 4 weeks, 8 weeks, and 16 weeks. All unused medication in the bottles will be counted and recorded on the Medication Compliance Form, and maintained in the patient's drug kit by the Research Coordinator. At the conclusion of the study, remaining medication from the study patients (and left-over medication study kits) can be destroyed at the clinical site.

Dose Titration:
We will perform measurements of FK-506 level 4 days after the initial dose & after every dose change. If the level is at goal, a second level check will be performed after 4 days. If the second level is at goal, levels will then be checked every 2 weeks. If the second level is not at goal, the dose will be modified. As before, 2 consecutive levels at goal are required before the level interval can be increased to 2 weeks (*see attached algorithm*).

Blood for FK-506 levels will be drawn and measured at a Labcore facility with a standardized method (ELISA). Results will be reported to Stanford per e-mail and fax to the FK506 dose adjustment committee (headed by Dr. Yon Sung).

Measurements of FK-506 levels:
Principle: Level will be determined 4 days after initiation or change of dose. If level is at goal, a second level check will be performed after 4 days. If level is at goal, levels will be checked every 2 weeks. If level is not at goal, the dose will be modified and again 2 consecutive levels at goal are required before the level interval can be increased to 2 weeks.

Algorithm for FK506 blood level testing



* Relevant Azole is defined as: Use of antifugal of azole class including but not limited to posaconazole, ketaconazole, voriconazole. Fluconazole does not require FK-506 dose reduction.

Tacrolimus dose modification formula:

$$\text{New Dose (mg)} = \frac{\text{Actual Dose (mg)} \times \text{Trough}_{\text{target}}(\text{ng/mL})}{\text{Actual Trough from lab (ng/mL)}}$$

*Round to the nearest 0.5mg capsule size
** Tacrolimus levels reported by the core lab as <2ng/ml should be entered into the calculator as 1ng/ml.

Chapter 5: Events and Data Collection

Overview:

BASELINE STUDY VISIT

- Vital signs including height and weight.
- Physical examination with WHO/NYHA functional class (part of routine clinical care).
- PFT with DLCO (if no PFTs within 4 weeks of baseline).
- Echocardiography (if not done within 4 weeks of baseline visit)
- Administer the 6MWT with Borg Dyspnea Index score (BDI score)
- Comprehensive Metabolic Panel (if not done within 4 weeks of baseline visit)
- CBC (if not done within 4 weeks of baseline visit)
- Uric acid
- NT-pro BNP
- Urine pregnancy test for female subjects of childbearing potential (if not done within 4 weeks of baseline visit)
- Research Biomarkers: Blood (ie Id1, eNOS, Apelin, IL-6, GDF-15)
- Note changes to medical history and medications.
- Randomization, dispense study drug (using the MEMS – Medication Event Monitoring System by Aardex) and dose titration advice (The dose will be adjusted according to a dose calculator developed for us by Dr. Janel Long-Boyle Pharm D, UCSF – *see attachment*)

  Remind subject to call the study coordinator or PI's promptly should an adverse event (AE) occur.

WEEK 2, 6, 10, 12Telephone Contact:

- Assess symptoms, medications use and compliance, any adverse events or subject concerns.

WEEK 4 STUDY VISIT

- Record vital signs & weight.
- Physical examination with WHO/NYHA functional class.
- Administer the 6MWT with BDI score.
- Comprehensive metabolic panel
- CBC
- NT-pro BNP
- 1 lavender tube for Stanford FK506 level
- Record any reported adverse events (AEs) since last visit
- Review medication compliance and any medication changes.
- Remind subject to call the study coordinator or PI promptly should an AE occur.

WEEK 8 STUDY VISIT

- Record vital signs & weight.
- Physical examination with WHO/NYHA functional class (part of routine clinical care).
- Administer the 6MWT with BDI score.

- Comprehensive metabolic panel
- CBC
- NT-pro BNP
- 1 lavender tube for Stanford FK506 level
- Record any reported adverse events (AEs) since last visit
- Review medication compliance and any medication changes.
- Remind subject to call the study coordinator or PI promptly should an AE occur.

WEEK 16 STUDY VISIT
- Record vital signs & weight.
- Physical examination with WHO/NYHA functional class (part of routine clinical care).
- Administer the 6MWT with BDI.
- Echocardiography (part of routine clinical care).
- PFT with DLCO
- NT-pro BNP
- Uric Acid
- Comprehensive Metabolic Panel (part of routine clinical care)
- CBC
- Research Biomarkers: Blood (ie. Id1, IL-6, GDF-15, eNOS, Apelin).
- Record any reported adverse events (AEs) since last visit
- Review medication compliance and any medication changes.
- All study medication will be returned

WEEK 18 FINAL VISIT (end of study)
- Post-study drug visit to assess symptoms, any adverse events, or subject concerns at approximately 2 weeks after study drug stopped.
- Record vital signs & weight.
- Physical examination with WHO/NYHA functional class
- Administer the 6MWT with BDI.
- NT-proBNP

SAEs should be followed until resolution or judged by the Investigator to no longer be clinically significant or for at least 4 weeks after the final study visit.

A	B	C	D	E	F	G	H	I	J	K	L	M
PROPOSED TIME LINE: TRANsFoRM PAH STUDY (version 4.0 04/17/12) - **All Events Combined**												
	Screening	**Baseline**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**	**Week 16**	**Week 18**
Visit #	0	1				2		3			4	5
Day #	-30 - 0	0	7 ± 3	14 ± 3	21 ± 3	28 ± 3	42 ± 3	56 ± 3	70 ± 3	84 ± 7	112 ± 7	126 ± 14
Telephone Contact				S			S		S	S		
Informed Consent	S											
History and physical exam												
Medical history	RC	RC										
Symptom assessment	RC	RC		S		S	S	S	S	S	RC	S
Medications	RC	RC		S		S	S	S	S	S	RC	S
Vital Signs & Weight	RC	RC				S		S			RC	S
Physical Exam including NYHA functional class		RC				S		S			RC	S
General Testing												
Comprehensive Metabolic Panel, CBC		RC				S		S			RC	
Serum HCG (if applicable)		S***										
FK-506 Level (according to algorithm*), estimate			S	S	S	S	S	S	S	S	S	
Endpoint assessment												
6 minute walk distance		S				S		S			S	S
NT-pro-BNP		RC				S		S			RC	S
Echocardiograms		RC***									RC	
PFT with DLCO		RC***									S	
Serum/Plasma for biomarkers		S									S	
Uric Acid		S									S	
Study Procedures												
Dispense study drug		S				S		S				
Dose titration advice (according to drug calculator**)		S	S	S	S	S	S	S	S	S	S	
Medication Compliance				S		S	S	S	S	S	S	
Adverse Events				S		S	S	S	S	S	S	S
Randomization		S										
Study Termination												S
RC = Standard practice at enrolling centers												
S = Study related procedure at enrolling centers												
* see attached algorithm of FK-level frequency: Level check after 4 days for 2 consecutive times until steady state is reached, then every 2 weeks; days and total number of FK506 levels vary between patients												
** see attached FK-dose calculator												
*** If not done within 4 weeks of baseline												

Timing of the Baseline/randomization Visits

The Baseline Visit (Visit #1a, 1b) must occur between 1 and 30 days after the Screening Visit. If a subject does not have the Baseline Visits within 30 days of the screening visit, the subject must be re-screened by the Research Coordinator for eligibility criteria and not randomized. If the subject is randomized longer than 30 days from the Screening Visit, it will be considered a protocol violation. The patient will remain in the study and undergo re-screening.

Scheduling and preparing for the Baseline Visits

The Research Coordinator will call the patient 1-2 days before the Baseline Visits to remind him or her:

1) to bring all medications with him or her to the study visit,
2) to wear loosely fitting clothing and comfortable walking shoes, and
3) to remind her/him where to go for the study visit.

The Research Coordinator must notify the echo and exercise technicians of the patient's scheduled visit and study times. The Research Coordinator should complete the header of each CRF for the Baseline Visit with the subject's initials and study ID in advance of the visit. The Research Coordinator should prepare all necessary laboratory materials before the visit.

The visits will require approximately 3.0 hours on one day. During this time, the subject must undergo a brief history (to make sure that no events since screening have made the patient ineligible), have phlebotomy performed, echocardiogram, a six minute walk test and have a brief physical examination. The patient will be randomized at this time.

Day of Baseline Visits
Sequence of procedures, including the randomization

This sequence of steps is followed for all subjects at the Baseline Visit:
• Perform a brief history
• Confirm eligibility and randomize the subject

If the subject is ineligible, the Research Coordinator thanks the subject for participating.

If eligibility criteria are not met which are temporary (or may resolve), the patient may be rescreened > 1 month later.

If the patient is eligible, the Research Coordinator performs these steps:
• Obtain patient vital signs
• Record the subject's current medications
• Accompany the patient to the lab
• Accompany the patient to the six minute walk test
• Take patient to Echo lab for echocardiogram
• Perform a detailed history and physical examination
• Provide the patient with the first two bottles of study treatment
• Confirm the next follow-up visit and give subject an Appointment Reminder Card
See the respective sections for details regarding CRF completion and test performance for each of the assessments. Details regarding the steps taken by the Research Coordinator are provided below.

The brief history performed by the Research Coordinator should include initiation of new PAH therapy, allergy or hypersensitivity to FK506, and current use of a non-FDA approved therapy for PAH.

After the Research Coordinator confirms eligibility of the patient with the brief history and collects vital signs, the patient will be randomized into one of the 3 treatment arms or Placebo arms.

The Research Coordinator will accompany the patient to the laboratory for blood draws (clinical labs as described above and research labs (2 PMBCs, 1 redtop, 1 lavender top) and will perform a 6 minute walk testing. Afterwards, the Research Coordinator should accompany the patient to the echocardiography laboratory. The Research Coordinator should bring the Echocardiogram Form with the subject.

After the conclusion of the echo, the Research Coordinator should collect the completed Echo Form and the subject will undergo a detailed medical history and physical examination, performed by the Research Coordinator or Investigator. The Research Coordinator should review all the medication bottles that the subject should have brought to the visit. If the patient has not brought all of the medications, the Research Coordinator should record the regimen of all medications taken during the previous two weeks recalled by the patient. In addition, the Research Coordinator should confirm the medication list by telephone after the visit in this

instance. If the participant brings a list of medications, instead of the medication containers, record all pertinent information from the list.

If the Research Coordinator has any doubt about the accuracy of the list, a follow-up telephone call should be scheduled to confirm what has been recorded.

The Research Coordinator records each medication which the subject is currently taking (administered in the previous two weeks) on the Concomitant Medication Form. Each medication uses its own form. Include all prescription medications and over-the-counter medications. If the subject cannot remember the date when a medication started, the date of the Baseline Visit may be used. Do not record non-therapeutic agents (e.g. mouthwash, cold packs) or therapeutic classes or drugs (e.g. antidepressant, antihypertensive).

The Research Coordinator may complete the Hemodynamics Form at this time, or may complete this before or after the Baseline Visit (but within five business days of the visit).

The Research Coordinator asks the subject about the presence of all of the symptoms listed on the Symptoms Assessment Form and completes the form. The subject should be asked about the presence of each of the symptoms in the previous 30 days. The Research Coordinator or Investigator examines the subject. Vital signs should be recorded on the Vital Signs/Physical Examination Form. The Research Coordinator should measure the subject's height and weight with his/her shoes off and record the weight rounded to the nearest whole kilogram and the height rounded to the nearest whole centimeter. Attempt to use the same scale for the subject for the entire study. The Investigator should assess and record the WHO functional class.

The Research Coordinator should verify the Contact Information Form with the subject, who may fill in missing information.

After assignment of the Study Drug number, the Research Coordinator will record this on the Dispense Drug Form. The Research Coordinator will write the subject's name on the bottles. The Research Coordinator will record the use of bottle on the Drug Accountability Form.

To measure patient's compliance, pill bottles with a monitoring device as the lid (MEMS) will be used. The MEMS device will be activated at the first visit and patients will be informed about the use of the MEMS device. Patients should take out his medication once daily and should not repetitively open the lid during the day. Opening of the device will leave a time stamp in the device.

The patient will then be provided with two bottles of tablets (FK506 1 mg and FK506 0.5 mg) with MEMS lid. The dispensation of the specific study drug bottles should be recorded by the Research Coordinator on the Drug Accountability Form (See Appendix G). The Research Coordinator should explain to the patient that he/she should take as many tablets as prescribed by the PI's depending on the allocated FK-506 blood level group. Patients in group FK506 3-5 ng/ml will start with 3 mg daily, patients in FK506 group 2-3 ng/ml will start with 1.5 mg daily and patients allocated to the group < 2 ng/ml will start with 1 mg daily. Patients will have their

FK506 levels measured after 4 days and dose adjustment recommendations will be made according to the pre-designed FK506 adjustment *algorithm.*

The Research Coordinator should remind the patient to record all non-routine medications (prescription or over the counter), as well as dates for changes in dose or regimen of medications.

The patient should be instructed to tell other health care practitioners that he or she is possibly taking FK506, so that the subject may be managed accordingly. The Research Coordinator should tell the patient that he/she will also send a letter to the patient's PAH physician and primary care doctor explaining that the patient may be taking FK506. The subject should be asked to contact the Research Coordinator if they undergo laboratory testing or have any adverse events during the study period.

The Research Coordinator should make sure the subject has had all of his/her questions answered, that he/she has the correct contact information for the study team, and that he/she understands how to proceed. The Research Coordinator should give the subject an Appointment Card for the next study visit. The Research Coordinator should record the date of the next scheduled study contact (Week 2 Telephone Call) on the Patient Scheduling Form.

After the visit
The Research Coordinator notifies the patient's PAH physician and primary care physician of the patient's participation in the study. The provided letter (See MOP Appendix E) should be faxed to the physicians' offices. The Research Coordinator should enter all of the CRFs from the Baseline Visit into the study binder within 5 business days of the visit.

Follow-up telephone calls (Week 2, 6, 10, 12)

Purpose
The purposes of the follow-up telephone calls are
• Assess side effects and adverse events
• Ask patient to assess if any change in weight of patient
• Assess compliance
• Assess patient clinical status
• Reinforce compliance with study medications and medication avoidance
• Confirm appointments for follow up visits

Timing of the telephone calls
The telephone calls must occur within a 6 day window of the designated time point from the baseline visit (i.e., Day 14 ± 3 and Day 42 ± 3). If the subject is not contacted within this time window, it will be considered a protocol violation.

Procedure for the telephone calls
The call will require approximately 15 minutes. During this time, the Research Coordinator will inquire about the presence of symptoms and whether the patient's clinical status is overall better,

the same, or worse from a PAH perspective (Symptoms Assessment Form- Week 2, 6, 10, 12). The subject should be asked about the presence of each of the symptoms since the last study visit. The subject should be asked about compliance with each study medication and the Research Coordinator should complete the second and third pages of the Symptoms Assessment Form.

The Research Coordinator should ask for the patient's weight to access if any changes are occurring.

The Research Coordinator should reinforce that the patient should take the amount of tablets as prescribed each day. The Research Coordinator should remind the patient to record all non-routine medications (prescription or over the counter), as well as dates for changes in dose or regimen of medications.

The Research Coordinator should make sure the subject has had all of his/her questions answered, that he/she has the correct contact information for the study team, and that he/she understands how to proceed. The Research Coordinator should record the date of the next scheduled study contact on the Patient Scheduling Form.

After the telephone calls

The Research Coordinator should enter all of the CRFs from the Telephone Calls into the study binder within 2 business days of the call.

Follow-up visits (Visits # 2-4)

Purpose

The purposes of the follow-up visits are

- Obtaining laboratory samples
- Recording changes in medication use
- Obtaining six minute walk testing
- Assessing side effects and adverse events
- Replenish study drugs (Visits #2-3)
- Confirming the appointment for the next visit

Timing of the follow-up visits

The follow-up visits must occur within a 7 day window of the designated time point from the baseline visit. (i.e. Visit 3 should be scheduled 56 ± 7 days from the day of Visit 1). If the subject has a visit which is outside of this time window, it will be considered a protocol violation.

Scheduling and preparing for the follow-up visits

The Research Coordinator should send the subject a letter (See MOP Appendix E) two weeks before the visit reminding the subject of the date and preparatory activities necessary before the

visit. The Research Coordinator should call the patient 1-2 days before the Baseline Visit to remind him or her
1) to not exercise heavily in the twelve hours before the study visit,
2) to bring all medications with him or her to the study visit (including the study medications),
3) to wear loosely fitting clothing and comfortable walking shoes, and
4) to remind her/him where to go for the study visit.

The Research Coordinator must notify the echo lab and exercise technicians of the patient's scheduled visit and study times. The Research Coordinator should complete the header of each CRF for the follow-up visits with the subject's initials and study ID in advance of the visits. The Research Coordinator should prepare all necessary laboratory materials before the visit.

The follow-up visits will require approximately 3 hours. During this time, the subject must have phlebotomy performed, perform a six minute walk test, a brief physical examination performed, and the study drugs replaced.

Sequence of procedures
This sequence of steps is followed for all subjects by the Research Coordinator:
• Accompany the patient to the six minute walk test
• Record any changes in the subject's previous medications
• Perform a detailed history and physical examination
• Collect the two bottles of study medications from the previous visit, and replace with the next two (except Visit 4)
• Confirm the next follow-up visit and give subject an Appointment Reminder Card

See the respective sections for details regarding CRF completion and test performance for each of the assessments. Details regarding the steps taken by the Research Coordinator are provided below.

The Research Coordinator should take the patient and the Six Minute Walk Test Form to the site where six minute walk testing will be performed.

The Research Coordinator or Investigators should perform a focused medical interview and physical examination. The Research Coordinator should review all the medication bottles that the subject should have brought to the visit. Any changes in medications or in dosages from the previous visit will be recorded by the Research Coordinator. The date when the previous medication dose was stopped should be recorded on the Concomitant Medication Form for that medicine, and a new Form for the same medicine with the new dose should be completed. Any new medications should be recorded by the Research Coordinator on a new Concomitant Medications Form. Include all prescription medications and over-the-counter medications. Do not record non-therapeutic agents (e.g. mouthwash, cold packs) or therapeutic classes or drugs (e.g. antidepressant, antihypertensive).

If the patient has not brought all of the medications, the Research Coordinator should record the regimen of all medications taken during the previous two weeks recalled by the patient and compare to the regimen recorded on the Concomitant Medications Forms from the previous visit.

In addition, the Research Coordinator should confirm the regimen by telephone after the visit in this instance. If the participant brings a list of medications, instead of the medication containers, review all pertinent information from the list. If the Research Coordinator has any doubt about the accuracy of the list, a follow-up telephone call should be scheduled to confirm what has been recorded.

The Research Coordinator and/or the pharmacy then performs pill counts and collects the pill bottles from the study drug. The Research Coordinator should complete the Medication Compliance Form.

New symptoms (or changes in old symptoms) should be recorded. New medical diagnoses should be recorded as adverse events using the Adverse Event Form or Serious Adverse Event Form as appropriate.

The Research Coordinator asks the subject about the presence of all of the symptoms listed on the Symptoms Assessment Form and completes the form. The Research Coordinator asks for the patient's assessment of his/her clinical status in reference to the previous visit. The Research Coordinator or Investigator examines the subject. Vital signs should be recorded on the Vital Signs/Physical Examination Form. The Research Coordinator should measure the subject's height and weight with his/her shoes off and record the weight rounded to the nearest whole kilogram and the height rounded to the nearest whole centimeter. Attempt to use the same scale for the subject for the entire study. The Research Coordinator or Investigator makes an assessment of the patient's clinical status since that last visit. Investigators should assess and record the WHO functional class.

The Research Coordinator should meet the patient at the Stanford laboratory. The Research Coordinator will bring the necessary labels for the clinical laboratories (marked with the patient's name and local medical record number) and labels for the study labs, marked with the date of patient visit and study identification number. Be careful that the labels for samples being sent to the hospital clinical laboratory do not have any study identifiers and that the labels for samples being stored for study laboratories do not have any patient identifiers (such as MRN). **The Research Coordinator brings the Phlebotomy and Sample Processing Forms to the laboratory.** See Section 17.1 for details of phlebotomy procedure and sample processing and labeling. The Research Coordinator collects the Phlebotomy and Sample Processing Forms when completed.

(Visits 2-3)
The patient will then be provided with two bottles of tablets, one containing morning dose of study drug and one containing evening does of study drug. The dispensation of the study drug bottles should be recorded by the Research Coordinator on the Dispense Drug Form and the Kit Drug Accountability Form (See Appendix E).

The Research Coordinator should remind the patient to record all non-routine medications (prescription or over the counter), as well as dates for changes in dose or regimen of medications.

End-of Study Drug Visit (Visit # 4)
Purpose
The purpose of this Visit is to
• Perform labs and final biomarkers
• Accompany the patient to the six minute walk test
• Take patient to Echo lab for echocardiogram
• Perform a detailed history and physical examination
• Assess side effects and adverse events
• Determine WHO functional class
• Fill out Study Completion Form
• Perform a 6-min walk
• Perform Pulmonary function test with DLCO
• Perform echocardiography

Timing of the end-of study drug visit
The final follow-up visit must occur within a 7 day window of the designated time point from the Baseline Visit. If the subject has a visit which is outside of this time window, it will be considered a protocol violation.

Scheduling and preparing for the end-of study drug visit
The visit may take place over two days. During this time, the subject must have chemistries and biomarkers taken, a 6 minute walk distance, an echo and history taken and a brief physical examination.

The Research Coordinator will call the patient 1-2 days before the final Follow-up Visit to remind him or her where he or she goes for the study visit.

Sequence of procedures
This sequence of steps is followed for all subjects by the Research Coordinator:
• Perform labs and final biomarkers
• Accompany the patient to the six minute walk test
• Take patient to Echo lab for echocardiogram
• Perform a detailed history and physical examination
• Assess side effects and adverse events
• Determine WHO functional class
• Fill out Study Completion Form
• Thank the subject for his or her participation

New symptoms (or changes in old symptoms) should be recorded. New medical diagnoses should be recorded as adverse events using the Adverse Event Form or Serious Adverse Event Form as appropriate. The Research Coordinator asks the subject about the presence of all of the symptoms listed on the Symptoms Assessment Form and completes the form. The Research

Coordinator asks for the patient's assessment of his/her clinical status in reference to the previous visit. The Research Coordinator or Investigator examines the subject. Vital signs should be recorded on the Vital Signs/Physical Examination Form. The Research Coordinator should measure the subject's height and weight with his/her shoes off and record the weight rounded to the nearest whole kilogram and the height rounded to the nearest whole centimeter. Attempt to use the same scale for the subject for the entire study. The Research Coordinator or Investigator makes an assessment of the patient's clinical status since that last visit. The Investigator should assess and record the WHO functional class.

Either one of PI's signs the Study Completion Form.

The patient should be told that the therapy portion of the study has concluded. If the patient desires to take FK506 for treatment of PAH, the Research Coordinator should refer the patient to his/her PAH physician. As FK506 is an FDA approved drug yet not approved for the use in PAH, the physician can prescribe the medication as off-label use. If asked for results from study testing or the identity of the study drugs administered, the study staff should explain that all drug assignments and study results (except clinical laboratories) are kept masked for the integrity of the study.

The Research Coordinator should make sure the subject has had all of his/her questions answered, that he/she has the correct contact information for the study team, and that he/she understands how to proceed.

The Research Coordinator checks the clinical laboratory results, completes the Laboratory Tests Form and notifies the patient's PAH physician and primary care physician (by fax) of the results.

The Research Coordinator will also call the subject after the visit to discuss whether any actions regarding the study medications or other medications are necessary based on the results. The Research Coordinator should enter all of the CRFs from the visit into the study binder within 5 business days of the visit.

After Visit #4 (week 16) or premature discontinuation of one or both study drugs, the Research Coordinator will send a letter to the patient's PAH physician (if different from the PI's) and primary care provider to inform them that the patient is no longer on study drug and to thank them for their cooperation throughout the study period. The Research Coordinator should thank the participant for his/her participation.

Visit windows, reminders, late/missed visits

Timing
There are several important visit windows which must be adhered to in the conduct of the trial.

Baseline Visit

This visit must occur between 0 and 30 days from the screening visit. This tight window must be adhered to as 1) The baseline visit must be within 30 days, since the inclusion criteria must hold at the time of study entry.

Remaining study visits
Other study visits may be conducted within ± 7 days of the scheduled time point of assessment (4 weeks, 8 weeks, and 16 weeks). The End-of Study drug visit (weeks 18) can be conducted within ± 14 days. The reference date for scheduling all visits is the date of the Baseline Visit (i.e., visits are scheduled 28, 56, and 112 days (± 7 days) from the baseline visit date).

Reminders
The Research Coordinator will send reminders by mail to subjects during the course of the trial (See MOP Appendix E). The Research Coordinator will call the patient 1-2 days before each scheduled visit to remind the patient to attend the study visit,

Late and missed visits
Despite the windows established for each study visit, it is possible that subjects may either come late for one of the scheduled assessments or miss the visit entirely. We will try to avoid this by emphasizing to the study subjects the importance of complying with all scheduled study visits. If this happens, the Research Coordinator will complete a Missed Visit Form. The Research Coordinator will contact the subject by phone. If this fails, we will send two letters by Fed Ex, one week apart. We will attempt to have the patient come to center as soon as possible for 1) study assessments and 2) replenishment of study drug. The data for these visits will be input into the Study Binder into the next assessment in the study sequence.

Methods to increase compliance
We will attempt to maximize compliance with study methods with phone calls before each visit.

Chapter 6: Safety and Outcome Assessment

Adverse Events
Definitions
Adverse Event (AE) **-** Any untoward medical occurrence in a patient, including worsening of existing conditions, that occurred during treatment and which may or may not have a causal relationship with the treatment. An adverse event is any unfavorable or unintended medical occurrence that is temporally associated with the study treatment, regardless of whether it is thought to have a causal relationship with the treatment. Temporal association with treatment means that the adverse event occurred after treatment was started, or either when or soon after it was stopped. Examples include the following:
• Sign or symptom (e.g., headache, dizziness, bleeding)
• Illness (e.g., flu)
• Abnormal laboratory findings
• Concurrent illness or accident

• Increase in severity or frequency of a medical condition occurring during the trial
• Increase in severity or frequency of signs or symptoms of a pre-existing illness

Serious Adverse Event (SAE) **-** Any adverse experience that results in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital abnormality/birth defect. An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Major bleeding would be considered a serious adverse event by this definition.

Unexpected serious adverse event
A serious event which has not been described in the package insert for aspirin or simvastatin, this protocol, or the informed consent document.

Reporting of adverse events and serious adverse events
For each identified adverse event, the Research Coordinator will complete an Adverse Event Form. Within 1 working day of learning of an SAE, the Research Coordinator and Investigator complete the AE Form and the Research Coordinator enters the Form into the Web-based database. Also within 1 working day, the Research Coordinator and Investigator complete the SAE Form (paper only) and then fax or email the Form to Chair of the DSMB. The Research Coordinator submits the SAE form and pertinent records to his/her IRB within 48 hours if the SAE is unexpected.

If a participant dies, the Investigator and Research Coordinator complete the Death Report Form, an Adverse Event Form, and a Serious Adverse Event Form and submit these to the DSMB within 1 working day of learning of the death. The Death Report Form and Adverse Event Form should be completed on paper and then submitted using the web-based database; The Serious Adverse Event Form is complete on paper, and then this form is faxed to the Chair of the DSMB. The PIs will prepare summary reports of adverse events to be distributed to the IRB yearly. PI's will be responsible for adhering to the policies of the IRB and for accurate documentation, investigation and follow-up of all possible study related adverse events.

<u>Drug interruption and stopping criteria</u>
Medical situations requiring permanent interruption
The occurrence of **an allergic reaction** or anaphylaxis after taking the study drugs warrants immediate and permanent interruption of the study drugs. Lung transplantation also requires permanent interruption of the study medications.
Although FK506 will be dosed in the low-immunosuppressive range, the occurrence of a **malignancy** (skin cancer, lymphoma or other solid tumors) warrants permanent discontinuation of the medication.

Posterior reversible encephalopathy syndrome (**PRES**) is a syndrome characterized by headache, confusion, seizures and visual loss. It may occur due to a number of causes, predominantly malignant hypertension, eclampsia and some medical treatments such as FK506. On MRI of the

brain, areas of edema (swelling) are seen. The symptoms tend to resolve after a period of time, although visual changes sometimes remain. While the recommendation after organ transplant is to reduce the dose or withholding the drug for a few days and then restarting it at a lower dose, as the risk of rejection without immunosuppression is taken into account, we will discontinue the drug for safety reasons if PRES is diagnosed.

In cases of severe sepsis/septic shock permanent interruption will be considered and guidance of the DSMB requested.

Of note, even with permanent discontinuation of the study medications, the patient should be strongly urged to complete all scheduled study visits and assessments, as long as considered safe by the patient's physician. After any of the these events, the Research Coordinator should complete the appropriate forms, including the Adverse Event Form with/without the Serious Adverse Event form, the Treatment Interruption Form, and the Patient Withdrawal Form (if applicable).

Medical situations not requiring permanent interruption
Other adverse and serious adverse events should not require permanent interruption. **Surgical or other invasive procedures** warrant temporary discontinuation of FK506/placebo therapy, however reinstitution of this therapy should be performed when determined to be safe from the clinician's point of view. The physician providing clinical care to the subject will assist in deciding whether the FK506 study drug should be temporarily interrupted or stopped.

Infections:

SIRS (Systemic Inflammatory Response Syndrome)	2 or more of the following criteria: - Temperature < 36 °C or > 38 °C - HR > 90 - RR > 20 or PaCO 2 < 32 - WBC > 12000 < 4000, or > 10% immature (band) forms
Sepsis	Documented infection together with 2 or more SIRS criteria above.
Severe Sepsis	Sepsis associated with organ dysfunction.
Septic Shock	Sepsis with refractory hypotension or hypoperfusion abnormalities in spite of adequate fluid resuscitation.

In case of SIRS/Sepsis/Severe Sepsis and Septic Shock, FK506 will be temporarily discontinued and only restarted if the infection has resolved and with the guidance of the DSMB.

Line Infections: Many PAH patients are on intravenous prostacyclin therapy with an inhered risk of infection. Some patients already have a history of line infections. A recommended diagnostic and treatment algorithm for catheter-related infections will be followed (Mermel et al. Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection: 2009 Update by the Infectious Diseases Society of America (IDSA) Clinical

Infectious Diseases 2009; 49:1–45).

Figure 1: Diagnostic Algorithm

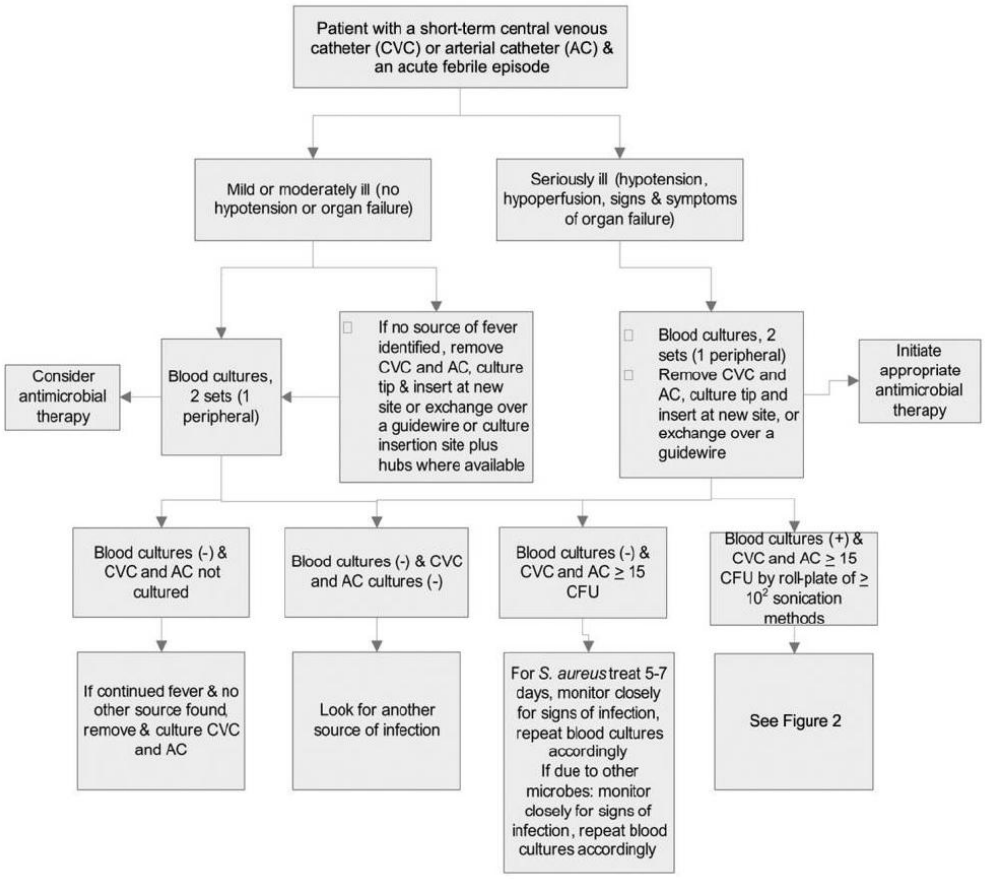
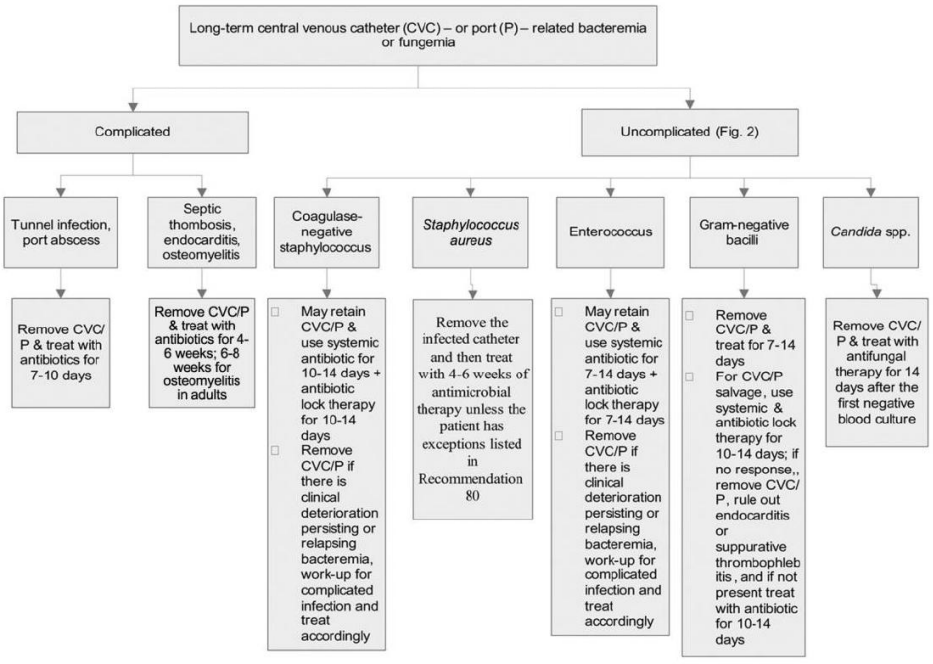


Figure 2: Treatment Algorithm



If a bloodstream infection, tunnel infection or pocket infection is present, FK506 will be temporarily discontinued until the infection is resolved with appropriate antibiotic treatment (see above) and most likely the removal of the catheter. An uncomplicated blood stream infection is defined as: fever which resolves in 72h, no intravascular hardware, no evidence of endocarditis or suppurative thrombophlebitis and for *S. aureus* is without active malignancy or immunosuppression.

Table 4. Commonly used clinical definitions of intravascular catheter-related infections.

Infection	Definition
Catheter colonization	Significant growth of ⩾1 microorganism in a quantitative or semiquantitative culture of the catheter tip, subcutaneous catheter segment, or catheter hub
Phlebitis	Induration or erythema, warmth, and pain or tenderness along the tract of a catheterized or recently catheterized vein
Exit site infection	
Microbiological	Exudate at catheter exit site yields a microorganism with or without concomitant bloodstream infection
Clinical	Erythema, induration, and/or tenderness within 2 cm of the catheter exit site; may be associated with other signs and symptoms of infection, such as fever or purulent drainage emerging from the exit site, with or without concomitant bloodstream infection[a]
Tunnel infection	Tenderness, erythema, and/or induration >2 cm from the catheter exit site, along the subcutaneous tract of a tunneled catheter (e.g., Hickman or Broviac catheter), with or without concomitant bloodstream infection[a]
Pocket infection	Infected fluid in the subcutaneous pocket of a totally implanted intravascular device; often associated with tenderness, erythema, and/or induration over the pocket; spontaneous rupture and drainage, or necrosis of the overlying skin, with or without concomitant bloodstream infection[a]
Bloodstream infection	
Infusate related	Concordant growth of a microorganism from infusate and cultures of percutaneously obtained blood cultures with no other identifiable source of infection
Catheter related	Bacteremia or fungemia in a patient who has an intravascular device and >1 positive blood culture result obtained from the peripheral vein, clinical manifestations of infection (e.g., fever, chills, and/or hypotension), and no apparent source for bloodstream infection (with the exception of the catheter). One of the following should be present: a positive result of semiquantitative (>15 cfu per catheter segment) or quantitative (>10^2 cfu per catheter segment) catheter culture, whereby the same organism (species) is isolated from a catheter segment and a peripheral blood culture; simultaneous quantitative cultures of blood with a ratio of >3:1 cfu/mL of blood (catheter vs. peripheral blood); differential time to positivity (growth in a culture of blood obtained through a catheter hub is detected by an automated blood culture system at least 2 h earlier than a culture of simultaneously drawn peripheral blood of equal volume). Note that this definition differs from the definition of central line–associated bloodstream infection used for infection-control surveillance activities.

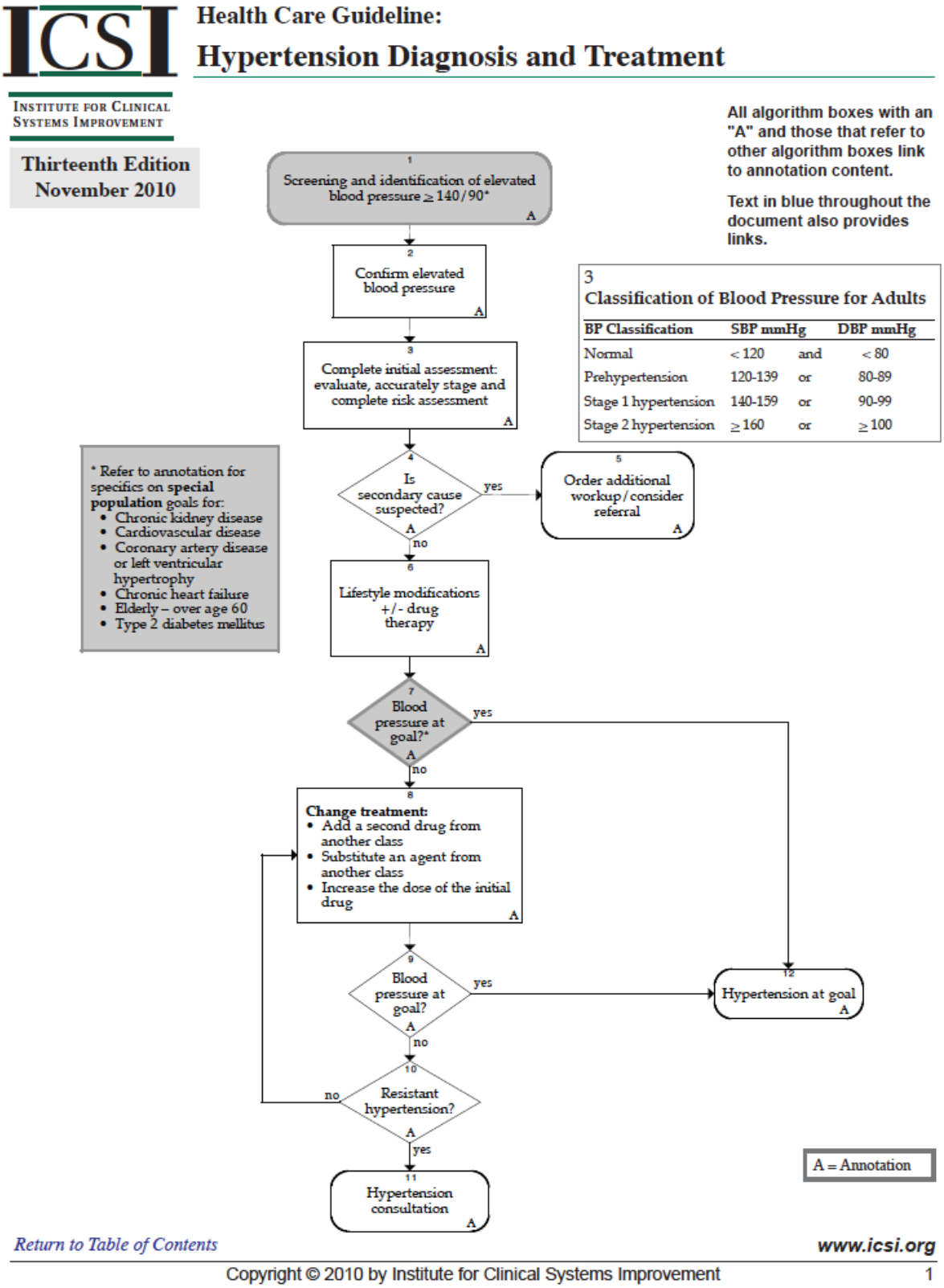
NOTE. Adapted in part from Pearson [18]. cfu, colony forming units.

[a] For surveillance purposes, patients with positive results of blood culture would be classified as having central line–associated bloodstream infection.

If just a phlebitis or exit site infection without fever or concomitant bloodstream infection is present, a culture swab will be taken and patients will be treated with antibiotics. FK506 will not be discontinued in this situation.

Hypertension: General guideline to treat hypertension will be followed. If the blood pressure is measured > 140/90 and confirmed once, it will be assessed whether other causes than the initiation of therapy with FK506 are suspected for the increased BP. If no, then lifestyle modification (reinforcement of sodium reduced diet) as well as an antihypertensive therapy will be initated. As an increased BP as a potential side effect of FK506 can be very well treated, we

would not discontinue the study medication unless malignant hypertension develops.



Neurological Symptoms such as numbness, tingling, tremor, insomia:
Neurological symptoms have been described in patients taking FK506, yet they are often related to the FK506 dose. As we use a low dose FK506 treatment in our study, we don't anticipate very frequent neurological symptoms. In the event we will discuss with the patients about the tolerability of the symptoms and will decide on a case by case basis as to whether to discontinue the study medication or not.

Pregnancy: Although patients are only included that use two barrier contraception, the study drug will not permanently interrupted should a pregnancy occur.

Serious adverse events which are not deemed to be drug-related will not necessarily be indications for drug interruption. For example, clinical **worsening of PAH** requiring hospital admission for intravenous diuretic medications may not warrant cessation study drug, despite being a serious adverse event. The Research Coordinator should complete an Adverse Event Form and a Serious Adverse Event Form within 1 business day for all such events. If the Serious Adverse Event is unexpected, the Research Coordinator submits the Forms and pertinent records to his/her IRB within 2 business days.

9.3.3 Study drug management after laboratory abnormalities
In the event of a laboratory abnormality, follow-up laboratories may be performed locally, if more convenient for the patient. All laboratory abnormalities should be considered adverse events, and the Adverse Event Form (See Appendix C) should be completed by the Research Coordinator with each episode.

9.3.3.1 Creatinine
Patients who develop increased creatinine levels (> 30% baseline resulting in a Cr level above 1.0) will be asked to come in for a clinic visit. Although given at a low dose, FK506 could worsen renal function. Yet, an increase in creatinine could also be associated with worsening pulmonary hypertension or overdiuresis. At the visit, an echocardiography will be performed to assess the RV function and fluid status. In addition the elevation of creatinine will be verified in the Stanford laboratory. Adjustments to the diuretic regimen will be made if appropriate. The study drug may be temporarily interrupted. Creatinine levels will be followed until the abnormalities return to normal. If persistent elevations occur over two weeks (three consecutive laboratory assessments) the FK506 drug will be permanently discontinued, if the elevations return to baseline, the study drug will be reinstituted and labs will be checked for two weeks.

9.3.3.2 Blood sugar
The use of tacrolimus has been identified as risk factors for Post Transplant Diabetes Mellitus (PTDM). Its incidence is up to 8% among a white European population and 10% in North American studies at 1 year following transplantation. The 10- and 20-year incidence of PTDM has been reported to be 15.0% and 22.0%, respectively. Increasing time after transplantation is also a risk factor. Conversion from Tac BID to Tac once daily has been shown to decrease the frequency of PTDM among stable renal transplant recipients receiving maintenance immunosuppressive therapy. We are aiming a low FK506 blood levels and will dose FK506 once

daily. So we don't anticipate that DM will be a common side effect of our study drug.

Patients will have a random blood glucose test performed at baseline, 4 weeks, 8 weeks and 16 weesk. If the random blood glucose levels are > 140 mg/dL, a fasting blood glucose test will be performed. If the blood glucose is between 70-100 mg/dL not further action is required. If you the fasting blood glucose test is 100-125mg/dL, it means that there is impaired fasting glucose, a type of prediabetes. A level of 126 mg/dL and higher is considered as diabetes. Patients will then be treated clinically according to the diabetes guideline. FK506 will not be interrupted in this event.

WBC count
Albeit rare, leukopenia is observed as a side-effect of FK506. After exclusion of other reasons for leukopenia (in specific infection), FK506 with be interrupted non-permanently when WBC count < 3000 and will be re-instituted once WBCs have recovered.

Anemia
Severe or acute anemia (Hct < 30% or an absolute change from screening > 6%) will be considered an emergent indication to interrupt treatment with FK506 study drug. Complete blood counts will be performed at reasonable intervals, based on the clinical scenario. The FK506 study drug may be re-instituted when the Hct > 30% and is stable and an evaluation has shown no evidence of active bleeding.

Potassium
FK506 my lead to hyperkalemia. Serum Potassium levels in the range of 3.5 - 5.0 mEq/L are considered normal. If Potassium Serum levels are > 5.1 mEq/L, we will recheck the Potassium level once.
- If still elevated and between 5.1 – 5.5 we will hold FK506 and check again after 3-4 days.
    - If hyperkalemia resolves then FK506 will be reinstituted.
    - If hyperkalemia has not resolved, then patient will be advised to go to the nearest ER
- If still elevated and >= 5.6, then patient will be told to go to the closed ER and FK506 will be advised to be held

Interruption of study drugs not occasioned by adverse events
Patient refusal to continue
Patients may decide at any time to stop the study therapies. The date of cessation of the study medication will be noted by the Research Coordinator using the Treatment Interruption and Reinstatement Form, and the patient will proceed as usual with the planned study assessments. If the patient wishes to partially or completely withdraw from the study, the Research Coordinator should complete the Patient Withdrawal Form (See Appendix C).

Patient compliance
The Research Coordinator will perform a pill count at each assessment and the compliance will be monitors via the MEMS monitoring system, which should give some indication of the compliance of the patient with the study therapy. The Research Coordinator will ask each patient about missed doses of study medication during the trial. The Research Coordinator will complete the Medication Compliance Form with this information.

Missed appointments
Patients may miss the scheduled study assessments. As study drug must be replenished at each study assessment, there is the possibility that the patient will run out of study drug in the event of missed visits. The Research Pharmacy will provide extra doses of study medication in each pill bottle for the eventuality that the patient may not attend the scheduled appointment within the appropriate window. While the patient still must come to the study center to replenish the medication supply, this at least ensures that the patient can continue on study drug until they can attend a study visit. If the patient cannot come to the center within the required time, he/she will have to stop study drug, until he/she can return to the center. Due to safety concerns, we will not ship medication to the subject if he or she does not attend the scheduled clinical visits.

Pill counts and MEMS showing lack of adherence to the protocol
If the patient reports missing doses since the last study visit, or the pill counts reflect one or more missed dosages, the Research Coordinator will record this on the Medication Compliance Form. The importance of compliance with the study protocol and study medications will be reinforced by the Research Coordinator at each contact.

Accidental dosing mistake
If patient accidentally takes medication twice or a different dose than prescribed, patient will need to have his/her blood checked again to document current FK506 level. According to the levels recommendations about up- or down-titrations will be made.

Interruption of therapy for planned procedures
When the subject is enrolled in the study, the Research Coordinator will notify the patient's PAH physician and primary care physician of the patient's participation in the study and the potential use of FK506. The Research Coordinator will fax or send a letter describing the study to these physicians (See MOP Appendix E).The Research Coordinator will instruct the patient at the baseline visit to tell other health care practitioners that he or she is possibly taking FK506, so that other providers may manage the subject accordingly. Planned invasive procedures may require interruption of therapy for safety or logistical reasons (i.e., NPO status). For planned dental, surgical, or diagnostic (e.g., right heart catheterization) procedures, we will leave the discontinuation of the FK506/placebo treatment arm to the discretion of the clinician performing the procedure. After the completion of the procedure (and when the caring physician feels it is safe to start FK506 therapy), the patient will restart the FK506 study drug, noting this as well in the Medication Diary. The Research Coordinator will encourage the patient (and the physician) to contact the study staff at any time with questions regarding management of either study drug. In the event of an unplanned or emergent procedure, we will again leave the decision regarding study medication in the hands of the clinician providing the clinical care of the patient. For example, an emergent abdominal surgery might require holding study medication for one week or greater. At the point when the patient has resumed taking medications by mouth, and treatment with FK506 is deemed safe by the clinician caring for the patient, the patient will restart the study medications. It should be remembered that even if the study drug is held, the patient should attend the study visits and evaluations as scheduled.

Notification requirements
The DSMB Committee should be notified about all decisions to permanently interrupt study medication. The Research Coordinator should complete the Treatment Interruption Form.

Outcomes:
Endpoint adjudication
There are several safety, tolerability, and possible efficacy endpoints for this clinical trial.

Primary Endpoints:

Safety of low-dose FK-506 in PAH

Cardiovascular death

We will define a cardiovascular death as:
1) Sudden death
or
2) Death preceded by:
a) cardiogenic shock (hypotension resulting in a failure to maintain normal renal or cerebral function for >15 minutes prior to death)
or
b) heart failure symptoms or signs requiring:
i) intravenous therapy or oxygen in the hospital or
ii) confinement to bed in the absence of secondary causes (such as systemic infection or dysfunction of intravenous or subcutaneous medication delivery devices) or alternative causes of death.

Non-cardiovascular death

A death which does not meet the criteria above will be considered a non-cardiovascular death.

Hospitalization for right-sided heart failure

A hospital admission due to right-sided heart failure will be defined as a hospitalization because of lower extremity edema or dyspnea refractory to outpatient increases in dose or frequency of diuretics or specific PAH medications.

Non-right-sided heart failure hospitalization

A hospital admission which does not meet the above criteria will be considered as a non-right heart failure hospitalizaiton.

Laboratory abnormalities

Changes in renal function and occurance of anemia, leukopenia, hyperkalemia, and hypomagnesemia will be recorded and reported.

Neurological symptoms

Occurance of rremor, tingling, numbness, insomnia, headaches, and weakness.

Systemic hypertension

As defined in previous section will be recorded and reported.

Rate of infections and infectious complications

As defined in previous section will be recorded and reported.

Rates of other unanticipated side effects

It is possible that other unanticipated side effects related to any organ system maybe observed and thus require recording and reporting. Free text section will allow reporting of a side effects based on a review of systems approach.

Secondary Objectives/Endpoints:

Combined Clinical Events/Time to Clinical Worsening @ 16 weeks:

- All cause mortality
- Transplantation
- Atrial septostomy
- Need for escalation of therapies as deemed by site investigator
- Worsening of NYHA/WHO classification by at least 1 point.
- Hospitalization for right heart failure.

Change in 6MWD at 16 weeks

Change in NT-Pro-BNP at 16 weeks

Change in Uric Acid at 16 weeks

Change in DLCO at 16 weeks

Change in novel RV parameters by transthoracic echocardiography: Change in RV size, RA size, RV function, TAPSE, RVSP

Adjudication procedures

Center procedure

The Research Coordinator and Investigators will be responsible for determining whether a safety or efficacy endpoint, or hospitalization or death has occurred. The first line of surveillance for these important end points will be the subject, who will be instructed by the Research Coordinator to report symptoms in real time to the Research Coordinator. Hospitalizations or other events should also be reported. The next line of surveillance will be the subjects' physicians, who will also be instructed to contact the study team if a substantial clinical change, hospitalization, or death has occurred. Last, the Research Coordinator or Investigator will question the subject about adverse events at each study contact (visits and telephone calls). When an adverse event is identified which includes bleeding or hospitalization, the Research Coordinator will obtain a detailed history regarding the event from the patient. This should include the time of onset, the duration, and the severity of the event. Whether hospitalization or a medical contact was required for the event should also be recorded. Any medical records resulting from the event (Emergency Room records, outpatient or inpatient records) should be

obtained by the Research Coordinator. In addition, any studies performed as a result of the event (e.g., an echocardiogram done during a hospitalization) should be obtained.
After the Research Coordinator has obtained all of the pertinent history and records, the Coordinator will black out any identifiable information (such as name, address, and medical record number) from all records. Each page of the medical records pertaining to the event should be noted with the study ID of the patient.

CCC procedure
These records will be stored by the Research Coordinator at Stanford University in a locked file cabinet in an office to which only the Coordinator has access. The Research Coordinator at Stanford University will photocopy the records after confirming that all identifiable information has been obscured and that only the study ID number identifies the records. Three copies of the records will be made and stored in a locked file cabinet.

Committee procedure
Every six months, the Stanford University Research Coordinator will distribute copies of all un-reviewed endpoint records to the Endpoint Adjudication Committee. The Chair of the Adjudication Committee will receive the records and a cover sheet for each event with fields for the study ID, date of event, Adverse Event sequence number, and major and minor bleeding, type of hospitalization, and type of death (See MOP Appendix C). The members of the Committee will be provided with the definitions of each type of event. The members of the Committee will review the records and convene by conference call or in person to adjudicate the events. The determination of the types of events will be by consensus. The Chair of the Committee will record the final determination on the cover sheet for each event. These completed cover sheets will be transmitted by fax or overnight mail to the CCC. The Committee members may return the copies of the medical records to the CCC or destroy them.

Chapter 7: Statistical Considerations

Data analysis

Primary and secondary endpoint analysis will follow the intent-to-treat principle.

Primary endpoint

The primary endpoint of the study is safety profile of FK-506 in PAH. As such, rates of side effects will be reported and compared across placebo versus intervention arms 1,2,&3. Due to the limited nature of the study and enrollment, we will undertake a qualitative reporting of side effect profile across different arms.

Secondary endpoints

While efficacy markers will be evaluated for the study, the primary aim of the project is to demonstrate safety and efficacy. As such, the study is not powered to meet efficacy parameters as outlined below. The secondary endpoints will be thus be used to inform future study design and are not intended to limit or prohibit whether future trials are indicated. A lack of secondary endpoint findings is thus non-informative for future efficacy and effectiveness studies.

TTCW @ 16 weeks

As defined previously, Time to clinical worsening (TTCW) at week 16 is a composite of multiple events. Analysis of this endpoint will be the direct comparison of combined event rate at week 16 between arms 1-4 and development of a Kaplan Meier analysis with determination of log-rank p value for event rates over course of study.

A secondary analysis is planned for similar evaluation of arm 1 (placebo) versus arms 2,3,4 combined.

Change in 6MWD at 16 weeks

Change in 6MWD from baseline to 16 weeks across all groups (1-4) will be analyzed using ANOVA with a Bonferroni post-test analysis.

Change in NT-Pro-BNP at 16 weeks

Change in NT-pro BNP from baseline to 16 weeks across all groups (1-4) will be analyzed using ANOVA with a Bonferroni post-test analysis.

Change in Uric Acid at 16 weeks

Change in Uric Acid from baseline to 16 weeks across all groups (1-4) will be analyzed using ANOVA with a Bonferroni post-test analysis.

Change in Percent predicted Diffusing Capacity for CO adjusted form hemoglobin at 16 weeks

Change in DLCOadj Hgb from baseline to 16 weeks across all groups (1-4) will be analyzed using ANOVA with a Bonferroni post-test analysis.

Change in novel RV parameters by transthoracic echocardiography: Change in RV size, RA size, RV function, and TAPSE.

RV Size, RA size, and TAPSE will be handled as continuous variables. Change in each specific parameter from baseline to 16 weeks across all groups (1-4) will be analyzed using ANOVA with a Bonferroni post-test analysis.

Changes in RV function will be determined by echocardiographer and subjectively reported over all 4 arms.

Chapter 8: Participant Safety and Confidentiality

Informed consent process

The Research Coordinator performs the formal process of obtaining informed consent, if the patient meets the inclusion and exclusion criteria (other than laboratory results which are checked over the next week).

Informed consent requires:

1) Disclosure of relevant information to prospective participants about the research;
2) The participant's comprehension of the information; and
3) The participant's voluntary agreement to research participation without coercion or undue influence.

Informed consent is a process that involves:

1) Providing patients with adequate information concerning the study procedures and scope;
2) Providing adequate opportunity for the patient to consider all available options;
3) Responding to the patient's questions and concerns;
4) Ensuring that each patient understands all information provided; and
5) Obtaining the patient's written voluntary consent to participate.

If the patient appears eligible from initial review of records, before the potential subject is given the consent form, the Research Coordinator or Study Investigators will discuss the nature of the study, randomization, blinding, study procedures, the importance of compliance with study procedures, potential risks and benefits, and the duration of the study. The potential subject should be told that he/she is not obligated to participate, that there will be no penalty for declining to participate, and that treatment will not be compromised if the patient does not participate or ceases participation at any time.

The potential subject should be told that even if study drug is withdrawn (or the subject requests that he/she be withdrawn from the study medication), the completion of the study visits and assessments as scheduled will be strongly recommended. The patient should be reminded that he or she is free to discontinue his/her participation in any portion of (or the entire) clinical trial at any point. Standardized recruitment text is provided below:

"You are invited to participate in a research study of the safety of FK506 in pulmonary hypertension and the effect that treatment with FK506 has on your pulmonary hypertension. There are scientific reasons why this medications may be beneficial for patients with PAH, but we don't know whether this medication helps patients with PAH or does not help. To see if this medication has a good effect and is safe, we are asking individuals who have PAH like you to join our study. I will go over what would be required to give you an idea about what to expect if you decide to participate.

It is important that you fully understand everything about the study before we go on to sign the agreement form, so if you have any questions please feel free to ask them and I will be more than happy to answer them.

I will go over the list of your medical problems and will take note of all the medications that you are taking on a regular basis. If it appears that you would be eligible for this study, you will read and sign an agreement to participate (informed consent form), a HIPAA (privacy) form. After that, you will have about 2 tablespoons of blood withdrawn for your first laboratory tests. You will be contacted by phone in the next day or two to tell you about your test results and, if they look okay for inclusion in the study, we will schedule a second visit, which must be less than 1 months from your visit today.

During the 16 weeks that you will be on the medication you will be seen in the pulmonary hypertension clinic in a routine fashion on a monthly basis and will have routine testing done as required by your pulmonary hypertension state. You will have monthly blood testing done to make sure your kidney tests remain normal while on either of the medications. During time of enrollment in the study, you will receive the same care, evaluation, and follow up of your pulmonary hypertension as you would have if you were not participating in the study. The study will not interfere with any needs that you may have for increasing or adding other medications.

You will be asked to undergo echocardiography and Pulmonary Function testing at the beginning and end of the study.

Blood collected during this study will be sent to our research laboratory and saved for future testing in accordance with the protocol of this study.

As patients metabolize FK506 differently, we will measure FK506 levels in your blood every 4 days until a steady-state blood level is achieved. Subsequently FK506 blood levels will be drawn every 2 weeks. We have made arrangements with Labcorp, who will draw your blood and determine the FK506 level. We will be notified about the levels and one of our physicians will determine a dose adjustment if necessary.

We may contact you about future studies that may be of interest to you.

You will come back here in 4 weeks, 8 weeks, 16 and 18 weeks and perform the tests described above and we will refill your pills during these visits. You will also receive a call from one of us at week 2, week 6, week 6, week 10 and week 12 to make sure you are doing well and not having any problems. Your final visit will be 16 18 weeks after the study begins when you will again go over the list of your medical problems with one of us and will tell us how you are feeling compared to when you first started the trial.

You should call us if you are having problems which may or may not be related to the study medicine.

It is possible for any drug to cause side effects. You need to know about the side effects that could occur in this study before you agree to take part. In addition to the risks below, there may

be risks that are currently unknown. If further risks are identified during the study, you will be told about them in a timely manner.

Note that all risks and side effects described below are in the context of Tacrolimus taken at a higher dose than used in this study. Tacrolimus is usually prescribed as an immunosuppressant after transplantation or to treat autoimmune diseases, which requires up to 3-10 times the dose of Tacrolimus that will be used in this study (depending on the treatment arm).

What is the most important information I should know about Tacrolimus?

Tacrolimus can cause serious side effects, including:

1. **Increased risk of cancer.** People who take Tacrolimus have an increased risk of getting cancer, including skin and lymph gland cancer (lymphoma). Most data on the risk of developing malignancies with Tacrolimus is available from patients on immunosuppressive therapy after organ transplantation.
   Studies have shown that in the first 10 years after transplantation the risk of developing **skin cancer** is around 14%. As we aim for low immunosuppressive Tacrolimus levels in our trial, as well as a short clinical trial duration of only 4 months, we would expect the risk to develop skin cancers to be much less. As usual for patients with increased risk for skin cancer, exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen with a high protection factor.
   The risk of **lymphoma** with Tacrolimus after transplantation depends on the type of the transplanted organ and appears to be related to the intensity and duration of the immunosuppression. The risk is lowest following renal transplantation (1%–2.3%), moderate after heart transplantation (1%–6.3%) and highest after lung transplantation (4.2%–10%), which requires the highest immunosuppression. As we aim for sub-immunosuppressive Tacrolimus levels in our trial, we would expect the risk to develop lymphomas to be < 1%

1. **Increased risk of Infection.** Tacrolimus is a medicine that affects the immune system. Tacrolimus can lower the ability of your immune system to fight infections. Serious infections can happen in people receiving Tacrolimus that can cause death. Call you doctor right away if you have symptoms of any infection such as:
   - Fever
   - sweats or chills
   - cough of flu-like symptoms
   - muscle aches
   - warm, red, or painful areas on your skin

What are the possible side effects of Tacrolimus?

- High blood sugar (diabetes):
- Kidney problems
- Nervous system problems
- High levels of potassium in your blood
- High blood pressure

- Heart problems (myocardial hypertrophy)

The most common side effects of Tacrolimus in people receiving liver transplants are:

- Tremors
- Headache
- Diarrhea
- High blood pressure
- Nausea
- Kidney problems
- Stomach pain
- Trouble sleeping
- Numbness or tingling in hands or feet
- Anemia
- Pain
- Fever
- Weakness
- High level of potassium in the blood
- Low level of magnesium in the blood

The most common side effects of Tacrolimus in people receiving heart transplants are:

- Kidney problems
- High blood pressure

RISKS TO PREGNANCY AND BREASTFEEDING:

The risks to the unborn fetus and newborn from tacrolimus are not known. Tacrolimus has been used during pregnancy in a limited number of cases, although neonatal hyperkalemia and renal dysfunction have been reported. Because tacrolimus may be secreted in breast milk, a nursing infant may also be exposed. Women who are pregnant or nursing a child may not participate in this trial. If you become pregnant during the trial, your therapy will be discontinued. It is necessary that every effort be made to avoid the possibility of pregnancy or fathering a child while receiving tacrolimus and for a minimum of 30 days after treatment. Women of child-bearing potential must have a negative pregnancy test prior to each month while in trial. It is important that individuals enrolled in this study who are of child-bearing potential (i.e. not post-menopausal or surgically sterile) use 2 medically acceptable form of birth control before beginning treatment. The study doctor will discuss appropriate birth control measures with you. If you suspect that you (or your partner) have become pregnant during the trial, you must notify the study doctor immediately.

<u>Other:</u>

As with all drugs, an allergic reaction as well as unexpected or yet unknown side effects may occur. Dr. Spiekerkoetter or Dr. Zamanian will tell you any new information that becomes known during the study. This information may affect your desire to continue participation in the study.

If you have any new symptoms or the symptoms you have now become worse, tell Dr. Spiekerkoetter or Dr. Zamanian or study coordinator as soon as possible.

Since we will be drawing several blood samples from you, there is a risk that you will develop minor bleeding and/or bruising. You may have done a six minute walk test before; this is a test where you will be asked to walk as far as you can during six minutes. While doing so, you may feel light-headed or other symptoms, however the risk of this test is minimal. You will receive an echocardiography and a Pulmonary Function Test at Baseline and after 16 weeks follow-up, which are test routinely performed in the Pulmonary Hypertension Clinic.

I would also like to tell you that even if you choose to participate in the study, you are free to decide to stop your participation at any time. Even if you have to stop the study medications, we will ask that you keep your follow-up visits with us as scheduled. Remember that the decision to participate is totally yours to make, and there are no penalties for not wanting to participate. Your medical care will not be affected if you do not join our study, and your medical care will not be affected if you decide to pull out from the study at any point in time.

During the study, you should continue your current PAH medications and/or can add new medications if your doctor determines this to be necessary.

Take enough time to go over what we have just discussed as well as the consent form and if you come up with any questions, I am here to answer them for you. If for any reason you are not able to read the consent, I will read it aloud to you.

We also have a HIPAA form for you to review. Signing this form allows us access to your medical records and other Protected Health Information, including information which could identify you, which is created by a healthcare provider, and relates to your past, present, or future health."

Potential subjects must then be given ample time to read and understand the consent form and to ask questions. If the potential subject cannot read for any reason, clinic staff must read the consent aloud to him or her.

After the subject (and the Research Coordinator and PI) signs the consent form and the HIPAA form, a copy is given to the patient for his/her records. The signed original and a second signed copy of all forms are kept by the Research Coordinator in a locked cabinet with other confidential patient information in a locked office.

Informed consent document

The approved informed consent documents are provided (Appendix B). If there is a change in any of the study procedures which may affect the participant, before institution of the procedural changes, both informed consent forms must be revised in an identical fashion, resubmitted to the local IRB, and approved at the IRB.

The Research Coordinator should be notified with all proposed changes to the consent form and will ensure that the consent forms remain consistent.

When a consent form is revised, all patients who are active in the treatment portion (between the Baseline Visit and ~~Visit #4~~ Week 18 Visit ~~(fourth month))~~ of the study must re-sign the revised form after IRB approval.

HIPAA requirements

The Health Insurance Portability and Accountability Act (HIPAA) requires that all research collecting identifiable health information on an individual person be in compliance with HIPAA standards and regulations. HIPAA regulations specifically apply to research studies collecting Protected Health Information (PHI).

PHI is defined by HIPAA as health information transmitted or maintained in any form or medium that:

1. Identifies or could be used to identify an individual; and
2. Is created or received by a healthcare provider, health plan or employer; and
3. Relates to past, present or future physical or mental health or condition of an individual.

Given that this study will obtain subjects' PHI, we must comply with the HIPAA regulations as they relate to research.

Compliance will require that each subject read and sign a form entitled, "HIPAA Authorization to Use and Disclose Individual Health Information for Research Purposes." This completed document must be submitted to each site's IRB for the purposes of fulfilling HIPAA regulations.

Following the informed consent process, each subject must read and sign the site specific HIPAA Authorization. Subjects must be given a copy of the signed authorization.

Data and Safety Monitoring Board

We have assembled a Data and Safety Monitoring Board (DSMB) to monitor the safety of all participants involved in the study and to ensure the validity and integrity of the data. DSMB activities are established in order to protect the safety of human participants and maintain and ensure the scientific integrity of the study. DSMB activities include a review of the protocol with emphasis on data integrity and patient safety issues, monitoring of adverse events, protection of the confidentiality of the data and monitoring results, and recommendations to the Principal Investigator regarding continuation or conclusion of a study.

A description of the roles and responsibilities of the Safety Officer and/or DSMB includes the following:

- review protocol, informed consent documents, plans for data safety and monitoring;
- evaluate study progress, including periodic assessments of data quality, participant recruitment and retention, participant risk versus benefit, site performance, and other factors that may affect study outcome;
- consider external factors such as scientific or therapeutic developments that may impact on the safety of the participants or the ethics of the trial;
- protect the safety and scientific progress of the trial;
- make recommendations to the Principal Investigator regarding continuation, termination, or other modifications to the study based on observed beneficial or adverse effects of the treatment under study;

- ensure data integrity;
- ensure the confidentiality of the trial data and the monitoring results; and
- assist the PI by providing recommendations on any problems with study conduct, enrollment, sample size, and/or data collection.

DSMB Members
Steven M. Kawut, MD, MS
Stephen C. Mathai, MD, MHS
Benjamin A. Goldstein, Ph.D

The three independent members are appointed for the length of the project.

19.2 Generic monitoring plans
The Chair will develop the DSMB charter, meeting agenda, request information for the meetings from the PI's and the study statistician, oversee the meetings and prepare reports and recommendations that are an accurate and complete record of the Board's deliberations. The primary responsibility of the DSMB will be to monitor the progress of the study and recommend modifying the study as appropriate. Concerns that might dictate modification or terminations of the study include patient safety, data quality, integrity, recruitment, and performance. To meet this responsibility, the DSMB members will familiarize themselves with the research protocol and consent forms, review interim reports of adverse incidents, review interim reports of trial participant accrual, review reports from related studies to determine whether those results indicate the need to change or terminate the present trial, and make recommendations to the investigators concerning continuation, termination, or modification of the trial. The PI's, study statistician and study staff will prepare the interim reports issued to the DSMB. Those reports prepared by the PI's and study staff include: reports of adverse events, reports of trial participant recruitment and follow-up, and reports from related studies. This Board will convene after the randomization of every eight subjects by conference call and more often if warranted by safety considerations. The first meeting will evaluate the clinical trial protocol and establish guidelines for monitoring the study. Subsequent meetings will be for the purpose of data and safety monitoring. All reports to be discussed at the meeting will be mailed to all Board members at least one week prior to the meeting. The format of the meetings consists of an open session, a closed session, and an executive session. Trial investigators, trial staff, staff of the institution may attend the open session, during which unblinded data will not be discussed. Issues to be discussed at the open session include reports of adverse events, recruitment progress, reports from related studies, proposed major modifications to the study protocol prior to implementation, and informed consent procedures. Voting members of the board and the study biostatistician (if invited) may attend the portions of the closed sessions. All discussions during these sessions will be deemed confidential. Issues to be discussed in the closed session will include performance. The executive session will only include the members of the DSMB. At the end of each meeting, the DSMB will make a recommendations regarding the status of the trial, specifically whether the trial should continue, whether the protocol should be modified, whether the frequency of meetings should be changed, and other issues. Between meetings of the DSMB, information regarding issues deemed critical to the trial or participants' safety will be provided by the PI's. As a result of receiving this critical information, a meeting to discuss the critical information

may be convened. Critical information includes: increases in morbidity or mortality potentially ascribable to the study interventions, serious adverse reactions potentially ascribable to the study intervention, suspicion of scientific fraud or misconduct, or any other issues which may warrant protocol changes or modifications. The local IRBs will perform supervision of the study site, requiring submission of all serious adverse events and reviewing the reports of the DSMB meetings. All patients will be assessed for toxicity and included in the safety analysis. This analysis will include summaries of the incidence and grade of toxicities. Safety interim analyses will be performed and reported at each DSMB meeting.

Study compliance

Objectives and general considerations

The objectives of the TrANsFoRM study compliance policy are:
• to protect subjects;
• to preserve the study's scientific integrity; and
• to identify problems and correct them quickly.

This policy is founded on the presumption that all study personnel want to conduct the study in accordance with accepted ethical and scientific standards. Therefore, these compliance procedures are not punitive toward study staff unless there is a clear pattern of violations that persists despite repeated attempts to correct the underlying problems.

Accordingly, the general approach to protocol violations is 1) to prevent them by thorough training and careful procedures, and 2) to detect and correct problems so as to prevent future violations.

Types of protocol violations

The following list gives the most serious and most likely protocol violations, although other violations are possible:
(a) Failing to obtain signed informed consent from a subject.
(b) Failing to obtain signed HIPAA Authorization of Disclosure.
(c) Breaching subject confidentiality.
(d) Failing to keep Institutional Review Board (IRB) approval up to date.
(e) Administering the wrong therapy to a subject.
(f) Randomizing ineligible subjects. The most likely instance is an error in assessing or recording medical conditions or concurrent use of medications that make the subject ineligible.

Items (a), (b) and (c) are grave ethical violations. Item (d) does not necessarily indicate that any subject's rights have been violated but it does point to a serious administrative failure. Items (a), (b), (c) or (d) are sufficiently serious that if they persist or are extensive in nature, the entire Study could be terminated. If these problems are extensive and severe enough, they could result in criminal or civil liability.

Items (e) and (f) are concerns mainly because they endanger the scientific integrity of the study.

Item (e) corrupts the randomization, which is the basis for attributing the study's result to the treatment. Item (f) tends to dilute any treatment effect that may be present. Also, if either of these violations is detected, it may be necessary to exclude the affected subjects from any analyses of study data, which reduces the study's power to detect treatment effects.

Procedures

How violations are detected

Routine data processing at the DCC will uncover some violations. The PI's monthly monitoring will detect consent irregularities, unsafe confidentiality practices and subjects who are ineligible because of errors in assessing or recording relevant medical conditions.

Site monitoring by the CCC Research Coordinator and the Chair of the DCC will also detect consent irregularities, unsafe confidentiality practices and, to a lesser extent, subjects who are ineligible because of errors in assessing or recording relevant medical conditions.

If IRB approval has been allowed to lapse at a Site, the affected IRB will contact the Site's PI. If protocol changes have been made at either center requiring new IRB approval, the Site's PI is responsible for obtaining this approval and site monitoring by the CCC Research Coordinator will include checking that the study has current approval from the IRB.

Procedure upon detecting a violation

If a protocol violation is detected, the person detecting the violation should report it the same day to the PI's and the Chair of the DCC and record the event on the Protocol Violation Log (See Appendix F). The CCC Research Coordinator maintains a log of violations.

Based on the severity of the violation, the PI's, and Research Coordinator will have a conference call with the DSMB Chair and any other appropriate personnel. The conference call's purpose is to identify procedural weaknesses that may have caused the violation and to propose corrective measures. The PI's then record the likely cause of the violation and any proposed corrective measures. This memo is copied to the Research Coordinator and the DCC Chair. It is then the PIs' responsibility to ensure that the corrective measures are implemented and, if unsuccessful, that further corrective measures are identified and implemented.

Procedure in case of a persistent problem

Any member may call for a conference call to consider a persistent problem. If the EC determines that its intervention is required, the EC will identify the problem or problems and propose and implement solutions. Solutions could range from ordering specific study personnel to be retrained or terminated from the study to terminating enrollment at a specific Site, depending on the severity and chronicity of the violation.

A problem that reaches this stage will generally be of such gravity as to present a clear and present danger to the study's completion, requiring immediate and drastic action.

Are you participating in any **other** research studies? _____ yes _____ no

PURPOSE OF THIS CONSENT FORM

Please read this consent form and ask as many questions as needed. You should not sign this form if you have any questions that have not been answered to your satisfaction.

You should understand the purpose of the study, how your participation may help you, any potential risks to you, and what is expected of you during the study.

Voluntary Participation

Your participation in this study is entirely voluntary. Your decision not to participate will not have any negative effect on you or your medical care. You can decide to participate now, but withdraw your consent later and stop being in the study without any loss of benefits or medical care to which you are entitled

INTRODUCTION TO THE STUDY

You are invited to participate in this study because you have pulmonary hypertension (PH) and are currently treated with one or multiple drugs for PH such as **phosphodiesterase type 5 inhibitors** (PDE-5 inhibitors) (sildenafil, tadalafil), prostacyclins (Flolan, Remodulin, Iloprost) and/or the endothelin antagonist Ambrisentan. While all these drugs are effective as vasodilators, new medications are sought that could reverse the cellular growth that occluds blood vessel in the lungs in PH. It is known that a certain genetic pathway, the **Bone morphogenetic protein receptor type II** (BMPR2) pathway, is impaired in PH. Animal studies done here at Stanford have shown that the immunosuppressive drug Tacrolimus (FK-506) activates the BMPR2 pathway, preventing and reversing pulmonary hypertension in animal models of PH.

PURPOSE OF THE STUDY

The purpose of this study is to test whether adding Tacrolimus to your PH treatment at a dose below the normal dose that is used for immunosuppression is safe and whether it will improve your pulmonary hypertension. Your heart function & PH symptoms will be assessed by cardiac echo, 6-min walk and the biomarker brain natriuretic peptide (NT-proBNP).

FUNDING OF THE STUDY:

The Study is sponsored by a grant from **Spectrum**, the Stanford Center for Clinical and Translational Education and Research, an initiative from the Stanford School of Medicine that supports translational research to generate new drugs and treatments (National Institutes of Health (NIH)CTSA award UL1 RR025744) as well as from a seed grant from the **Vera Moulton Wall Center** for cardiopulmonary disease at Stanford.

DESIGN OF THE STUDY

This study is open to adult PH patients who are on any PH medication except Tracleer (bosentan). Patients on Tracleer are not eligible to enroll in this study because Tacrolimus increases Tracleer levels. The study drug will be given in addition to your baseline PH therapy. This study is only available at Stanford. We hope to enroll 40 subjects.

You will be allocated to one of the drug options through a process called randomization. Randomization means that the study drug you will receive is selected by chance (like the flip of a coin). The study drug options for this study are placebo (a pill which contains no active ingredients), and 3 different doses of Tacrolimus (goal blood levels: < 2.0, 2.0-3.0, and 3.0-5.0 ng/ml. The randomization for this study is 1:3 which means you have a 75% chance of receiving treatment with Tacrolimus.

Patients on Tacrolimus after transplantation have goal blood level of 5-15 ng/ml).

STUDY MEDICATION

Tacrolimus is an Food and Drug Administration (FDA)-approved immunosuppressive drug used after organ transplantation and to treat autoimmune diseases. As the absorption and metabolism of Tacrolimus differs quite widely from patient to patient, dosing is directed by measuring the drug level in your blood. During the study you will have your blood drawn at a local Labcore facility. Results will be reported to Stanford and your Tacrolimus dose will be changed as needed. In this study we aim for a much lower drug level (see above) than the immunosuppressive level (5-15 ng/ml). Other lab work required for the study will be drawn at Stanford. See study time line for details.
You will receive the study drug for free for the duration of study. The drug will be delivered in a special device that allows monitoring of drug intake. This device is called a Medication Event Monitoring System (MEMS). You will be taught how to use the device.

DURATION OF THE STUDY

Your participation in the study will last for approximately 18 weeks. You will take the study medication for 16 weeks. During this time, you will be required to visit Stanford approximately 6 times.

STUDY PROCEDURES

If you agree to take part in this study, you will first sign this consent form. You will receive a copy of the consent form.

Screening

- Your medical history, including your medications and previous test results (right heart catheterization, pulmonary function tests etc.) will be reviewed.
- You will be asked about current participation in research studies.
- If you meet the study entry criteria you will be scheduled to return within 4 weeks for your baseline study visit.

Baseline Visit

- Your vital signs including height and weight will be recorded.
- Any change in medical history or medication will be noted
- You will have a physical examination
- You will have a 6 Minute Walk Test (6MWT) with Borg Dyspnea Index score (BDI score
- You will have pulmonary function tests (if not done within 4 weeks of baseline)
- You will have an echocardiograph (if not done within 4 weeks of baseline)
- Labs will be drawn at Labcore and Stanford (approximately 50 cc/ 3-4 tablespoons of blood) including a pregnancy test if you are a woman of child-bearing potential applicable.

- After all of these procedures are complete and if you qualify for the study, you will be randomized and the study drug will be dispensed.
- You will receive your MEMS medication device and you will take your first dose of study drug the morning after your baseline visit.
- We will remind you to call the study coordinator or PI promptly should you notice any side-effects

Week 2, 6, 10, 12 Telephone Contact:

- We will assess any change in your symptoms, medication use, compliance and any side - effects or other concerns you might have.

Week 4 Visit

- Your vital signs & weight will be recorded
- You will have a physical examination
- You will have the 6MWT with BDI score
- You will report to us any adverse events since the last visit
- We will review your medication compliance using the MEMS Medication device and any medication changes
- Labs will be drawn at Labcore and Stanford approximately 15cc/1 tablespoons)
- We will remind you to call the study coordinator or PI promptly should you notice any side effects

Week 8 visit

- Your vital signs & weight will be recorded
- You will have a physical examination
- You will have a 6MWT with BDI score
- You will report to us any adverse events (AEs) since the last visit
- We will review your medication compliance using the MEMS device and any medication changes
- Labs will be drawn at Labcore and Stanford approximately 15cc/1 tablespoons of blood).
- We will remind you to call the study coordinator or PI promptly should you notice any side-effects

Week 16 visit Your vital signs & weight will be recorded

- You will have a physical examination
- You will have a 6MWT with BDI
- You will have an echocardiography (part of routine clinical care)
- You will have pulmonary function tests
- We will remind you to call the study coordinator or PI promptly should you notice any side-effects

- We will review for your medication compliance using the MEMS device and any medication changes
- Labs will be drawn at Labcore and Stanford approximately 50 cc/ 3-4 tablespoons of blood).

You will stop taking the study medication at this visit

Week 18 visit (End of Study)

- Your vital signs & weight will be recorded
- You will have a physical examination
- You will have a 6MWT with BDI

You will have blood drawn at Stanford approximately 7cc/1/2 tablespoon of blood)

- You will report to us any side-effects noticed since the last visit

SUBJECT RESPONSIBILITIES

It is your responsibility to come to Stanford for all the study visits and follow instructions given to you by the study team.

If you decide to be in this study, there are certain rules you must follow before during and after the study period such as:

- If you are female and able to bear children, you must avoid becoming pregnant during this study and for 30 days afterward by using 2 methods of birth control.
- Tell the study staff all the information you know about your health and medications you take throughout the study period. If you do not tell the staff everything, you may be putting your health at risk.
- Return all the used study drug bottles and unused study drug.
- Follow all the instructions of Dr. Spiekerkoetter and Dr. Zamanian and study staff. If you are not able to follow the instructions, the doctors above may discontinue you from the study. If you are unsure about what you are supposed to do, ask Dr. Spiekerkoetter and Dr. Zamanian or a member of the study staff.

RISKS AND DISCOMFORTS

It is possible for any drug to cause side effects. You need to know about the side effects that could occur in this study before you agree to take part. In addition to the risks below, there may

be risks that are currently unknown. If further risks are identified during the study, you will be told about them in a timely manner.

Note that all risks and side effects described below are in the context of Tacrolimus taken at a higher dose than used in this study. Tacrolimus is usually prescribed as an immunosuppressant after transplantation or to treat autoimmune diseases, which requires up to 10 times the dose of Tacrolimus that will be used in this study.

What is the most important information I should know about Tacrolimus?

Tacrolimus can cause serious side effects, including:

1. **Increased risk of cancer.** People who take Tacrolimus have an increased risk of getting cancer, including skin and lymph gland cancer (lymphoma). Most data on the risk of developing malignancies with Tacrolimus is available from patients on immunosuppressive therapy after organ transplantation.
   Studies have shown that in the first 10 years after transplantation the risk of developing **skin cancer** is around 14%. As we aim for sub-immunosuppressive Tacrolimus levels in our trial, we would expect the risk to develop skin cancers to be much less. As usual for patients with increased risk for skin cancer, exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen with a high protection factor.
   The risk of **lymphoma** with Tacrolimus after transplantation depends on the type of the transplanted organ and appears to be related to the intensity and duration of the immunosuppression. The risk is lowest following renal transplantation (1%–2.3%), moderate after heart transplantation (1%–6.3%) and highest after lung transplantation (4.2%–10%), which requires the highest immunosuppression. As we aim for sub-immunosuppressive Tacrolimus levels in our trial, we would expect the risk to develop lymphomas to be < 1%

1. **Increased risk of Infection.** Tacrolimus is a medicine that affects the immune system. Tacrolimus can lower the ability of your immune system to fight infections. Serious infections can happen in people receiving Tacrolimus that can cause death. Call you doctor right away if you have symptoms of any infection such as:
   - Fever
   - sweats or chills
   - cough of flu-like symptoms
   - muscle aches
   - warm, red, or painful areas on your skin

STUDY

What are the possible side effects of Tacrolimus?

- High blood sugar (diabetes):
- Kidney problems
- Nervous system problems
- High levels of potassium in your blood
- High blood pressure
- Heart problems (myocardial hypertrophy)

The most common side effects of Tacrolimus in people receiving liver transplants are:

- Tremors
- Headache
- Diarrhea
- High blood pressure
- Nausea
- Kidney problems
- Stomach pain
- Trouble sleeping
- Numbness or tingling in hands or feet
- Anemia
- Pain
- Fever
- Weakness
- High level of potassium in the blood
- Low level of magnesium in the blood

The most common side effects of Tacrolimus in people receiving heart transplants are:

- Kidney problems
- High blood pressure

RISKS TO PREGNANCY AND BREASTFEEDING:

The risks to the unborn fetus and newborn from tacrolimus are not known. Tacrolimus has been used during pregnancy in a limited number of cases, although neonatal hyperkalemia and renal dysfunction have been reported. Because tacrolimus may be secreted in breast milk, a nursing infant may also be exposed. Women who are pregnant or nursing a child may not participate in this trial. If you become pregnant during the trial, your therapy will be discontinued. It is necessary that every effort be made to avoid the possibility of pregnancy or fathering a child

STUDY

while receiving tacrolimus and for a minimum of 30 days after treatment. Women of child-bearing potential must have a negative pregnancy test prior to each treatment with tacrolimus. It is important that individuals enrolled in this study who are of child-bearing potential (i.e. not post-menopausal or surgically sterile) use 2 medically acceptable form of birth control before beginning treatment. The study doctor will discuss appropriate birth control measures with you. If you suspect that you (or your partner) have become pregnant during the trial, you must notify the study doctor immediately.

Other:
As with all drugs, an allergic reaction as well as unexpected or yet unknown side effects may occur. Dr. Spiekerkoetter or Dr. Zamanian will tell you any new information that becomes known during the study. This information may affect your desire to continue participation in the study.

If you have any new symptoms or the symptoms you have now become worse, tell Dr. Spiekerkoetter or Dr. Zamanian or study coordinator as soon as possible.

How should I take the study drug?
- You should take the study medication in the morning at approximately the same time each day.
- You can take it with or without food, try to be consistent.
- Do **not** take the study drug with grapefruit juice as this might increase Tacrolimus levels in the blood
- The Tacrolimus blood test should be taken **before** your dose, ideally at the same time of the day.

RISKS/DISCOMFORTS OF STUDY PROCEDURES:

Echocardiography, pulmonary function tests as well as the 6-min walk are all standard of care for PH patients.

NEW FINDINGS

Dr. Spiekerkoetter and Dr. Zamanian will tell you of any information learned during the course of the study that might cause you to change your mind about taking part in the study. You may contact the doctors at any time after your participation ends to find out if any new information about this study has become available.

STUDY

POSSIBLE BENEFITS OF THE STUDY

There is no guarantee that you will receive personal benefit from participating in this study. Research studies such as this one are a way for doctors to determine if a drug is useful in treating a medical condition. By participating in this study you will help to provide information regarding the safety and efficacy of low dose FK-505 in the treatment of pulmonary hypertension. The information that you will provide may help in the treatment of PH by providing increased knowledge and information to the scientists, doctors, and the community. In addition, during your participation you will have close medical monitoring of your health condition by blood tests and other evaluations during study visits.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOU WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

PAYMENT FOR PARTICIPATION

You will not be paid for taking part in the study. You may be reimbursed for some travel expenses associated with study participation. In some cases, you may be asked to provide documentation and/or receipts. Payments may only be made to U.S. citizens, legal resident aliens, and those who have a work eligible visa.
If you decide to withdraw from the study early, you will be paid only for expenses for completed study visits.

COST OF TREATMENT

The study drug, Tacrolimus or placebo will be given to you at no cost. All study visits, professional, diagnostic, and laboratory fees that are part of this study will be provided at no cost to you. However, you or your usual health care payer will be responsible for any other health care costs related to the routine treatment of your PH and complications of the disease.

Financial Disclosure

Drs. Spiekerkoetter and Zamanian and the study staff will be paid by the grants to conduct this research study.

ALTERNATIVE TREATMENT OPTIONS

You do not have to participate in this study to receive treatment for your condition. If you choose not to take part in or decide to quit this study, you may choose another treatment for your

condition. You should talk about other treatment options with Dr. Spiekerkoetter and Dr. Zamanian. Make sure that you understand all of your choices before you decide to take part in this study.

CONFIDENTIALITY AND RELEASE OF MEDICAL RECORDS

The purpose of this research study is to obtain data or information on the safety and effectiveness of Tacrolimus in pulmonary hypertension patients; the results will be provided to the sponsor and the Food and Drug Administration and other federal and regulatory agencies as required. The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your identity will not be disclosed**.** Your identity will be kept as confidential as possible as required by law. As required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your research records may be disclosed outside of Stanford, but in this case, you will be identified only by a unique code number. Information about the code will be kept in a secure location where access is limited to research study personnel. The US Food and Drug Administration (FDA) and other regulatory or governmental agencies and the Stanford Independent Review Board (IRB) may review study subjects medical and study records. A court of law could order medical records shown to other people, but that is unlikely. Therefore, absolute confidentiality cannot be guaranteed.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed consent form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

STUDY

This research study will provide information on the safety and efficacy (how well it works) of low dose Tacrolimus in pulmonary hypertension therapy. Information gained in this study will be provided to the sponsor (Spark at Stanford, Wall Center for pulmonary vascular research at Stanford) and others as outlined below.

Do I have to sign this authorization form?
You do not have to sign this authorization form. But if you do not, you will not be able to participate in this research study or be able to receive the study medication. Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?
If you decide to participate, you are free to withdraw your authorization regarding the use and disclosure of your health information (and to discontinue any other participation in the study) at any time. After any revocation, your health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must write

to: Dr. Spiekerkoetter or Dr. Zamanian at Pulmonary and Critical Care Medicine, Stanford University, 300 Pasteur Drive, Stanford, CA 94305-5236.

What Personal Information Will Be Used or Disclosed?
Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, your medical history, information about your pulmonary hypertension, laboratory results, vital signs, your medications you are taking, and other health-related information.

The purposes for the collection and sharing of your health information include: checking your suitability to take part in the study, monitoring your treatment with

the study drug, comparing and pooling your treatment results with those of other subjects in clinical studies, supporting the development of the study drug, and supporting the licensing application for regulatory approval of the study drug anywhere in the world.

Who May Use or Disclose the Information?
The following parties are authorized to use and/or disclose your health information in connection with this research study:

- The Protocol Director, Dr. Spiekerkoetter
- The Protocol Co-Director, Dr. Zamanian
- The Stanford University Administrative Panel on Human Subjects in Medical Research (IRB) and any other unit of Stanford University as necessary
- Research Staff
- Other healthcare providers (eg your primary doctor)

Who May Receive or Use the Information?
The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- Food and Drug Administration

Your information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?
Your authorization for the use and/or disclosure of your health information will expire on May 31, 2112.

Will access to my medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or billing decision about you (e.g., if included in your official medical record).

_____ _____

Signature of Subject Date

COMPENSATION FOR STUDY-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. You will be responsible for any associated co-payments or deductibles as required by your insurance.

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

You do not waive any liability rights for personal injury by signing this form.

QUESTIONS, CONCERNS OR COMPLAINTS

For the duration of the study, you will be under the care of Dr. Spiekerkoetter and Dr. Zamanian.

- If you have any questions, concerns or complaints about this research study, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, Dr. Spiekerkoetter. You may contact her now or later at (650) 724-1493 or (650) 739-5031. You should also contact her at any time if you feel you have been hurt by being a part of this study.
- Independent Contact: If you are not satisfied with how this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a participant, please contact the Stanford Institutional Review Board (IRB) to speak to someone independent of the research team at (650)-723-5244 or toll free at 1-866-680-2906. You can also write to the Stanford IRB, Stanford University, Stanford, CA 94305-5401.
- Appointment Contact: If you need to change your appointment, please contact Val Scott at (650) 725-9861or Patricia Del Rosario at (650) 721-2408Alternate Contact: If you cannot reach the Protocol Directors, please page another member of the research team - Val Scott or Patricia Del Rosario or the pulmonary fellow on call. All these staff members can be reached via the Stanford page operator at (650) 723 6661.

Once you have had all your questions answered and you are comfortable participating in this study, please sign below.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

As a research participant you have the following rights. These rights include but are not limited to the participant's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;
- be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated consent form; and

- be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING CONSENT, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Printed Name of Subject

_____ _____

Signature of Subject Date

PERSON OBTAINING CONSENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied – that the subject has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the subject and explained to him or her in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected.

_____ _____

Signature of Person Obtaining Consent Date

STUDY

☐☐☐ **Subject #** ☐☐☐ **Subject Initials**

Date of patient's withdrawal/study termination __ __ /__ __ / 2 0 __ __
month day year

☐ Partial withdrawal: Study medication stopped, patient willing to complete final study visit.
☐ Complete withdrawal: Study medication stopped, patient unwilling to have final study visit.

Reason for withdrawal (check all that apply)

☐ Adverse event.

☐ Protocol violation

☐ No longer interested in taking the study medication.

☐ Found study procedures, schedule or requirements too demanding.

☐ Relocated.

☐ Withdrew consent but gave no reason.

☐ Physician recommended study withdrawal.

☐ Death.

☐ Other.

Date of last dose of study medication __ __ /__ __ / 2 0 __ __
month day year

FURTHER EXPLANATION/ COMMENTS:

_____

_____

_____

_____

Initials _____

All Placebo will start on 1.5 mg daily

	Blood Draw #	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Placebo 1	Check date	T0	d4	d4	d4	d4	2wk	d4	d4	2wks	2wk	2wk	2wk	2wk	d4	2wk	
	action	1.5	down, 1	up, 1.5	up, 2	stay, 2	up, 2.5	down, 2	stay, 2	stay, 2	stay, 2	stay, 2	stay, 2	down, 1.5	stay, 1.5	down, 1	
Placebo 2		T0	d4	d4	2wk	2wk	d4	d4	2wk	d4	2wks	2wks	2wks	2wks	d4		
		1.5	up, 2	stay, 2	stay, 2	up, 2.5	up, 3	stay,3	reduce, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	up, 2	stay, 2		
Placebo 3		T0	d4	2wk	2wk	4d	2wk	4d	2wk	4d	4d	2wk	2wk	2wk			
		1.5	stay, 1.5	stay, 1.5	down, 1	stay, 1	up, 1.5	stay, 1.5	down, 1	up, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	stay, 1.5			
Placebo 4		T0	d4	d4	2wk	d4	2wk	d4	2wk	d4	d4	2wk	d4	2wk	2wk		
		1.5	up, 2	stay, 2	up, 2.5	stay, 2.5	down, 2	stay, 2	up, 2.5	up, 3	stay, 3	up, 3.5	stay, 3.5	stay, 3.5	stay, 3.5		
Placebo 5		T0	d4	d4	d4	2wk	2wk	d4	2wk	2wk	2wk	d4	2wk	d4	2wk		
		1.5	up, 2	down, 1.5	stay, 1.5	stay, 1.5	down, 1	stay, 1	stay, 1	stay, 1	up, 1.5	stay, 1.5	up, 2	stay, 2	stay, 2		
Placebo 6		T0	d4	d4	d4	2wk	d4	2wk	d4	d4	2wk	2wk	d4	2wk	2wk	d4	
		1.5	up, 2	up, 2.5	stay, 2.5	up, 3	stay, 3	down, 2.5	down, 2	stay, 2	stay, 2	down, 1.5	stay, 1.5	stay, 1.5	up, 2	stay, 2	
Placebo 7		T0	d4	d4	d4	2wk	2wk	d4	2wk	2wk	d4	2wk	d4	2wk	d4	d4	
		1.5	down, 1	up, 1.5	stay, 1.5	stay, 1.5	down, 1	stay, 1	stay, 1	up, 1.5	stay, 1.5	down, 1	stay, 1.5	up, 2	up, 2.5	up, 3	
Placebo 8		T0	d4	2wk	2wk	d4	2wk	4d	2wk	2wk	4d	4d	2wk	4d	4d	4d	
		1.5	stay, 1.5	stay 1.5	down, 1	stay, 1	down, 0.5	stay, 0.5	stay, 0.5	up, 1	up, 1.5	stay, 1.5	down, 1	down, 0.5	own, 0.5 qd	up, 0.5	
Placebo 9		T0	d4	d4	d4	2wk	2wk	d4	2wk	d4	2wk	2wk	2wk	2wk	2wk		
		1.5	down, 1	up, 1.5	stay, 1.5	stay, 1.5	down, 1	stay, 1	up, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	stay, 1.5	stay, 1.5		
Placebo 10		T0	d4	2wk	2wk	d4	d4	d4	2wk	2 wk	d4	2wk	d4	d4	d4	2wk	2wk
		1.5	stay, 1.5	stay, 1.5	down, 1	up, 1.5	up, 2	stay, 2	stay, 2	up, 2.5	stay, 2.5	down, 2	up, 2.5	down, 2	stay, 2	stay, 2	down, 1.5

LEGEND:

Expected End 16 wk