C O N F I D E N T I A L

Autoimmunity Centers of Excellence

Protocol # ASC01

A Randomized, Double-blind, Placebo-Controlled, Phase II Multicenter Trial of a Monoclonal Antibody to CD20 (Rituximab) for the Treatment of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH)

Short Title: Rituximab for Treatment of SSc-PAH

BB-IND 105,863

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INVESTIGATOR SIGNATURE PAGE

Protocol Title: A Randomized, Double-blind, Placebo-Controlled, Phase II Multicenter Trial of a Monoclonal Antibody to CD20 (Rituximab) for the Treatment of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH)

Protocol Number: Protocol # ASC01

Protocol Version: Version 8.0 (24 April 2017)

Study Sponsor: Division of Allergy, Immunology, and Transplantation (DAIT)
National Institute of Allergy and Infectious Diseases
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Please print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the SACCC.

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As a Principal Investigator on this protocol, I agree to conduct "A Randomized, Double-blind, Placebo-Controlled, Phase II Multicenter Trial of a Monoclonal Antibody to CD20 (Rituximab) for the Treatment of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH)" by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the DAIT/NIAID.

_____
Principal Investigator (Print)

_____
Principal Investigator Signature

_____
Date

PROTOCOL SYNOPSIS

Title of the Protocol: A Randomized, Double-blind, Placebo-Controlled, Phase II Multicenter Trial of a Monoclonal Antibody to CD20 (Rituximab) for the Treatment of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension (SSc-PAH)

ACE Protocol Number: ASC01

Protocol Chair(s): Mark Nicolls, MD; David Badesch, MD

IND Holder: DAIT/NIAID/NIH

Primary Objective: The primary objective of this study is to compare patients with SSc-PAH treated with rituximab to those on placebo for change in exercise capacity, as determined by six minute walk distance (6MWD).

Secondary Efficacy Objectives:

- To compare patients treated with rituximab to those on placebo for change in exercise capacity, as determined by 6MWD, at time points other than 24 weeks.
- To compare patients treated with rituximab to those on placebo for change in PVR as assessed by right heart catheterization.
- To compare patients treated with rituximab to those on placebo for time to clinical worsening, defined as first occurrence of:
    a. death,
    b. hospitalization for SSc-PAH,
    c. lung transplantation,
    d. atrial septostomy,
    e. addition of other PAH therapeutic agents, or
    f. worsening of 6MWD by > 20% and a decrease in functional class.
- To compare patients treated with rituximab to those on placebo who changed or added other PAH therapeutic agents.
- To compare patients treated with rituximab to those on placebo for change in quality of life as determined by the short form 36 (SF-36) and the disability index of the Scleroderma Health Assessment Questionnaire (SHAQ).
- To compare patients treated with rituximab to those on placebo for change in number of new digital ulcers.
- To compare patients treated with rituximab to those on placebo for change in severity of Raynaud phenomenon as measured by the VAS scale of the SHAQ.
- To compare patients treated with rituximab to those on placebo for change in DLCO and room air oxygen saturation at rest.

A secondary objective of this study is to determine whether the effects on clinical disease progression are paralleled by changes in selected biomarkers.

Secondary Safety Objectives:

- To assess the safety and tolerability of rituximab for the treatment of SSc-PAH in patients receiving stable background medical treatment with prostanoid, endothelin receptor antagonist, PDE-5 inhibitor therapy, and/or guanylate cyclase stimulators.

Mechanistic Study Objectives:

- CD19 Studies
    - To determine whether the extent and/or duration of CD19+ B cell depletion correlates with treatment response as measured by exercise capacity (6MWD), PVR (right heart catheterization), and quality of life (SF-36 and SHAQ).
    - To monitor and correlate the reconstitution patterns of B cell subpopulations with treatment response
- To determine if the biomarkers anti-U1 RNP, anti-U3 RNP, anti-B23, anti-cardiolipin, anti-Th/To, anti-CENP-B, RF, and other autoantibodies, and quantitative immunoglobulin levels, including IgG subclasses, correlate with treatment response as measured by PVR (right heart catheterization),

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exercise capacity (6MWD), and quality of life (SF-36 and SHAQ). • To determine if the biomarkers BNP/NT-proBNP, serum Na^+, IL-1, IL-6, and other cytokines correlate with treatment response as measured by PVR (right heart catheterization), exercise capacity (6MWD), and quality of life (SF-36 and SHAQ). • To determine if B cell clones are present in SSc-PAH and what impact rituximab may have on these clones. • To determine if B cell clones present in SSc-PAH are correlated with clinical outcomes. **Exploratory Objectives**: • To evaluate agreement between PVR calculated by site-based standards and a standardized Fick-based calculation of PVR. • To assess sensitivity of the secondary efficacy analysis to standardization of the PVR calculation. • To assess the utility of a standardized derived pulmonary artery capacitance as a primary efficacy endpoint. • To evaluate the relationship between 6MWD, PVR, and other clinical measures.
Study Arms: Subjects will be randomized in a 1:1 ratio to receive either: ▪ 2 infusions of rituximab, 1000 mg each, given 2 weeks apart, or ▪ 2 infusions of placebo given 2 weeks apart
Study Design: This is a prospective, double-blind, placebo-controlled, multi-center, randomized trial to evaluate the effect of rituximab on disease progression in subjects with SSc-PAH receiving concurrent stable-dose standard medical therapy with a prostanoid, endothelin receptor antagonist, phosphodiesterase 5 (PDE-5) inhibitor, and/or guanylate cyclase stimulators. The study will focus on assessment of clinical response and safety measures longitudinally. In addition, the effects of treatment with rituximab on the underlying immune mechanisms associated with B-cell dysregulation and pathogenic autoantibody response in this disease will be investigated. Subjects with SSc-PAH with elevated mean pulmonary artery pressure as measured by right heart catheterization will be enrolled. The diagnosis of SSc-PAH should be confirmed by a rheumatologist experienced in the diagnosis and treatment of systemic sclerosis in conjunction with a pulmonologist or cardiologist specializing in management of PAH. Both specialists will be part of the study team at each site. Rituximab/placebo will be administered as two IV infusions of 1000 mg each, given two weeks apart at Day 0 and Week 2. All subjects will receive 40mg of prednisone orally the night before and morning of each infusion, as well as 100 mg methylprednisolone or equivalent corticosteroid intravenously thirty minutes prior to each infusion, with diphenhydramine and acetaminophen orally thirty to sixty minutes prior to each infusion of rituximab or placebo. Subjects will remain on their baseline standard medical regimen. Sixty eligible subjects will be accrued. Each potential study subject will provide written informed consent prior to screening procedures. All inclusion and exclusion criteria (Section 4, *Selection of Subjects*) must be met at time of randomization prior to receipt of the first dose of rituximab/placebo (Day 0, Treatment Initiation). Clinical assessments and sample collection will occur regularly through Week 48 with telephone follow-up conducted intermittently. If a subject has not recovered B cells by Week 48, B cell studies will be conducted quarterly until recovery is documented or for 2 years after initial treatment (whichever occurs first). Recovery is defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available then recovery is defined as a B cell level of at least 90% of the lower limit of normal. During this quarterly monitoring period of B cell recovery, AEs and SAEs will be assessed, providing the subject has not withdrawn consent, to capture any infectious event ≥ Grade 3 using the National Cancer Institute—Common Terminology Criteria for Adverse Events (NCI-CTCAE). No additional study-related data will be collected. The primary efficacy endpoint is the change in 6MWD from baseline to 24 weeks after treatment initiation. Hemodynamic measures will be assessed at baseline and Week 24, contributing to the understanding of the relationship between PVR and clinical endpoints. Time to clinical worsening will be assessed as a secondary outcome measure through Week 48, contributing to the understanding of the relationship between PVR and

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clinical endpoints. Initiation of new therapy due to disease worsening prior to Week 24 will prompt an endpoint visit and right heart catheterization prior to initiation of the new therapy. B cell recovery for all will be monitored by serial assessments of B cell counts as determined by flow cytometry.

The total duration of the study is anticipated to be approximately 8 years.

Study Population: Any individual 18 to 75 years of age, who has been diagnosed with SSc-PAH within the past 5 years (as defined by a clinical diagnosis of SSc with a mPAP ≥ 25 mmHg), a NYHA Functional Class of II, III, or IV, a mean PVR of > 3 Wood units, and who meets all entry criteria is eligible for randomization.

Primary Efficacy Endpoint: Change in exercise capacity, assessed by 6MWD, from baseline to 24 weeks after treatment initiation.

Secondary Efficacy Endpoints:
Secondary efficacy endpoints include the following clinical endpoints:

1. Change in PVR measured by right heart catheterization, from baseline to 24 weeks after treatment initiation.
2. Exercise capacity, as determined by 6MWD, assessed longitudinally over the 48 weeks following treatment initiation.
3. Assessment of time to clinical worsening, censored at 48 weeks, defined as first occurrence of:
    a. death,
    b. hospitalization for SSc-PAH,
    c. lung transplantation,
    d. atrial septostomy,
    e. addition of other PAH therapeutic agents, or
    f. worsening of 6MWD by > 20% and a decrease in functional class.
4. Percentage of subjects who changed or added PAH therapeutic agents at 24 and 48 weeks.
5. Change in quality of life as determined by the SF-36 and disability index of the SHAQ from baseline to 24 and 48 weeks after treatment initiation.
6. Number of new digital ulcers assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
7. Severity of Raynaud phenomenon, as measured by the VAS scale of the SHAQ, assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
8. DLCO and oxygen saturation at rest on room air assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
9. Change in PVR from baseline to 24 weeks after treatment initiation as a percentage of the baseline value.

Secondary efficacy endpoints also include the following mechanistic endpoints:

1. CD19 Studies
    a. CD19+ B cells (absolute number and percentage of gated lymphocytes) at time of B cell depletion and longitudinally over the 48 weeks following treatment initiation, where B cell depletion is defined as <5 CD19+ cells/μL.
    b. Time to repopulation of CD19+ B cells, defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available, then recovery is defined as a B cell level of at least 90% of the lower limit of normal.
    c. Reconstitution pattern of B cell subpopulations assessed longitudinally over the 48 weeks following treatment initiation.
2. Level of anti-U1 RNP, anti-B23, anti-cardiolipin, anti-CENP-B, RF autoantibodies, and immunoglobulins, including IgG subclasses, measured by ELISA and assessed longitudinally over the 48 weeks following treatment initiation.
3. Presence or absence of anti-U3 RNP, anti-Th/To, and other autoantibodies assessed longitudinally over the 48 weeks following treatment initiation.
4. Level of BNP/NT-proBNP, serum Na^+, IL-1, IL-6, and other cytokines assessed longitudinally over the 48 weeks following treatment initiation.
5. Presence or absence of B cell clones assessed at baseline and longitudinally over the 48 weeks

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following treatment initiation.

Secondary Safety Endpoints:

1. All NCI-CTCAE Grade 3-5 AEs, which are defined as possibly, probably, or definitely related to rituximab infusion.
2. All NCI-CTCAE Grade 3-5 adverse events.
3. All infection related events.
4. Treatment-related mortality, defined as death occurring at any time after randomization and possibly, probably, or definitely resulting from treatment given in the study. Study investigators will make an initial determination of the cause of death of any subject and if uncertainty remains, a final decision will be made by an independent blinded panel after reviewing all clinical and, if available, autopsy data.
5. All-cause mortality, defined as any death occurring at any time after randomization.
6. CD19+ B cell levels post treatment (note that analyses will be performed regularly per Table 6.1 through 48 weeks and quarterly thereafter as documented in Section 3.1, *Description of Study Design*).
7. Peripheral blood cell counts, liver function, and creatinine levels after treatment.

Exploratory Endpoints:
Exploratory analysis of standardization of the PVR calculation will be based on PVR calculated from a standardized Fick calculation of cardiac output, which is a function of hemoglobin, arterial and mixed-venous saturations, and oxygen consumption according to the La-Farge and Miettinen formula. The cardiac output calculation is a function of assumed oxygen consumption according to the La-Farge calculation of values normalized for age, gender, heart rate. Calculation of a standardized Fick cardiac output will also allow for determination of the pulmonary artery capacitance (PACf). See Appendix E, *Exploratory Analysis: Standardized Fick-based Pulmonary Vascular Resistance and Pulmonary Artery Capacitance* for details. Additionally, an exploratory analysis of the relationship between change in PVR and change in 6MWD will be addressed. Relationships with other clinical endpoints, like change in NTproBNP, will also be explored.

Sample Size: The study will include 30 subjects treated with rituximab and 30 subjects treated with placebo.

Data Analyses: The primary efficacy analysis of change in 6MWD from baseline to 24 weeks will be performed on the intention-to-treat (ITT) population. and will be tested using a repeated measures random effects model to model change from baseline in 6MWD as the primary outcome and treatment as the primary predictor, controlling for visit day, limitations by musculoskeletal disease, baseline levels of 6MWD, and a treatment by visit day interaction. A random slope and intercept will be fit for each subject. An unstructured covariance matrix will be assumed for the random effects. Additional covariates will be considered for this model as part of secondary analysis.

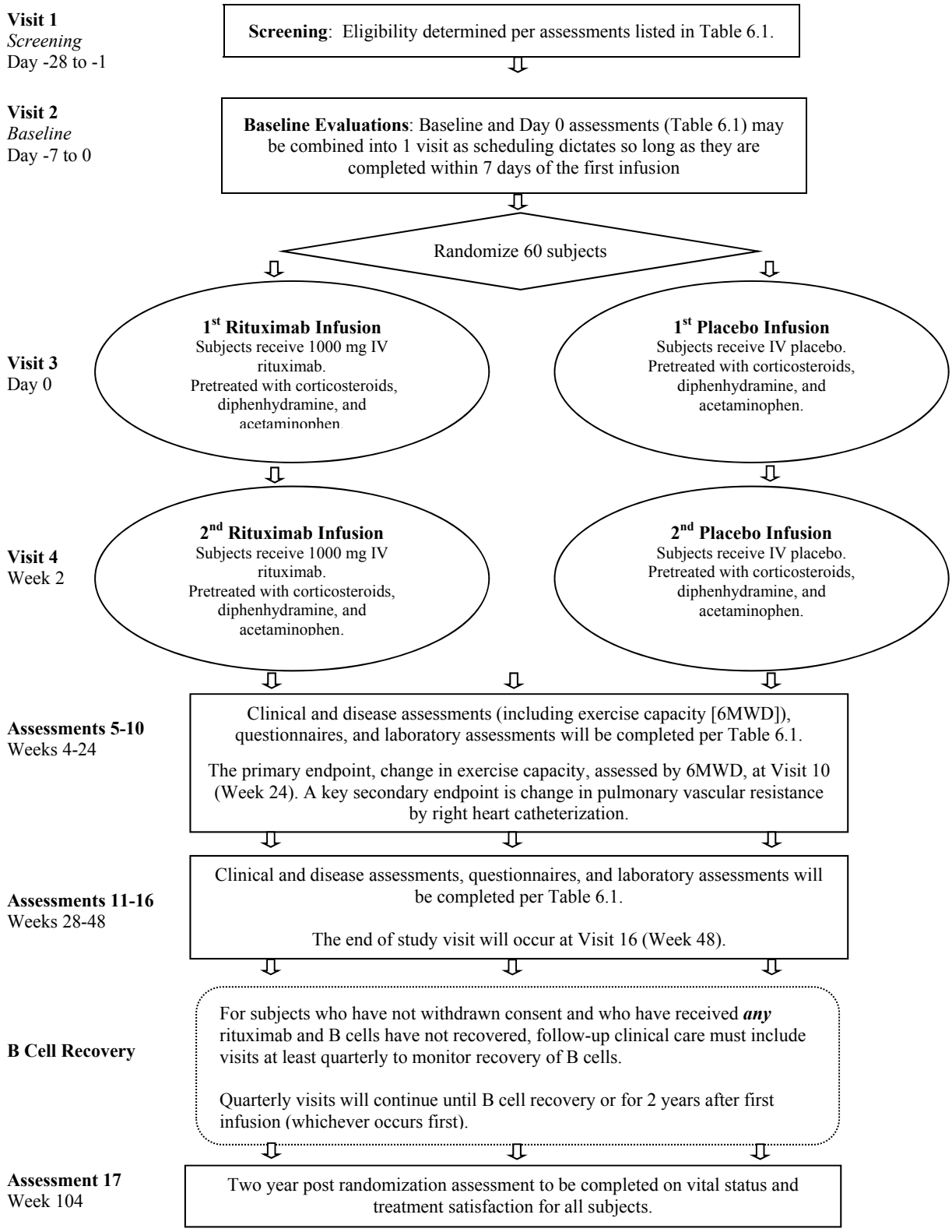
Secondary analyses will include repeating the primary analysis in the per protocol population, subgroup analyses of the primary outcome, safety analyses, exploratory analyses of the secondary endpoints, and exploratory analyses of the mechanistic endpoints.

Lay Summary:

Sixty eligible adults between the ages of 18 and 75 who have been diagnosed with SSc-PAH within the past 5 years and meet all entry criteria will be enrolled in this study. Participants will receive 2 infusions (1000 mg) of either rituximab or placebo 14 days apart.

This study will compare patients treated with rituximab to those on placebo for change in six minute walk distance (6MWD). The secondary objectives of this study are to compare treatment groups for other measures of clinical disease progression and to determine whether the effects on clinical disease progression are paralleled by changes in selected biomarkers. Additionally, the safety and tolerability of rituximab for the treatment of SSc-PAH in patients receiving stable background medical treatment with prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators therapy will be assessed.

FLOW DIAGRAM OF PROTOCOL



ABBREVIATIONS

6MWD	6-Minute Walk Distance
6MWT	6-Minute Walk Test
ACE	Autoimmunity Centers of Excellence
ADCC	Antibody-dependent Cellular Cytotoxicity
ADCT	Autoimmune Diseases Clinical Trials
AE	Adverse event
AECA	Anti-Endothelial Cell Antibody
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANAs	Serum Antinuclear Antibodies
ANCOVA	Analysis of Covariance
Anti-B23	Antibody to the B23 antigen
Anti-CENP-B	Antibody to the CENP-B (centromere) antigen
Anti-Th/To	Antibody to the Th/To antigen
APAH	Associated Pulmonary Arterial Hypertension
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration
AST	Aspartate Aminotransferase
ATG	Anti-thymocyte Globulin
BMPR2	Bone Morphogenetic Protein Receptor Type 2
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
BUN	Blood Urea Nitrogen
C	Celsius
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CI	Cardiac Index
CMRI	Cardiac Magnetic Resonance Imaging
CO2	Carbon Dioxide
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTGF	Connective Tissue Growth Factor
CXR	Chest X-ray
CY	Cyclophosphamide
DAIT	Division of Allergy, Immunology, and Transplantation
DE	Delayed Enhancement
DHHS	Department of Health and Human Services
DLCO	Carbon Monoxide Diffusing Capacity
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ERA	Endothelin Receptor Antagonist
ET-1	Endothelin-1
F	Fahrenheit
Fc	Fragment, crystallizable

FDA	Food and Drug Administration
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
HACA	Human Anti-chimera Antibody
HBV	Hepatitis B Virus
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
Hg	Mercury
HIMC	Human Immune Monitoring Center
HIV	Human Immunodeficiency Virus
HHV8	Human Herpesvirus-8
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
IgG	Immunoglobulin G
IJ	Internal Jugular
IL	Interleukin
IND	Investigational New Drug Application
IPAH	Idiopathic Pulmonary Arterial Hypertension
IR	Immune Reconstitution
IRB	Institutional Review Board
ITT	Intention-to-Treat or Intent-to-Treat
ITP	Idiopathic Thrombocytopenic Purpura
IUD	Intrauterine Device
IV	Intravenous
IVIG	Intravenous Immunoglobulin
kD	Kilo Dalton
LOCF	Last Observation Carried Forward
LON	Late Onset Neutropenia
LPS	Lipopolysaccharide
LV	Left Ventricle
MAP	Mean Arterial Pressure
MCP	Monocyte Chemotactic Protein
MI	Myocardial Infarction
MID	Minimally Important Difference
MOP	Manual of Procedures
mPAP	Mean Pulmonary Arterial Pressure
MRI	Magnetic Resonance Imaging
MTX	Methotrexate
Na^+	Sodium
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NS	Normal Saline
NT-proBNP	N-Terminal Pro Brain Natriuretic Peptide

NYHA	New York Heart Association
OHRP	Office of Human Research Protection
PA	Posteroanterior
PAAT	Pulmonary Arterial Acceleration Time
PACf	Fick-based calculation of Pulmonary Artery Capacitance
PAH	Pulmonary Arterial Hypertension
PAP	Pulmonary Arterial Pressure
PASP	Pulmonary Arterial Systolic Pressure
PCR	Polymerase Chain Reaction
PCWP	Pulmonary Capillary Wedge Pressure
PDE-5	Phosphodiesterase 5
PE	Physical Examination
PFTs	Pulmonary Function Tests
PI	Principal Investigator
PK	Pharmacokinetics
PL	Placebo
PP	Per Protocol
PPD	Purified Protein Derivative
PML	Progressive Multifocal Leukoencephalopathy
PVR	Pulmonary Vascular Resistance
PVRf	Fick-based calculation of Pulmonary Vascular Resistance
qPCR	Quantitative Polymerase Chain Reaction
RA	Rheumatoid Arthritis
RHC	Right Heart Catheterization
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RV	Right Ventricle
RVEDVI	Right Ventricular End Diastolic Volume Index (mL/m^2)
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Sprague Dawley
SF-36	Short Form 36
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SHAQ	Scleroderma Health Assessment Questionnaire
SLE	Systemic Lupus Erythematosus
SP	Safety Population
SSc	Systemic Sclerosis
SSc-PAH	Systemic Sclerosis-Associated Pulmonary Arterial Hypertension
SUHX	SU5416-hypoxia
SV	Stroke Volume
TB	Tuberculosis
TGF	Transforming Growth Factor
Th	T Helper

TLC	Total Lung Capacity
TLS	Tumor Lysis Syndrome
TNF	Tumor Necrosis Factor
VAS	Visual Analog Scale
TTE	Transthoracic Echocardiography
WHO	World Health Organization

TABLE OF CONTENTS

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Systemic Sclerosis – General Disease Concepts

***Background**:* Systemic sclerosis (SSc) is a systemic connective tissue disease characterized by fibrosis and atrophy of the skin, joints and tendons, skeletal muscles, and internal organs (e.g., lungs, heart, gastrointestinal tract, and kidney) as well as by immunological disturbances [1]. SSc is a rare disease which is diagnosed in approximately 67 male patients and 265 female patients per 100,000 people each year in the United States. Internationally, SSc is estimated to occur in 20 people per million annually but is relatively rare in Japan and China. Overall, a substantial female predominance exists, with a female-to-male ratio of 3-6:1 [2]. The limited cutaneous form of SSc has a more striking female predominance, with a female-to-male ratio of 10:1. SSc usually appears in women aged 30-40 years and in slightly older men. In roughly 85% of cases, SSc develops in individuals aged 20-60 years. Cases are also found among children and the elderly.

The clinical manifestations of SSc include Raynaud phenomenon and skin fibrosis and, in its severest form, sclerosis of multiple internal organs with vascular inflammatory and fibrotic components. Of the numerous manifestations that affect multiple organ systems, pulmonary involvement produces some of the most common and serious complications of the disease. The major pulmonary manifestations of SSc are interstitial lung disease and systemic sclerosis-associated pulmonary arterial hypertension (SSc-PAH). Estimates of the prevalence of PAH in patients with SSc have varied widely based on the definition of PAH and the method of obtaining the measurements (i.e. cardiac catheterization or echocardiography) but is thought to be between 8 and 12%. A conservative estimate of PAH prevalence of 10% among patients with SSc in the United States would indicate a prevalence of 24 individuals per million, which may actually be higher than the number of patients affected by idiopathic PAH. Further, patients with SSc-PAH tend to have a worse prognosis compared to all other forms of PAH. One year survival rates for SSc-PAH patients range from 50-81%, which is considerably lower than the 88% one year survival rate for idiopathic PAH patients [3-5]. One study documented survival rates for individuals with SSc-PAH of 81%, 63% and 56% at 1, 2, and 3 years from diagnosis respectively [4]. A more recent study again suggested a 3-year survival rate of 52% in patients with SSc-PAH [6].

SSc-PAH assumes three basic forms: 1) severe SSc-PAH accompanying limited cutaneous SSc, 2) SSc-PAH accompanying (secondary to) interstitial lung disease, and 3) a more indolent form of SSc-PAH which reflects vascular pathology of SSc [7]. Although there appear to be different subtypes of SSc-PAH, it is not known if and how the pathogenesis differs with each form. In all patients with SSc, SSc-PAH significantly worsens survival and is the leading cause of mortality in these patients.

Treatment for this disease is currently limited to the same vasodilator therapy employed in all forms of PAH. An evaluation of 91 patients with PAH treated with prostanoids revealed that

SSc-PAH patients had the worst survival of all subgroups analyzed [8]. SSc-PAH is a life-threatening illness for which there is currently no cure.

SSc-PAH Pathology*:* Lungs from patients with SSc-PAH exhibit a characteristic vascular pathology, the plexiform lesion, which is structurally similar to plexiform lesions found in other PAH conditions such as idiopathic PAH and HIV-associated PAH [9]. In SSc-PAH, both plexiform and concentric obliterative lesions stain positively for factor VIII-related antigen consistent with abnormal endothelial cell proliferation. Macrophages, T and B cells are noted clustering in and around the vascular lesions. Interestingly, the primary proliferative abnormalities within the pulmonary arterial walls are similar to those found in SSc digital arteries. Both digital and pulmonary arteries have medial and advential fibrosis that lead to structural luminal narrowing.

Pathogenesis of SSc and SSc -PAH*:* Endothelial cell apoptosis may be the first event in the pathogenesis of the disease [10]. Anti-endothelial cell antibodies (AECA) are found in the circulation of SSc patients and have been posited to play a role in the development of vascular disease found in SSc, including PAH [11]. An instigating injury to endothelial cells in SSc that may trigger such autoantibody formation may be a viral infection [12]. A number of investigators have found evidence for viral infections, such as Epstein-Barr virus, parvovirus B19 and hepatitis C, E, and G in patients with SSc [13-18]. Although the role of cytomegalovirus (CMV) in the pathogenesis of SSc is debated [19], indirect evidence for a role of CMV-specific antibodies in the development of this disease has also been presented [20, 21]. Not only are absolute lymphocyte counts reduced in SSc [22, 23], but SSc patients also have relatively fewer $CD4^{+}CD25^{+}$ cells in the peripheral circulation compared to healthy controls [24]. In this setting of diminished regulatory T cells, a dysregulation of B cells is also observed [25]. Of note is that CD19 expression is increased by ≈20% on B cells in patients with SSc, while other B cell markers CD20, CD22 and CD40 are normally expressed [26]. Several different autoantibodies have been implicated in disease pathogenesis; anti-topoisomerase I (anti-Scl-70) and anticentromere antibodies, for example, are relatively specific for SSc [27], whereas an antibody to Cu/Zn superoxide dismutase and anti-topoisomerase II have been associated with localized scleroderma [28, 29]. In summary, SSc is an autoimmune disorder that has been associated with viral infection, endothelial damage, diminished regulatory T cells, dysregulated B cells, abundant mast cells, and serum autoantibodies.

Specific Concepts Concerning SSc-PAH Pathogenesis*:* Very little is known about the pathogenesis of SSc-PAH, but it appears to be a complex and multifactorial process. Unlike familial PAH, SSc-PAH has not been found to be associated with a mutation of bone morphogenetic protein receptor type 2 (BMPR2), a member of the TGF-β superfamily of receptors [30]. However, it has been recognized that altered expression of TGF-β superfamily receptors, interacting proteins, or downstream signaling molecules occurs in SSc [31-33]. Defects in the balance of vasoconstrictors and vasodilators have been another research focus in SSc-PAH with a notable elevation of endothelin-1 (ET-1), a potent vasoconstrictor [34].

In addition to interest in TGF-β superfamily alterations and abnormal vascular tone, there is significant work focusing on dysregulated immunity in the pathogenesis of SSc and SSc-PAH. Plexiform lesions found in the arterial walls of SSc PAH patients include an inflammatory infiltrate [35] consisting of macrophages, T cells, B cells, and mast cells [36, 37].

Figure 1 presents a model which integrates what is known generally about SSc pathogenesis, including the concept of abnormal Treg behavior, and speculates how this immune dysregulation could culminate in SSc-PAH.

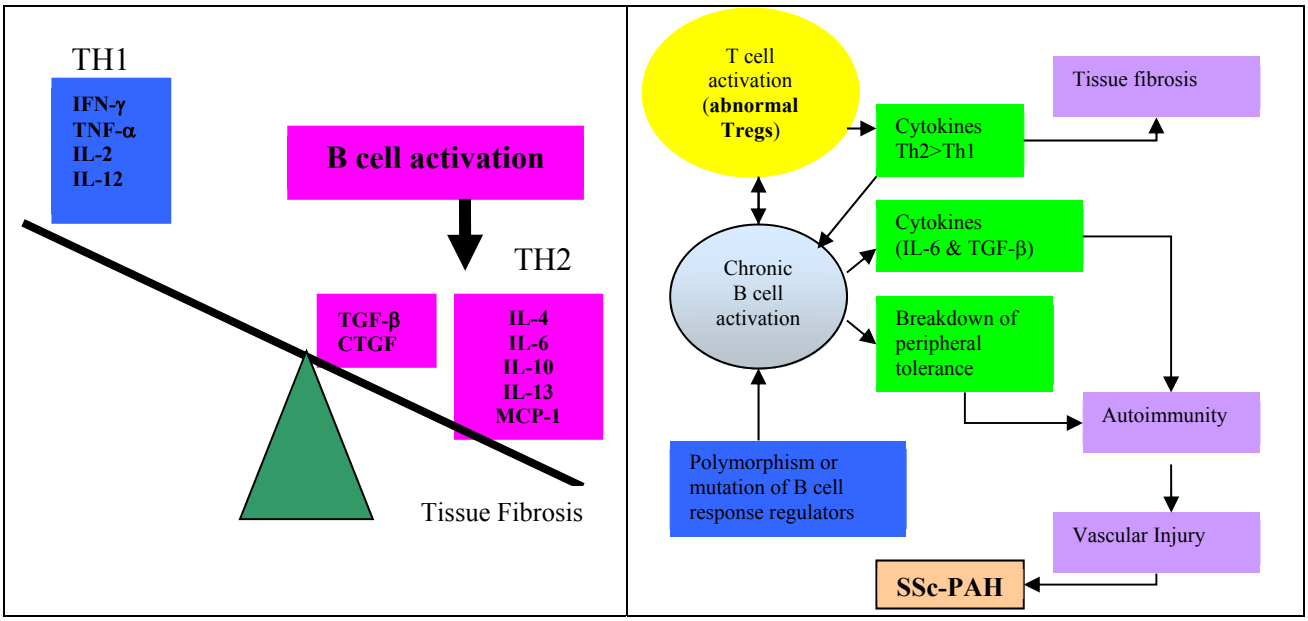


Figure 1. Immune Dysregulation in SSc (Adapted from Hasegawa et al., 2005 [38]) The panel on the left demonstrates that patients with SSc have a preponderance of Th2 cytokines which work in concert with growth factors to promote tissue fibrosis. The panel on the right is a model showing the putative relationship between systemic autoimmunity (possibly contributed to by abnormal Treg activity) and tissue fibrosis in SSc and how this could culminate in vascular injury and SSc-PAH.

Endothelial alterations may lead to a cascade of stimulatory changes that involve many cells, including T cells, macrophages, mast cells, and fibroblasts. Once activated, these cells secrete a variety of substances, including enzymes and their inhibitors and cytokines and their soluble receptors. These substances lead to changes in the extracellular matrix proteins, including fibronectin; proteoglycans; and collagen types I, III, V, and VII which results in fibroproliferative changes [39]. Activation of the immune system appears to be of paramount importance in the pathogenesis of SSc. Antigen-activated T cells infiltrate the skin and produce the profibrotic cytokine IL-4. B cells may contribute to fibrosis, as deficiency of CD19, a B-cell transduction molecule, results in decreased fibrosis in animal models as described below.

The chemokine CXCL4 was recently found to provide a link between plasmacytoid dendritic cell activity and the evolution of SSc. A proteome-wide screen showed CXCL4 to be the only chemokine that predicted the risk and progression of SSc. This landmark study demonstrated that high plasma CXCL4 levels were associated both with skin and lung fibrosis, as well as PAH. For this reason, we have added serial measurement of CXCL4 to the current study [40].

1.1.1 Current Treatment for Systemic Sclerosis-Associated PAH

Standard treatment for PAH includes therapeutics that address three major pathways involved in the abnormal proliferation and contraction of the smooth-muscle cells of the pulmonary artery including the endothelin pathway, nitric oxide pathway and prostacyclin pathway (**Figure 2**) [41]. Chronic prostacyclin infusion therapy does not provide a cure for PAH [42].

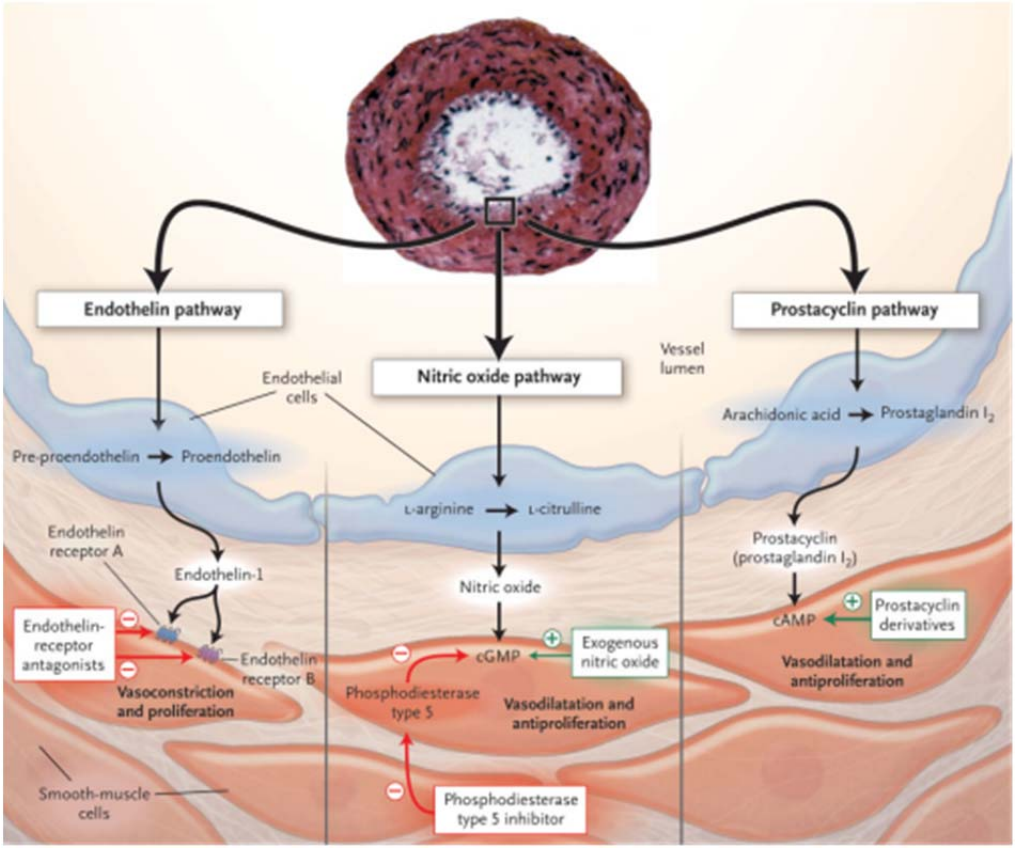


Figure 2. The Three Major Pathways Targeted by Conventional PAH Therapies [41].

Previously, the continuous infusion of the potent vasodilator prostacyclin has been considered maximum therapy for severe PAH [43-47]. More recently, the concept of combination therapy, such as an endothelin receptor blocker, or a phosphodiesterase 5 inhibitor, with prostacyclin, is being evaluated. In addition, patients receive anticoagulation

and right heart failure is frequently treated with diuretics and digitalis [44]. Aside from rare attempts to give prednisone or cytoxan, immunotherapy has rarely been used or systematically studied in SSc-PAH [46-49]. Unfortunately, currently offered medical therapy will usually fail in time and can only serve as a bridge to lung transplantation. Another major limitation of current medical therapies is cost, which can be more than $100,000 per year. Thus, the need for a new therapeutic regimen in SSc-PAH is significant.

1.2 Summary of Pre-Clinical and Clinical Studies

1.2.1 Pre-Clinical Studies

CD19, a critical cell-surface signal transduction molecule of B cells, is the most positive response regulator of B cells and has been implicated in SSc. Transgenic mice which overexpress CD19 lose self tolerance and generate autoantibodies spontaneously [50]. CD19 overexpression in the tight-skin mouse model of SSc results in increased autoantibody production [51] whereas CD19 deficiency significantly decreases skin fibrosis [52]. B cell depletion in the tight-skin mouse model was recently noted to be effective in reducing skin fibrosis and autoimmunity during the initiation of disease, but was not effective in established disease [53]. This has led Dr. Tedder and colleagues to hypothesize that chronic B cell activation due to augmented CD19 signaling leads to skin fibrosis and enhanced autoimmunity in SSc [54, 55].

The preclinical studies from our group, which were an impetus for the development of the current trial, broadly describe the phenomena of autoimmunity in PAH [56]. Inbred athymic nude rats treated with the Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitor, SU5416, develop significant vascular remodeling and severe PAH at Denver altitude [57]. Our experimental model of PAH occurs *because of the absence of regulatory T cell activity*. Unlike most published experimental models, this model does not require hypoxic conditions for disease initiation and progression.

However, athymic animals uniquely developed severe PAH following SU5416 treatment when exposed to chronic hypoxia. The data shown in **Figure 3** demonstrate that T cells play a protective role following vascular injury. Immune reconstitution of T cells with regulatory function prevents the development of PAH in athymic nude rats treated with SU5416 as well as the accumulation of B cells and anti-endothelial cell antibodies in the lungs of these animals.

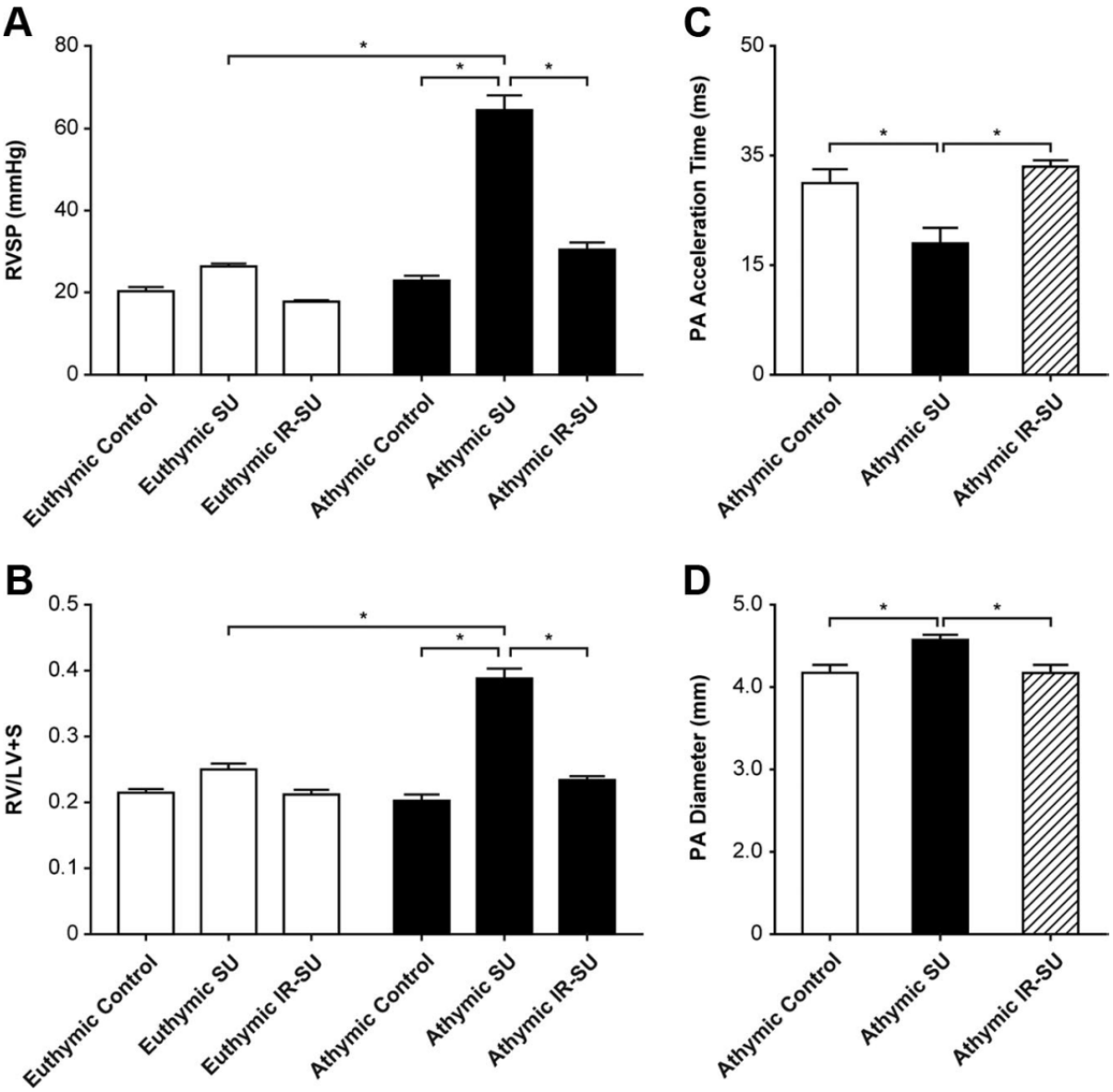


Figure 3. Immune reconstitution of athymic rats prevents PAH development.
Panel A: RVSP measurements in athymic and euthymic rats treated with SU5416 at d21 and vehicle-treated rats (athymic control, euthymic control). Immune reconstitution (IR) prior to SU5416 administration attenuates PAH development. Euthymic animals with normal immune systems regulate inflammation through the action of regulatory T cells (Tregs). Panel B: RVH measurements as assessed by RV/(LV+S) ratio in athymic and euthymic rats treated with SU5416 at d21 and vehicle-treated control rats (n=10–16 per group). Panels C and D: Sequential echocardiography of athymic controls, athymic SU, and athymic IR-SU at d21 (n=4 per group). Data are shown as means with error bars representing SEM. *P0.05.

In this pre-clinical model, B cells and AECAs may be critical effectors for the development of experimental PAH [58]. **Figure 4** demonstrates that in the absence of normal Treg activity, inflammation with macrophages and B cells are significant, and systemic inflammation is detectable in the peripheral circulation.

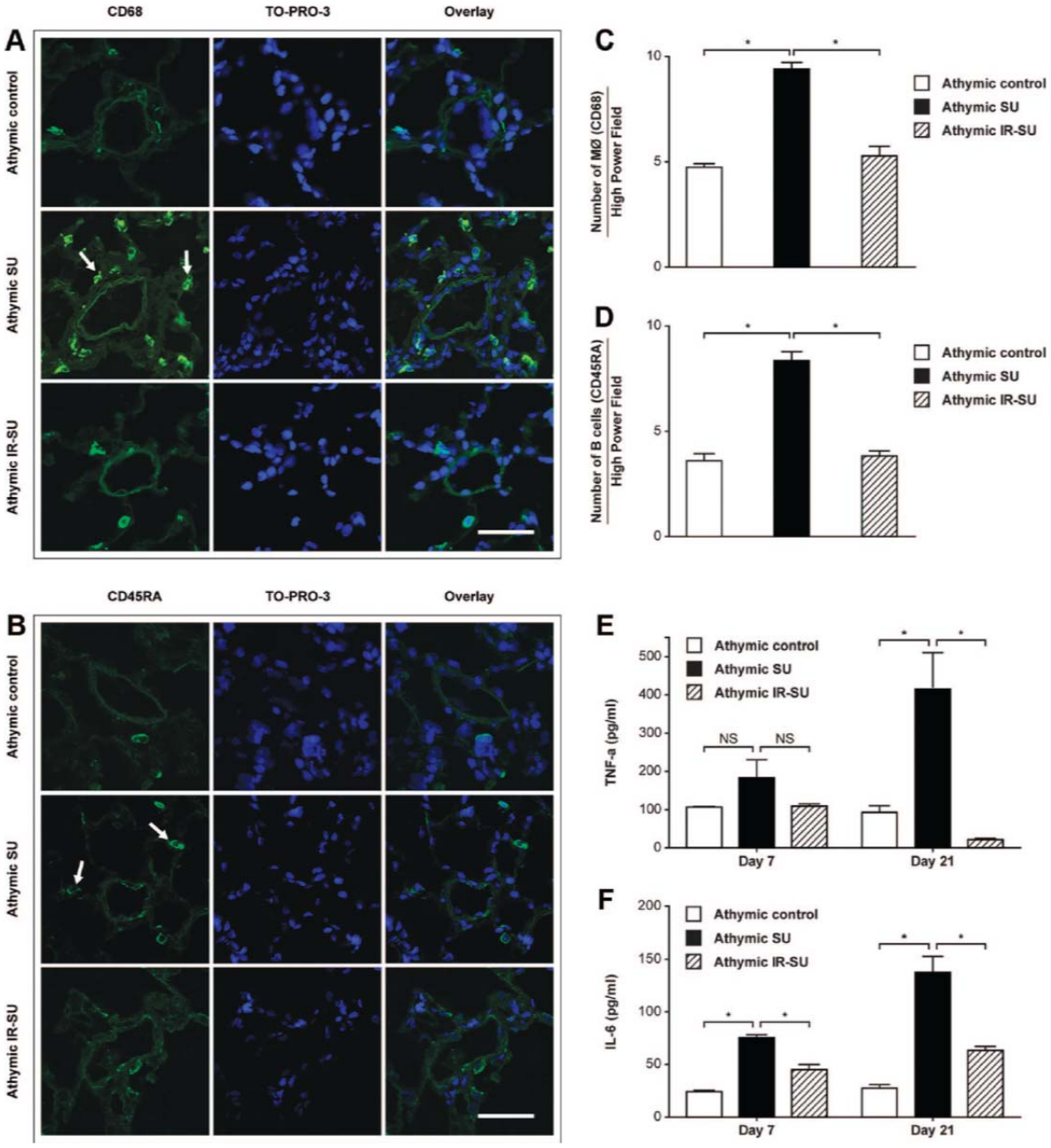


Figure 4. Evidence of anti-inflammatory effect of immune reconstitution (IR) at d7 after SU5416 administration. A and B, Immunofluorescent images of lung sections from athymic SU5416-treated animals stained with CD68 (arrows) for macrophages, and with CD45RA (arrows) for B cells at d7 after SU5416 administration (n=8 per group). C and D, Morphometric analysis of macrophages (CD68) and B cells (CD45RA) in lung sections at d7 after SU5416 administration (n=4 per group). E and F, Serum TNF- and IL-6 evaluated by ELISA at d7 and d21 (n=6–8 per group). Data are shown as means with error bars representing SEM. *P0.05. Scale bars: (A, B) 50 μm.

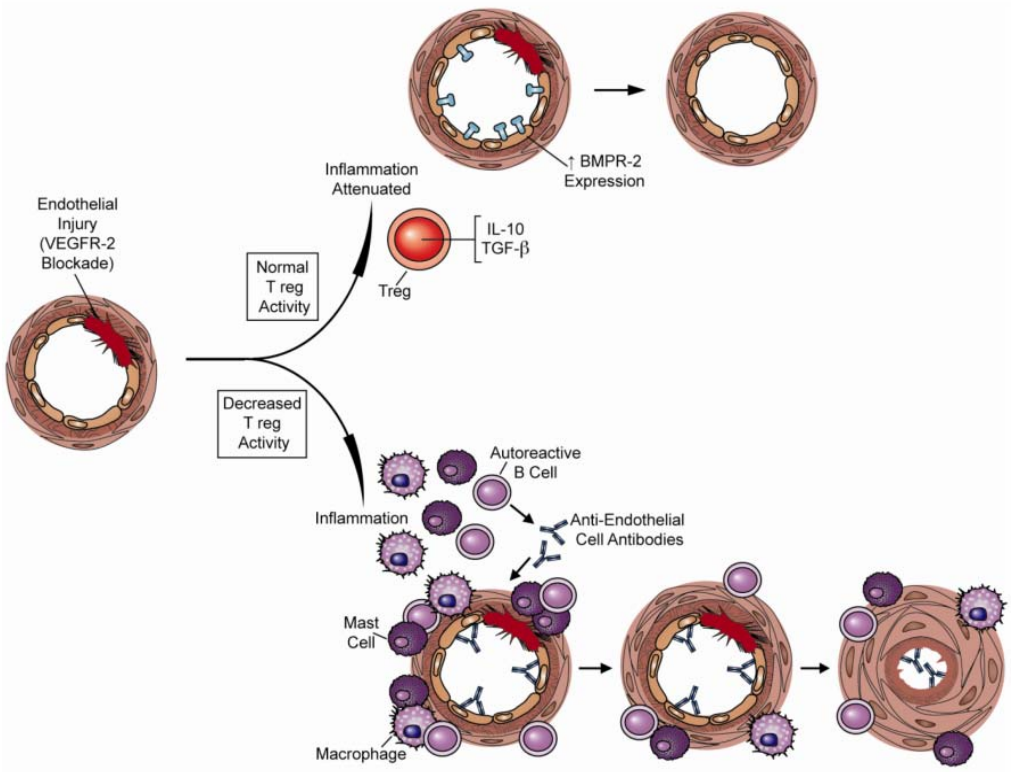


Figure 5. Model of immune regulation contributing to vascular health and immune dysregulation favoring vascular disease following endothelial injury. In this model of PAH, endothelial injury, causes a local immune response. With normal immune regulation, vascular injury does not culminate in pulmonary vascular remodeling and PAH. The absence of normal immune regulation results in an inappropriately exuberant inflammatory response, accelerated endothelial cell apoptosis, smooth muscle hypertrophy, and increased pulmonary vascular resistance.

To summarize, in T-cell deficient animals an accumulation of B cells and macrophages was noted within one week of SU5416 administration. These findings were not seen in athymic nude rats that underwent immune reconstitution prior to SU5416 administration. These results are concordant with the fact that the loss of self-tolerance in animals missing normal Treg populations is associated with the appearance of various autoantibodies and autoimmune disease [59, 60]. With complete elimination of $CD4^{+}CD25^{hi}$ cells, systemic autoimmunity occurs as manifested by multiorgan inflammation and autoantibody production [61]. Thus a loss of Treg-mediated self tolerance leads not only to a loss of T cell tolerance but also to a breakdown in B cell tolerance. This latter principle suggested that a therapy which addressed autoreactive B cells and possibly self-directed antibodies could be effective in PAH associated with immune dysregulation. As noted, this finding is relevant in SSc-PAH patients who likely have abnormal Treg functionality.

Dysregulated B cell immunity, as a direct consequence of diminished regulatory T cell control in the T-cell deficient athymic rat model, is important because at this time B cells can be more easily targeted with a therapeutic intervention than can abnormal Treg activity. To

this end, the effect of B cell depletion is currently being characterized in the athymic rat model using SU5416. Other animal models relevant to SSc-PAH have already been treated with B cell depletion, and results are promising. In addition to the studies of B cell depletion in the tight skinned mouse discussed above, at the 2011 American Thoracic Society Meeting in Denver, Dr. Norbert Voelkel, an internationally-recognized PAH investigator, presented data showing that anti-CD20 therapy ameliorates severe PAH in a unique inflammatory model of PAH. In this model, PAH is induced by exposing immunocompetent rats to ovalbumin sensitization and vascular injury with a VEGF receptor antagonist. This regimen leads to significant inflammatory PAH associated with Th2 inflammation. This exciting new model may be directly relevant to SSc-PAH because of the noted Th2 skewing. Data using this model of PAH shows that B cell depletion can be significantly protective against severe PAH [62].

1.2.2 **Clinical Studies**

Considered generally, therapies for localized scleroderma have tended to yield more positive results than those for SSc. However, treatments for localized scleroderma have also been less vigorously investigated in randomized, controlled trials [63]. Molecular analysis demonstrates the involvement of CD20-positive cells in SSc [64] although histochemical evidence has been more inconsistent [64, 65]. Investigators comparing skin biopsy samples from four scleroderma patients with samples from matched controls by micro-array gene analysis found that B-lymphocyte-specific genes were up-regulated in skin from patients with SSc [64]. Immunohistochemistry of patients' skin showed a predominance of CD20-positive cells in a perieccrine distribution in one of the patients. In contrast, stains for CD3 and CD138 were negative, indicating a relative absence of T cells and plasma cells, respectively.

Lafyatis et al. treated 15 SSc patients with diffuse skin thickening with rituximab. They showed that, as expected, peripheral blood B cells were depleted, but also that the modest B cell infiltrates present in most of the skin biopsies at baseline were markedly reduced or completely absent on repeat skin biopsy 6 months after treatment (mean B cell score declined from 10.4 to 3.4). Thus, rituximab was able to deplete B cells not only in peripheral blood but also in affected tissues [66].

There is very little published data on the use of rituximab in SSc lung disease. A compelling case report of SSc-PAH was recently published [67]. The patient presented with interstitial lung disease, but on closer review of the case the principal and potentially life- threatening manifestation was SSc-PAH. This patient improved dramatically after rituximab therapy and would likely have qualified for the current trial. In these and other unpublished cases, the effect of B cell depletion has been striking with reversal of advanced and life-threatening disease. While a specific antigenic target has not been identified in SSc-PAH, platelet-derived growth factor (PDGF)-specific antibodies have recently been identified as highly prevalent in SSc [68]. SSc patients who received rituximab had lower titers of anti-PDGF antibodies, less skin fibrosis, significantly improved health assessment questionnaire scores, decreased Type I collagen production, and decreased production of fibroblast-reactive

oxygen species in vitro. Further, skin biopsies from 11 patients treated with rituximab in a Phase I study showed no progression of fibrosis; sequential biopsies were remarkable for a significant reduction of dermal myofibroblasts [69].

In PAH, even partial reversal of disease has the potential to be life-saving. In this respect, some patients have shown clinical improvement in their PAH with rituximab. In several unpublished cases (e.g. a case of Waldenstrom's macroglobulinemia-associated PAH in Denver, Colorado), patients have enjoyed significant clinical improvement following rituximab therapy. The following is an example of one such case from Stanford University Medical Center: A 30 year old patient with SLE presented with Class IV heart failure symptoms, along with necrotizing digital ulcers. An echocardiogram revealed an RVEF of 10% and an LVEF of 35% along with evidence of myocarditis. While the patient's left ventricular function improved with diuresis and pulse steroids, the patient's right heart systolic pressures were systemic. The patient did not respond to cyclophosphamide and was subsequently treated with several doses of rituximab. Within weeks, the patient's symptoms improved dramatically with a fall in the pulmonary artery pressures from the 80s-90s to 25 mmHg and Class II heart failure symptoms (Dr. Roham Zamanian; personal communication). Another illustrative case at Stanford involved a woman with PAH secondary to the antiphospholipid antibody syndrome. The patient had suffered thromboembolic disease, and despite having undergone thromboendarterectomy twice, continued to have worsening symptoms of PAH. The patient was anticoagulated with coumadin and was treated with prostacyclin. The patient also suffered from severe thrombocytopenia and developed intracranial hemorrhages necessitating discontinuation of coumadin. The patient was treated with rituximab and enjoyed a significant improvement in her clinical findings with normalization of her platelet counts and downgrading to Class II symptoms. This improvement, attributed to rituximab, led to the conversion from intravenous prostacyclin to oral therapy. In summary, while there has been relatively little published to date on the use of rituximab in SSc, there appears to be a strong scientific and clinical basis for exploring this potential therapy, which is well studied in other diseases, in SSc patients who otherwise do not have much hope for long-term survival.

1.3 Rituximab/Treatment Background

1.3.1 Current Licensing of Rituximab

In 1997, rituximab was approved for treatment of subjects with relapsed or refractory low-grade or follicular, B cell non-Hodgkin's lymphoma. Rituximab is also approved for retreatment of lymphoma patients who have relapsed following initial rituximab therapy. Approved dosing is 375 mg/m^2 each week for 4 or 8 doses. In March 2006, rituximab in combination with methotrexate was approved for use in the treatment of moderately to severely active rheumatoid arthritis (RA) patients who have had an inadequate response to one or more tumor necrosis factor (TNF) antagonist therapies. The approved dosing for RA is two infusions of 1000 mg given two weeks apart. In April 2011, the indications for rituximab (with dosing similar to non-Hodgkin's lymphoma) were expanded to include

Wegener's granulomatosis and microscopic polyangiitis in adult patients in combination with glucocorticoids. (See product label and prescribing information at http://www.Rituxan.com/rituxan/pi.)

1.3.2 **Other Diseases in Which Rituximab Use Has Been Described**

This IgG1 chimeric monoclonal antibody was originally developed and approved for the treatment of non-Hodgkin's lymphoma. In addition, rituximab has been used in the treatment of chronic lymphocytic leukemia [70] and Waldenström's macroglobulinemia [71-73]. Post-approval experience has been estimated to include more than 2,484,131 (Genentech, 2011) patient exposures. Although reactions frequently occur with the first infusion, most are Grade 1 toxicity and respond to slowing or temporarily interrupting the infusion. In general, the drug has proven to be well tolerated, and serious reactions are very rare [74]. The period of B cell depletion with standard regimens of rituximab frequently averages 4 to 7 months [75, 76]. A total of 8.4% of patients from the All Exposure RA population demonstrated a CD19 count < lower limit of normal for 2 years or more following their last rituximab exposure. 1.5% of these patients had CD19 counts below the lower limit of quantification for at least 2 years. These patients are generally representative of the RA population with long-standing disease, previous use of multiple DMARDs, and high use of oral corticosteroids. Of note is the proportion of patients with low peripheral B-cell counts at baseline, prior to rituximab treatment, which correlates with those patients whose counts showed prolonged B cell depletion. The clinical relevance of prolonged peripheral B-cell depletion beyond two years following rituximab treatment is unknown; no safety signal has been identified in the prolonged B cell monitoring trials. The pattern of peripheral B cell depletion and repletion in granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) patients included in the RAVE study was similar to that previously observed in patients with RA [77]. Effects on total immunoglobulin (Ig) levels have been much less striking, and evidence of immunodeficiency related to hypogammaglobulinemia has been rare. While rituximab results in the near-complete depletion of circulating B cells, the exact mechanism(s) of this effect remains poorly understood. Proposed mechanisms include induction of apoptosis, cellular clearance by the reticuloendothelial system through bound antibodies, complement mediated cell lysis, and antibody-dependent cellular cytotoxicity (ADCC) via Fcγ-bearing effector cells. One of the Fc receptors that may be involved in anti-CD20 mediated ADCC is Fc gamma receptor 3A (FcγRIIIA), and *FCGR3A* polymorphisms have been shown to influence the response to rituximab [78, 79]. In studies of patients with lymphoma and SLE, homozygosity for the 158V allele predicted more complete depletion than homozygous 158F or heterozygous 158VF alleles.

In addition to the treatment of hematologic malignancies, rituximab has been used to treat a variety of autoimmune disorders. Rituximab has been licensed for use in rheumatoid arthritis refractory to TNF antagonists in combination with methotrexate after several large clinical trials demonstrated a reduction in disease activity [80-82]. Rituximab has recently been investigated as a treatment for SLE (renal and extrarenal) in randomized, double-blind, placebo controlled trials. However, these studies failed to meet their primary efficacy

endpoints [83, 84]. Small studies and case series have examined the use of rituximab in a variety of the autoimmune diseases including SLE [85-88], autoimmune hemolytic anemia [89], immune or idiopathic thrombocytopenic purpura (ITP) [90, 91], and Sjogren's syndrome [86, 92-99]. Early phase uncontrolled studies have also reported on the use of rituximab in other types of autoimmune disease, including, pemphigus vulgaris [100-103], dermatomyositis [104], polymyositis [105, 106], and others not reviewed in this document. In small, uncontrolled clinical studies investigating the use of rituximab in a total of 420 patients with pemphigus vulgaris overall clinical response rates ranging from 30-90% (with variable definitions of response have been reported [77]. A recent report of a phase II trial in relapsing–remitting multiple sclerosis suggested that rituximab had beneficial effect even though traditionally MS is not felt to be primarily an autoantibody mediated disease [107]. Recently, rituximab has been demonstrated to be efficacious in inducing disease remission in anti-neutrophil cytoplasmic autoantibody (ANCA) associated vasculitis [108, 109].

1.4 Known and Potential Risks of Rituximab Treatment

The label for rituximab includes four "black box" warnings: infusion reactions, severe mucocutaneous reactions, progressive multifocal leukoencephalopathy (PML), and hepatitis B virus reactivation.

1. *Infusion reactions*: Rituximab is associated with infusion-related/acute infusion reactions, which may be due to release of cytokines and/or other chemical mediators. Rituximab-induced reactions and sequelae include urticaria, hypotension, angioedema, hypoxia, bronchospasm, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, anaphylactoid events, or death. Severe infusion-related reactions with fatal outcome have been reported during clinical trials and post-marketing use in oncology patients at a rate of approximately 0.04-0.07% (4 to 7 patients per 10,000). These deaths have primarily been related to the large B cell burden in oncology patients with resultant tumor lysis and cytokine storm after rituximab treatment. Approximately 80% of fatal reactions occur after the first infusion of rituximab, with time to onset of 30-120 minutes. While approximately 32% of Rituxan-treated patients in RA placebo-controlled trials experienced an adverse infusion reaction during or within 24 hours following their first infusion, less than 1% were serious reactions. Since rituximab was first approved in February 2006, there have been four cases of fatal infusion-related reactions in RA patients treated with this agent.
2. *Severe mucocutaneous reactions*: Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, paraneoplastic pemphigus, and toxic epidermal necrolysis have been reported in patients treated with rituximab. These reactions are rare, and the causal relationship to rituximab is difficult to determine, especially given the multiple medications these patients receive and the various autoimmune syndromes associated with non-Hodgkin's lymphoma [110]. Reports of these events show variable onset time relative to rituximab exposure, with onset occurring as early as the day of first infusion. Some mucocutaneous reactions have been fatal. These reactions

have been reported very rarely in patients with autoimmune diseases treated with rituximab. A fatal outcome was reported in less than 1 in 100,000 patients in the post-marketing setting [77].

3. *Progressive multifocal leukoencephalopathy (PML):* PML has been reported in patients with autoimmune disease after receiving rituximab. PML is a demyelinating disease caused by the JC virus, a human polyoma virus. Approximately 80% of the population is seropositive for JC virus, which persists in a latent form but can reactivate in immunosuppressed patients. PML has been reported in a variety of immunosuppressed or immunodeficient patients including transplant patients, patients receiving chemotherapy, and HIV-infected patients independent of treatment with rituximab, and most recently in patients receiving natalizumab [111, 112]. Fatal cases of PML have also been reported in patients with hematologic malignancies and a variety of autoimmune diseases, including systemic lupus erythematosus (SLE, lupus), RA, and vasculitis following rituximab treatment. In general, the patients with autoimmune diseases had prior or concurrent immunosuppressive therapy. Most cases of PML were diagnosed within 12 months of the last infusion of rituximab. There have been 3 fatal reports of PML in patients with RA who had received rituximab (3 reports in 100,000 patients with RA exposed to rituximab), with one case in a patient exposed to rituximab who had not received prior TNF antagonist therapy but had been treated with leflunomide. The role of rituximab alone in the development of PML is unclear. The risk of development of PML is unknown for individuals with SSc-PAH treated with rituximab. Recognition of PML requires a high index of suspicion. Patients who are considering or who have taken rituximab should be informed about the risks of developing PML, including that there is no effective treatment for PML. Patients who have been treated with rituximab should contact their doctor if they present with new neurological manifestations (including, but not limited to: confusion, dizziness, loss of balance, difficulty talking or walking or visual problems). Consultation with a neurologist, brain MRI, and lumbar puncture should be considered as clinically indicated.
4. *Hepatitis B Virus Reactivation*: HBV reactivation has been reported in patients treated with CD20-directed cytolic antibody drugs, which include rituximab. In some cases this has resulted in fulminant hepatitis, hepatic failure, and death. Cases are not limited to hepatitis B surface antigen (HBsAg) positivity, but have also been reported in patients who are HBsAg negative but hepatitis B core antibody (anti-HBc) positive. Additionally, reactivation has occurred in patients with apparent resolved hepatitis B infection (HBsAg negative, anti-HBc positive and hepatitis B surface antibody [anti-HBs] positive).

In addition to risks communicated explicitly by the “black box” warnings, the package insert for rituximab reports adverse reactions observed in controlled trials. Events that seem to be specific to use in malignancy are Tumor Lysis Syndrome (TLS), severe renal toxicity, and bowel obstruction/perforation. Cases of acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, and hyperphosphatemia, some fatal, have been reported within 24 hours after first infusion. The risk of TLS correlates with number of circulating malignant cells or high

tumor burden. Abdominal pain, bowel obstruction, and perforation have occurred in patients receiving rituximab in combination with chemotherapy. Some of these cases were fatal.

Adverse reactions observed in trials with RA patients have been reported separately from those observed in malignancy trials. Information to date reflects experience in 2578 RA patients treated with rituximab in controlled and long-term studies (total exposure of 5014 patient-years). RA patients are likely to be more similar to the patients with SSc-PAH studied in this protocol due to the autoimmune association of the disease. Adverse events (AEs) occurring at a rate > 2% in RA patients who were given rituximab (or 1% greater than placebo), per the package insert are listed below:

1. The common adverse events occurring in rituximab treated patients (greater than 10%) included infusion reactions, upper respiratory infection, nasopharyngitis, urinary tract infection, and bronchitis.
2. Infusion reactions – In studies enrolling subjects with RA, 32% of rituximab-treated patients experienced an adverse reaction during or within 24 hours following the first infusion, compared to 23% of placebo-treated patients. This frequency decreased to 11% for rituximab-treated patients and 13% for placebo-treated patients for adverse events occurring within 24 hours following the second infusion. Serious acute infusion reactions were experienced by < 1% of patients in either the rituximab or placebo treatment groups. Acute infusion reactions requiring dose modification (stopping, interrupting, or slowing the infusion) occurred in 10% of rituximab treated patients and 2% of placebo treated patients. Acute infusion reactions (fever, chills, rigors, pruritis, urticaria/rash, angioedema, sneezing, throat irritation, cough, and/or bronchospasm with or without hypotension or hypertension) occurred with the first infusion in 27% of the rituximab treated patients and 19% of the placebo treated patients. Severe infusion reactions typically occurred during the first infusion with time to onset 30 to 120 minutes after starting the infusion. These reactions may include hypoxia and cardiovascular collapse, angioedema or bronchospasm, pulmonary infiltrates including acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. Milder infusion reactions include fever and chills, flushing, fatigue, pallor, headache, rhinitis, nausea and vomiting, tachycardia, tachypnea, dyspnea, chest pressure, and back pain. These reactions are similar to those described for IV immunoglobulin (IVIG). With IVIG, some patients are more sensitive to infusion reactions but “sensitivity” and “non-sensitivity” are relative terms since slow infusions are tolerated by “sensitive” individuals [113]. Moreover, “sensitive” patients can become desensitized to the infusion reaction, i.e. if several infusions were given over a short period of time the infusion reaction disappeared or at least became less severe. There are some data indicating that prophylactic glucocorticoids may ameliorate these reactions [114, 115].
3. Infections – In the pooled studies, infections occurred in 39% of rituximab treated patients and 34% of placebo treated patients. Serious infections occurred in 2% of rituximab treated patients and 1% of control patients. The most common infections were nasopharyngitis, upper respiratory tract infections, urinary tract infections, bronchitis, and sinusitis. The numbers of upper respiratory tract and rhinitis infections were slightly higher in the rituximab group (showed an absolute increase over placebo of at least 1%).

In the experience with rituximab in RA patients the most common serious infections (≥ 0.5%) were pneumonia or lower respiratory tract infections, cellulitis, and urinary tract infections. Fatal serious infections included pneumonia, sepsis and colitis [116].

4. Cardiac events – Serious cardiovascular events occurred in 1.7% of rituximab treated patients and 1.3% of placebo treated patients (pooled data). Cardiovascular deaths occurred in 0.4% of rituximab treated patients and 0% of placebo treated patients. Three cardiovascular deaths occurred during RA studies (all rituximab regimens) as compared to none in the placebo group. Myocardial infarction (MI) rates were consistent with rates in the general RA population. Patients with significant PAH may have baseline hypotension. In addition, the therapies used to treat PAH can cause hypotension. Exacerbation of hypotension in subjects with SSc-PAH treated with rituximab should be anticipated given the potential for the infusion itself to precipitate cardiac events.
5. Hypophosphatemia and hyperuricemia – In the pooled data, newly occurring hypophosphatemia was observed in 12% of patients receiving rituximab compared to10% of patients on placebo. Newly occurring hyperuricemia was observed in 1.5% of patients receiving rituximab and 0.3% of patients on placebo. The RA specific data showed newly-occurring hypophosphatemia in 21% of patients, with newly occurring hyperuricemia in 2% of patients. The majority of observed hypophosphatemia occurred at the time of the infusions and was transient.
6. Immunogenicity – Approximately 11% of rituximab treated RA patients tested positive for human anti-chimera antibody (HACA). Of 8 patients who had a serious infusion reaction, 2 were HACA positive. Approximately 12% of HACA positive patients who were re-treated with rituximab had a subsequent infusion reaction.

The implications of these observed events call for further discussion. For example, determining the effect of rituximab on infections is problematic since patients treated with rituximab often have concomitant immunodeficiency and/or are on immunosuppressive drugs. In patients treated for B cell lymphomas, the incidence of serious infections (grade III or IV including sepsis) is about 2%. One reassuring finding has been the observation in several studies that total serum IgG and levels of protective IgG antibodies are generally maintained after treatment with rituximab [117, 118]. Cases of hypogammaglobulinemia have been reported [119-122]. Criteria supporting the use of IVIG should probably be similar to those used for other patients with secondary humoral immunodeficiency, for example: (i) low levels of protective antibodies to encapsulated organisms, viruses, or bacterial toxins without a response to immunization; (ii) severe hypogammaglobulinemia (serum IgG < 200 mg/dL); and (iii) increased rate, severity, or duration of infections, especially infections of the respiratory system or with encapsulated organisms including, but not limited to, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitides* [123, 124].

Serious viral infections, either new, reactivated, or exacerbated, have been reported after use of rituximab including CMV, herpes simplex virus, West Nile virus, and hepatitis C. Some of these infections resulted in fatal outcomes up to 1 year following discontinuation of rituximab [116]. Most of these serious viral infections occurred in patients with hematologic malignancies receiving combination chemotherapy with rituximab.

An expected complication of rituximab therapy is failure to respond to vaccines. This was originally described in non-human primates treated with rituximab but has been confirmed in lymphoma patients, patients on dialysis, and patients with SLE [125-128]. Additional studies are needed to determine when this effect resolves. Inhibition of humoral immune responses poses a special problem with unusual pathogens including, but not limited to, parvovirus B19 or infections where toxins are important. Subjects in this trial will be vaccinated against pneumococcal pneumonia before entry. Subjects enrolled in this trial will continue to receive annual influenza vaccines and will be informed that the vaccine may not be fully protective. Vaccination with live vaccines is not recommended, as the safety of immunization with live vaccines following rituximab treatment has not been studied. Non-live vaccinations should be given at least 4 weeks prior to administration of rituximab.

Although neutropenia and thrombocytopenia have been associated with rituximab, cases are generally transient and do not cause clinically significant problems. Late onset neutropenia (LON) with rituximab has been described in patients treated with aggressive chemotherapy for hematological malignancies, but no such cases have been reported in patients with autoimmune disease.

LON may also predispose one to infections. There are a number of reports of LON after treatment of lymphomas with rituximab [129]. In a recent report, LON was investigated in 130 patients with B cell lymphoma receiving chemotherapy with or without rituximab [130]. LON was detected in 6 of 76 patients receiving rituximab and 0 of 54 patients not receiving rituximab. The median nadir neutrophil count for the patients who developed LON was 200; the lowest counts were 23 and 32. Onset occurred at an average of 175 days (77-204 days) post treatment and lasted an average of 14 days (11-16 days). One patient developed a buccal cellulitis; the other 5 patients were asymptomatic. Neutropenia responded to growth factors. Preliminary data suggests an association between B cell recovery and granulocyte decline. These changes in B cell recovery and granulocyte decline were also correlated with changes in the level of SDF-1, a chemokine affecting both B cell and granulocyte homeostasis. Since blood counts were only checked every 3 months, the authors estimated that they probably missed more cases of neutropenia than they found. Whether LON will be seen in non-malignant disease treated with rituximab is unclear, but until we have more data, neutrophil counts should be carefully monitored.

Rituximab is a humanized antibody but its variable regions are still of mouse origin. Therefore an immune response against rituximab (HACAs) can be seen. The clinical relevance of HACAs as a result of treatment with rituximab is unclear. Infusion reactions and rapid clearance have been linked to HACAs against other humanized monoclonal antibodies, including infliximab [131]. With infliximab, higher dose and/or combination with methotrexate reduced the occurrence of HACAs. However, 2 studies have been performed suggesting that subjects who develop serum antibodies to infliximab bind unique epitopes that do not cross-react with other antibodies, including rituximab [132, 133]. Anti-rituximab HACAs are rare among patients with lymphomas treated with this drug, and these HACAs are generally of low titer [134]. However, in a phase I/II dose escalation study of rituximab for SLE, high titer HACAs were detected in 6 of 17 patients [86]. Several factors may have

contributed to this unusual rate of HACA formation including the low dose of rituximab in the first two dose groups, the use of oral rather than IV steroids as prophylaxis for infusion reactions, and the use of low dose or no immunosuppressive drugs in many of the patients. In summary, while it is possible that recipients of rituximab may develop hypersensitivity, which may extend to other proteins of mouse origin, and thus may not be able to use other chimeric monoclonal antibodies or mouse-derived antibodies in the future, it has not been definitively shown that hypersensitivity to one chimeric molecule precludes someone from receiving another. For additional information on AEs observed to date with study drug and active controls, see the Rituxan Prescribing Information at http://www.rituxan.com/rituxan/pi.

Rare serious adverse events (SAEs) have occurred in patients following completion of rituximab infusions: arthritis, disorders of blood vessels (vasculitis and lupus-like syndrome), lung disorders (including pleuritis, scarring of the lung and bronchiolitis obliterans), and eye disorders (uveitis and optic neuritis). Patients with pre-existing cardiac conditions, including arrhythmia and angina pectoris, have had recurrences of these cardiac events during rituximab infusions. Abdominal pain, bowel obstruction, and perforation have been reported in patients receiving rituximab in combination with chemotherapy for malignancy.

Serum sickness, a syndrome consisting of one or more of the following has been reported: fever, joint pain, skin rash or hives, hematuria, or hypocomplementemia. The syndrome may occur as long as several weeks after the first infusion. This syndrome is believed to be caused by deposition in tissues of rituximab-anti-rituximab immune complexes [135-138].

Rituximab is a Category C drug. Reproduction studies in primates at human maternal exposure dose levels showed no teratogenetic effects. There are no adequate and well-controlled studies of rituximab in pregnant women. Postmarketing data indicate that B cell depletion can occur in infants exposed in-utero to rituximab, which generally resolves in less than six months. Rituximab has been detected in the serum of infants exposed in-utero.

1.5 Rationale for Study

The most effective treatments for SSc target the vascular complications and inflammatory processes that characterize this disease [139]. One of the most feared complications of SSc, SSc-PAH, demonstrates both vascular and inflammatory pathology [140], and yet, a randomized clinical trial specifically directed at this process has not been performed. Of note, the findings of a major study have just been published demonstrating that patients with SSc-associated interstitial lung disease enjoy a modest but significant beneficial effect with cyclophosphamide treatment [141]. SSc-PAH is associated with disabling dyspnea as well as significant mortality. An increasing body of experimental and clinical evidence is implicating dysregulated B cells and pathogenic autoantibodies in the pathogenesis of SSc [25, 54, 55, 142]. A recently described animal model of PAH has shown that autoimmune injury, characterized by pulmonary B cell accumulations and anti-endothelial cell antibody deposition on pulmonary vasculature, results in severe PAH that is similar to the human condition [57]. In another related preclinical model, correcting immune dysregulation

attenuates pulmonary B cell accumulation and antibody deposition, and greatly ameliorates PAH [143]. Finally, in clinical practice, rituximab has been anecdotally reported to be effective in several forms of PAH [144, 145] including SSc-PAH [67]. Given the strong scientific evidence implicating autoimmune vascular injury in disease pathogenesis, the expanding number of cases where rituximab has been highly effective in autoimmune-associated PAH, the poor overall prognosis of patients with SSc-PAH, the acceptable risk-benefit ratio of rituximab in this patient population, and the newly emerging and promising pre-clinical data, there is strong rationale for a trial testing the efficacy of B cell depletion in SSc-PAH.

Change in 6MWD has been chosen as the primary efficacy endpoint for this study as it is a commonly used functional measure of disease severity that has been previously used by the FDA for licensure which will make it more comparable to past trials. For more information about the change in primary endpoint from pulmonary vascular resistance (PVR) to 6MWD, see Section 8.1, *Sample Size*. A recent analysis was performed by Mathai (2012) to find the minimally important difference in 6MWD at 16 weeks. An analysis of 405 subjects enrolled in the Pulmonary Arterial Hypertension and Response to Tadalafil trial showed the estimated consensus minimally important difference (MID) at 16 weeks was 33m in subjects with PAH; therefore 33m was chosen as a minimally important distance for this trial. This study is also of longer duration than many previous trials in the field. Unlike vasodilating agents, B cell depletion may not have an immediate effect on PAH. Twenty four weeks after B cell depletion, vascular remodeling may have occurred resulting in disease stabilization or improvement or skin involvement could be improved, which could result in an increased 6MWD. Measurement of the clinical endpoints at this time, distant from study drug administration, may permit detection of the largest difference in the study endpoints between the 2 arms. In addition, measurement of the clinical and hemodynamic endpoints at 24 weeks will allow an assessment of durability of response to the study intervention. A relative increase of 20% in 6MWD or decrease of 30% in PVR (i.e. compared to placebo-treated controls) would suggest disease remission and could translate into improved survival.

1.5.1 **Rationale for the Entry Criteria**

The objective of this trial is to determine if rituximab has a beneficial effect on clinical disease progression with an acceptable level of toxicity in subjects with SSc-PAH.
The inclusion criteria are designed to ensure that all subjects have well documented, relatively recent onset PAH.

- Early in disease, the vascular lesion is assumed to have an inflammatory component potentially responsive to rituximab. It is unclear how long the inflammatory component persists. However, there is persistent B cell dysregulation as evidenced by the persistence of autoantibodies. Because lung disease in these patients may be heterogeneous with some areas being more actively inflamed than others, it is possible that B cell depletion may impact individual patients in a heterogeneous fashion. Patients that have more advanced and possibly irreversible disease in certain areas of their lungs may also have actively inflamed regions in other anatomic

pulmonary locations; these latter areas may be more susceptible to benefit from B cell depletion.

- In order to optimize the ability to detect a treatment effect, subjects should have sufficient disease burden attributable to SSc-PAH to cause at least mild symptoms manifested by some limitation of physical exertion with maximal effort. However, the need to also include subjects with early SSc-PAH implies that an upper limit of the six minute walk distance should not be defined. The entry criteria attempt to balance the need to include subjects with early SSc-PAH and the need to detect a treatment effect of rituximab in addition to standard therapy. Therefore, subjects with Class II as well as Class III and IV heart failure are included in this trial. In an attempt to include subjects with early disease but with definitive SSc-PAH and poor prognosis, the study entry mPAP has been defined as a lower limit of $\geq$ 25 mmHg.
- NYHA functional class IV subjects have been included in a number of previous clinical trials in PAH. These patients are by definition severely impaired functionally, and tend to have a poor prognosis. Most subjects with functional class IV disease who are being considered for enrollment in this study would likely have failed other currently available therapies. Even understanding that such patients may be at increased risk for complications during or immediately following the infusion of rituximab, the risk/benefit ratio in this setting is acceptable, especially in light of the additional precautions being taken to optimize the safety of these severely ill patients.
- Subjects must be on stable doses of standard of care to ensure the changes seen during the study do not reflect the confounding effect of changes in concurrent PAH therapy, but rather use of the study drug.
- Subjects should be competent adults able to understand the risks of participation in the study and capable of making medical decisions.
- The upper age of the subjects permitted to enter this study is in line with what has been deemed safe in other rituximab trials in RA, but has been limited to 75 in order to avoid comorbidities of advanced age (for a population already at greater risk due to pre-existing cardiorespiratory compromise) [116].

The exclusion criteria are designed to protect the subjects' safety by excluding those who are at risk for adverse events either because of the severity of their disease, their predisposition to infection or to the presence of co-morbid disease and to ensure a uniform study population.

- Subjects with longer-standing disease (i.e. beyond 5 years) are excluded because it is hypothesized that the late fibroproliferative component of SSc may be less responsive to immunotherapy than the early inflammatory vascular lesions. In order to evaluate a change within 6 months of entry, subjects with steadily progressing disease are required.
- Subjects must have PAH secondary to SSc only. Interstitial lung disease is a common pulmonary manifestation of SSc that can be associated with "secondary" pulmonary hypertension for reasons likely distinct from those factors causing "primary" SSc-PAH. Therefore subjects with significant interstitial lung disease (ILD) resulting in secondary PAH are excluded from this trial. Subjects with mild ILD are permitted in the study. Diagnostic criteria for mild ILD have been developed. Subjects with other

diseases resulting in PAH are excluded from this trial, as are subjects with elevated pulmonary capillary wedge pressures whose PAH may be the result of left heart failure, pulmonary venous occlusion, or mitral valve disease and not SSc.

- Because rituximab is an immunocompromising agent, subjects who have received recent live vaccines within 2 months, have any evidence of severe bacterial infections as defined in the entry criteria or chronic infections (HIV, TB, Hepatitis B or C), will be excluded from this trial.
- Rituximab has been associated with cardiovascular deaths, and cardiac disease is a warning specifically mentioned in the prescribing information. Therefore, a recent history of non-SSc-PAH-related heart disease excludes patients from entry into this study.
- Patients with a prior history of anaphylaxis or IgE-mediated hypersensitivity to murine proteins or any component of rituximab will be excluded. In addition, in order to keep the population uniform in terms of disease status, the trial is targeting subjects who have not received prior lymphocyte depleting agents.
- Subjects who are at increased risk for adverse events based on the presence of co-morbid disease will be excluded from this trial including renal disease even if secondary to scleroderma, recent malignancy, pregnancy, and blood dyscrasias.

Subjects who are unable to comply with the demands of this protocol for either physical or psychiatric reasons are also excluded.

1.5.2 **Rationale for Mechanistic Studies**

Currently serum biomarkers are not available to reliably follow the clinical course of SSc-PAH. The ASC01 mechanistic studies aim to evaluate several markers of disease and the change associated with rituximab treatment: biochemical indicators of heart failure severity (BNP/NT-proBNP), B cell numbers, and inflammatory markers. Because hyponatremia has recently been demonstrated to predict right heart failure and poor survival in PAH [146], we will monitor serum sodium levels throughout the trial. BNP/NT-proBNP is already an established marker useful in following heart failure in SSc-PAH [147] and will be used to follow response to therapy in this trial. Because CD19 is co-expressed on CD20 B cells, we expect numbers of CD19+ cells will fall following rituximab therapy. CD19+ cells will be measured to track the repopulation of B cells [148, 149] as well as to determine if B cell clones are present in SSc-PAH and what impact rituximab may have on these clones. With falling B cells, it is possible that serum immunoglobulins will also fall, and therefore the decline and eventual return of immunoglobulin subclasses will be monitored in this trial. With regard to following inflammatory biomarkers, both autoantibodies and serum cytokines will be measured on a regular basis. Anti-U1RNP, anti-U3RNP, anti-B23, anti-cardiolipin, anti-CENP-B have been reported to be elevated in SSc-PAH [150, 151] and will be followed at several time points to correlate disease activity with autoantibody levels. Because no single autoantibody has proven sufficient to track SSc-PAH activity, we will determine whether a panel of autoantibodies provides a more specific and sensitive measure. Finally, serum IL-1

and IL-6 are known to be elevated in severe primary pulmonary arterial hypertension [152] and may similarly be elevated in SSc-PAH. Other pro-inflammatory cytokines may be similarly elevated. Pilot studies done at Stanford University were performed using a multiplex analysis of serum. Preliminary evidence pointed to distinct inflammatory profiles in patients with PAH as compared with healthy controls, as well as sub-groups of PAH etiologies as compared to each other. For this reason, a similar multiplex approach for SSc-PAH patients could identify an important inflammatory signature, as well as provide biomarkers of inflammatory resolution following B-cell depletion. Thus, measures of both an autoantibody panel and a panel of cytokines may provide a peripheral (and therefore accessible) assay of systemic inflammation. The mechanistic studies are designed, in a straightforward manner, to measure B cells, heart failure and inflammatory changes over the course of the clinical trial.

2 STUDY OBJECTIVES AND PURPOSE

The objective of this trial is to determine if rituximab has a marked beneficial effect on clinical disease progression relative to placebo, with minimal toxicity, in subjects with SSc-PAH.

2.1 Primary Objective

- To compare patients with SSc-PAH treated with rituximab to those on placebo for change in exercise capacity, as determined by 6-minute walk distance (6MWD).

Hypothesis: The treatment group will demonstrate improvement or stabilization relative to the placebo arm in 6MWD from baseline to 24 weeks post-treatment initiation.

2.2 Secondary Objectives

Secondary Efficacy Objectives:

- To compare patients treated with rituximab to those on placebo for change in exercise capacity, as determined by 6MWD, at time points other than 24 weeks.
- To compare patients treated with rituximab to those on placebo for change in PVR as assessed by right heart catheterization.
- To compare patients treated with rituximab to those on placebo for time to clinical worsening, defined as first occurrence of:
    a. death,
    b. hospitalization for SSc-PAH,
    c. lung transplantation,
    d. atrial septostomy,
    e. addition of other PAH therapeutic agents, or
    f. worsening of 6MWD by > 20% and a decrease in functional class.
- To compare patients treated with rituximab to those on placebo who changed or added other PAH therapeutic agents.

- To compare patients treated with rituximab to those on placebo for change in quality of life as determined by the short form 36 (SF-36) and the disability index of the Scleroderma Health Assessment Questionnaire (SHAQ).
- To compare patients treated with rituximab to those on placebo for change in number of new digital ulcers.
- To compare patients treated with rituximab to those on placebo for change in severity of Raynaud phenomenon as measured by the VAS scale of the SHAQ.
- To compare patients treated with rituximab to those on placebo for change in DLCO and room air oxygen saturation at rest .

A secondary objective of this study is to determine whether the effects on clinical disease progression are paralleled by changes in selected biomarkers.

Secondary Safety Objectives:

- To assess the safety and tolerability of rituximab for the treatment of SSc-PAH in patients receiving stable background medical treatment with prostanoid, endothelin receptor antagonist, PDE-5 inhibitor therapy, and/or guanylate cyclase stimulators.

Mechanistic Study Objectives:

- CD19 Studies
  - To determine whether the extent and/or duration of CD19+ B cell depletion correlates with treatment response as measured by PVR (right heart catheterization), exercise capacity (6MWD), and quality of life (SF-36 and SHAQ).
  - To monitor and correlate the reconstitution patterns of B cell subpopulations with treatment response.
- To determine if the biomarkers anti-U1 RNP, anti-U3 RNP, anti-B23, anti-cardiolipin, anti-Th/To, anti-CENP-B, RF, and other autoantibodies, and quantitative immunoglobulin levels, including IgG subclasses, correlate with treatment response as measured by PVR (right heart catheterization), exercise capacity (6MWD), and quality of life (SF-36 and SHAQ).
- To determine if the biomarkers BNP/NT-proBNP, serum Na^+, IL-1, IL-6, and other cytokines correlate with treatment response as measured by PVR (right heart catheterization), exercise capacity (6MWD), and quality of life (SF-36 and SHAQ).
- To determine if B cell clones are present in SSc-PAH and what impact rituximab may have on these clones.
- To determine if B cell clones present in SSc-PAH are correlated with clinical outcomes.

2.3 Exploratory Objectives

- To evaluate agreement between PVR calculated by site-based standards and a standardized Fick-based calculation of PVR.

- To assess sensitivity of the secondary efficacy analysis to standardization of the PVR calculation.
- To assess the utility of a standardized derived pulmonary artery capacitance as a primary efficacy endpoint.
- To evaluate the relationship between 6MWD, PVR, and other clinical measures.

3 STUDY DESIGN

3.1 Description of Study Design

This is a prospective, double-blind, placebo-controlled, multi-center, randomized trial to evaluate the effect of rituximab on disease progression in subjects with SSc-PAH receiving concurrent stable-dose standard medical therapy with a prostanoid, endothelin receptor antagonist, phosphodiesterase 5 (PDE-5) inhibitor, and/or guanylate cyclase stimulators. The study will focus on assessment of clinical response and safety measures longitudinally. In addition, the effects of treatment with rituximab on the underlying immune mechanisms associated with B-cell dysregulation and pathogenic autoantibody response in this disease will be investigated.

Subjects with SSc-PAH with elevated mean pulmonary artery pressure as measured by right heart catheterization will be enrolled. The diagnosis of SSc-PAH should be confirmed by a rheumatologist experienced in the diagnosis and treatment of systemic sclerosis in conjunction with a pulmonologist or cardiologist specializing in management of PAH. Both specialists will be part of the study team at each site.

Rituximab/placebo will be administered as two IV infusions of 1000 mg each, given two weeks apart at Day 0 and Week 2. All subjects will receive 40mg of prednisone orally the night before and morning of each infusion, as well as 100 mg methylprednisolone or equivalent corticosteroid intravenously approximately thirty minutes prior to each infusion with diphenhydramine and acetaminophen orally approximately thirty to sixty minutes prior to each infusion of rituximab or placebo. Subjects will remain on their baseline standard medical regimen.

Sixty eligible subjects will be accrued. Each potential study subject will provide written informed consent prior to screening procedures. All inclusion and exclusion criteria (Section 4, *Selection of Subjects*) must be met at time of randomization prior to receipt of the first dose of rituximab/placebo (Day 0, Treatment Initiation).

Clinical assessments and sample collection will occur at regularly scheduled visits through Week 48 with telephone follow-up conducted in the interim. If a subject has not recovered B cells by Week 48, B cell studies will be conducted quarterly until recovery is documented or for 2 years after initial treatment (whichever occurs first). Recovery is defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available then recovery is defined as a B cell level of at least 90% of the lower limit of

normal. During this quarterly monitoring period of B cell recovery, AEs and SAEs will be assessed, providing the subject has not withdrawn consent, to capture any infectious event ≥ Grade 3 using the National Cancer Institute - Common Terminology Criteria for Adverse Events (NCI-CTCAE). No additional study-related data will be collected. Vital status on all subjects will be evaluated ≥ 2 years after randomization.

The primary efficacy endpoint is the change in 6MWD from baseline to 24 weeks after treatment initiation. Hemodynamic measures will be assessed at baseline and Week 24, contributing to the understanding of the relationship between PVR and clinical endpoints. Initiation of new PAH therapy due to disease worsening prior to Week 24 will prompt an endpoint visit and right heart catheterization prior to initiation of the new therapy. B cell recovery for all will be monitored by serial assessments of B cell counts as determined by flow cytometry.

The total duration of the study is anticipated to be approximately 8 years.

3.1.1 **Stratification, Randomization, and Masking**

Subjects will be randomized in a 1:1 ratio to receive 2 infusions 14 days apart of either rituximab 1000 mg (treatment arm) or placebo (control arm). The automated randomization system will implement an adaptive randomization procedure to maintain balance between treatment groups overall, by study site, with respect to disease severity as measured by PVR at baseline, and with respect to time since diagnosis of SSc-PAH.

To maintain study blind, the appearance of the packaging and solutions used to administer study drug/placebo will be identical in both study arms.

All clinical and immunology laboratory staff will be masked to the treatment assignments until the completion of the study. In addition, immunology laboratory staff will not have access to any clinical results and clinical staff members, including the investigators, will not have access to any immunology lab results until completion of the study. B cell recovery status and the need for quarterly monitoring visits as defined in Section 6.5.4.12, *Visit 16 [Week 48]* will be communicated to clinical staff upon completion of Visit 16 or Early Endpoint visit. If B cells have not recovered by two years after treatment initiation, B cell levels will be provided to the clinical staff.

Individual treatment assignments will not be unmasked until all subjects have completed the study. In the event a treatment assignment needs to be unmasked for safety reasons, the site PI will notify the SACCC, who will consult with the DAIT medical monitor. The DAIT medical monitor will review the circumstances and grant approval as indicated. In the event of a true medical emergency necessitating immediate unmasking during off-hours, the site pharmacist will confer with the study PI or designee prior to release of the treatment assignment to emergency treating personnel.

3.2 Description of Endpoints

3.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint is change in exercise capacity, assessed by 6MWD, from baseline to 24 weeks after treatment initiation.

3.2.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following clinical endpoints:

1. Change in PVR measured by right heart catheterization from baseline to 24 weeks after treatment initiation.
2. Exercise capacity, as determined by 6MWD, assessed longitudinally over the 48 weeks following treatment initiation.
3. Assessment of time to clinical worsening, censored at 48 weeks, defined as first occurrence of:
    a. death,
    b. hospitalization for SSc-PAH,
    c. lung transplantation,
    d. atrial septostomy,
    e. addition of other PAH therapeutic agents, or
    f. worsening of 6MWD by > 20% and a decrease in functional class.
4. Percentage of subjects who changed or added PAH therapeutic agents at 24 and 48 weeks.
5. Change in quality of life as determined by the SF-36 and disability index of the SHAQ from baseline to 24 and 48 weeks after treatment initiation.
6. Number of new digital ulcers assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
7. Severity of Raynaud phenomenon, as measured by the VAS scale of the SHAQ, assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
8. DLCO and oxygen saturation at rest on room air assessed longitudinally over both the 24 and 48 weeks following treatment initiation.
9. Change in PVR from baseline to 24 weeks after treatment initiation as a percentage of the baseline value.

Secondary efficacy endpoints also include the following mechanistic endpoints:

1. CD19 Studies
    a. CD19+ B cells (absolute number and percentage of gated lymphocytes) at time of B cell depletion and longitudinally over the 48 weeks following treatment initiation, where B cell depletion is defined as < 5 CD19+ cells/μL.
    b. Time to repopulation of CD19+ B cells, defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available then recovery is defined as a B cell level of at least 90% of the lower limit of normal.

c. Reconstitution pattern of B cell subpopulations assessed longitudinally over the 48 weeks following treatment initiation.

2. Level of anti-U1 RNP, anti-B23, anti-cardiolipin, anti-CENP-B, RF autoantibodies, and immunoglobulins, including IgG subclasses, measured by ELISA and assessed longitudinally over the 48 weeks following treatment initiation.
3. Presence or absence of anti-U3 RNP, anti-Th/To, and other autoantibodies assessed longitudinally over the 48 weeks following treatment initiation.
4. Level of BNP/NT-proBNP, serum Na^+, IL-1, IL-6, and other cytokines assessed longitudinally over the 48 weeks following treatment initiation.
5. Presence or absence of B cell clones assessed at baseline and longitudinally over the 48 weeks following treatment initiation.

3.2.3 **Secondary Safety Endpoints**

The safety endpoints will include the following:

1. All NCI-CTCAE Grade 3-5 AEs, which are defined as possibly, probably, or definitely related to rituximab.
2. All NCI-CTCAE Grade 3-5 adverse events.
3. All infection related events.
4. Treatment-related mortality, defined as death occurring at any time after randomization and possibly, probably, or definitely resulting from treatment given in the study. Study investigators will make an initial determination of the cause of death of any subject and if uncertainty remains, a final decision will be made by an independent blinded panel after reviewing all clinical and, if available, autopsy data.
5. All-cause mortality, defined as any death occurring at any time after randomization.
6. CD19+ B cell levels post treatment (note that analyses will be performed regularly per Table 6.1 through 48 weeks and quarterly thereafter as documented in Protocol Section 3.1, *Description of Study Design*).
7. Peripheral blood cell counts, liver function, and creatinine levels after treatment.

3.2.4 **Exploratory Endpoints**

Exploratory analysis of standardization of the PVR calculation will be based on PVR calculated from a standardized Fick calculation of cardiac output, which is a function of hemoglobin, arterial and mixed-venous saturations, and oxygen consumption according to the La-Farge and Miettinen formula. The cardiac output calculation is a function of assumed oxygen consumption according to the La-Farge calculation of values normalized for age, gender, heart rate. Calculation of a standardized Fick cardiac output will also allow for determination of the pulmonary artery capacitance (PACf). See Appendix E, *Exploratory Analysis: Standardized Fick-based Pulmonary Vascular Resistance and Pulmonary Artery Capacitance* for details.

Additionally, an exploratory analysis of the relationship between change in PVR and change in 6MWD will be addressed. Relationships with other clinical endpoints, like change in NT-proBNP, will also be explored.

3.3 Subject Disposition

3.3.1 Definition of Subject Completion

A subject is considered to have completed the study if he/she has completed Visit 16 (Week 48).

3.3.2 Discontinuation of Protocol-Specified Treatment Requirements

Protocol-specified requirements for the treatment of subjects, including regimens for investigational, concomitant, or rescue medications, as well as restrictions for disallowed medications, **will** be discontinued for any individual subject under the following conditions:

1. Any protocol-specified treatment requirement will be discontinued immediately at any time during the study at the request of the subject.
2. Investigator or DAIT/NIAID will discontinue any protocol-specified treatment requirement if the subject's health, safety, and/or well-being are threatened.
3. Protocol-specified treatment requirements for rituximab will be discontinued and replaced with appropriate therapy for any subject who experiences any of the following:
    - Significant cardiopulmonary disease **PROGRESSION** as manifested by new or worsening **Grade 3 or higher** AEs including:
        - Incompletely controlled or life threatening arrhythmias
        - Vasovagal episode occurring either during or within 12 hours following an infusion
        - Sinus bradycardia unresponsive to atropine treatment as detailed in Section 5.4.3.2, *Cardiovascular*
        - Chest pain consistent with angina or evidence of myocardial infarction
        - Cardiopulmonary arrest
        - Sustained systemic hypertension requiring additional therapy above baseline medications
        - Sustained hypotension
        - Congestive heart failure worsening from baseline level
        - Pericardial effusion or pericarditis
        - Pneumonitis progressing above baseline level
        - Bronchospasm
        - Dyspnea progressing above baseline level
        - Stridor or other indicators of respiratory compromise
    - New symptomatic pericarditis Grade 2 or higher
    - Toxicities of rituximab, if required as per Sections 5.3.5, *Criteria for Discontinuing Rituximab Infusion* and 5.4.3, *Management of Infusion Reactions/Known Toxicities to Rituximab*
4. Disease progression requiring change in Concurrent Medication as per Section 5.7, *Concurrent Medications and Therapy.*

3.3.2.1 **Follow-up Requirements for All Treated Subjects**

Independent of whether treated subjects have completed the full course of rituximab, the following procedures will apply:

1. If prohibited medications as defined in Section 5.6, *Prohibited Medications and Treatments* are not needed for treatment of progressive PAH, the subject will complete all scheduled study visits including all exams, procedures, assessments, and tests according to the protocol.
2. If prohibited medications as defined in Section 5.6, *Prohibited Medications and Treatments* (i.e. addition of new or a change in the dose of background PAH therapies [prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators] or surgical therapy) are needed for treatment of progressive PAH prior to the Week 24 visit, or if the subject withdraws consent, he/she will be asked to complete an Early Endpoint Visit as detailed in Section 6.5.5, *Early Endpoint Visit*.
3. In addition, if the subject has not withdrawn consent and has not achieved B cell recovery, follow-up clinical care must include visits at least quarterly to monitor recovery of B cells. Quarterly monitoring visits will continue until B cell recovery or for 2 years after initial treatment (whichever occurs first). Recovery is defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available then recovery is defined as a B cell level of at least 90% of the lower limit of normal. During this monitoring period, AEs and SAEs will be assessed, providing the subject has not withdrawn consent, to capture any infectious event ≥ Grade 3 using the NCI-CTCAE. No additional study-related data will be collected.

Subjects who have discontinued their concurrent medications prior to Week 24 for toxicity only, and not disease progression, will continue to participate in the study. They will be permitted to change medication for PAH in order to maintain control of their disease and will complete all exams, procedures, assessments, and tests according to the protocol.

3.3.3 **Subject Withdrawal from the Study**

When a subject is withdrawn from the study after initiating treatment but prior to B cell follow-up, protocol-specified treatment requirements are discontinued and study-related visits, exams, procedures, assessments, tests and data collection are terminated after completion of an Early Endpoint Visit as detailed in Section 6.5.5, *Early Endpoint Visit*. However, individual subjects will be withdrawn from the protocol under the following conditions:

1. The subject withdraws consent.
2. The investigator or DAIT/NIAID believes it is in the best interest of the subject.
3. The study is terminated.

Subjects who are lost to follow-up will also be regarded as withdrawn from the protocol.

3.3.3.1 Procedures for Subject Withdrawal

Unless a subject withdraws consent and refuses, all subjects who are being withdrawn or elect to withdraw from the study after initiating treatment will undergo an Early Endpoint Visit as detailed in Section 6.5.5, *Early Endpoint Visit*. In addition, unless consent is withdrawn, B cell monitoring will be performed as outlined in Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects,* #3.

3.3.4 Subject Replacement

Randomized subjects who withdraw from the trial prior to starting rituximab infusion will be replaced. Subjects who discontinue study treatment or withdraw from the trial after initiating the first treatment will not be replaced.

4 SELECTION OF SUBJECTS

Written informed consent must be obtained prior to the subject undergoing any study-related procedure, including screening tests and washout periods for prohibited medications, when applicable.

4.1 Inclusion Criteria

Subjects who meet all of the following criteria are eligible for enrollment into the study:

1. Subject has provided written informed consent.
2. Subject must be between the ages of 18 and 75, inclusive at the time of randomization.
3. Clinical diagnosis of systemic sclerosis (either limited or diffuse cutaneous disease).
4. Diagnosis of SSc-PAH within the past 5 years, with a mean pulmonary arterial pressure (mPAP) of ≥ 25 mmHg at entry.
5. Mean PVR of > 3 Wood units.
6. Screening 6MWD of at least 100 meters.
7. NYHA Functional Class II, III, or IV.
8. Subject must be able to maintain O_2 saturation ≥ 90% at rest (with or without oxygen). Oxygen use is permitted.
9. Subject must be vaccinated with the pneumococcal vaccine at least 4 weeks prior to initiation of therapy, unless subject was vaccinated within 5 years of study entry. If vaccination occurred greater than 5 years prior to study entry, the subject must be revaccinated at least 4 weeks prior to initiation of therapy.
10. Subjects must have been treated with background medical therapy for PAH (prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators) for a minimum of 8 weeks and have been on stable dose(s) of those medical therapy(ies) for at least 4 weeks prior to randomization with no expectation of change for 24 weeks after randomization.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria are disqualified from enrollment in the study:

1. Documented PAH for greater than 5 years at the time of randomization defined as:
    - Measurement of a mPAP > 25 mmHg by right heart catheterization (RHC) at least 5 years previously, OR
    - Treatment with targeted background PAH therapy for > 5 years.
2. Pulmonary Capillary Wedge Pressure (PCWP) > 15 mmHg or Left Ventricular End Diastolic Pressure > 15 mmHg.
3. Persistent hypotension with systolic blood pressure (SBP) < 90 mmHg.
4. Treatment with cyclophosphamide within 4 weeks of randomization.
5. Treatment with immunocompromising biologic agents (including, but not limited to TNF inhibitors, anakinra, abatacept, and tocilizumab) within 4 weeks prior to treatment initiation or treatment with infliximab within 8 weeks prior to treatment initiation.
6. If being treated with a non-biologic immunosuppressive or immunomodulating drug (including, but not limited to, methotrexate, azathioprine, mycophenolate, cyclosporine, hydroxychloroquine, penicillamine, and/or prednisone at ≤ 10mg daily), changes in dosage within 4 weeks prior to randomization. Subjects taking prednisone or equivalent corticosteroid > 10mg daily are excluded.
7. Previous exposure to any lymphocyte depleting agent (Campath, ATG, etc.).
8. Prior treatment with rituximab or other B cell depleting agents.
9. PAH for any reason other than SSc including congenital heart disease, coronary left heart disease, chronic obstructive pulmonary disease, chronic thrombotic and/or embolic pulmonary vascular disease, or sleep apnea.
10. History of coronary artery disease, with any of the following events within 3 years of randomization: significant ventricular tachy-arrhythmia, stent placement, coronary artery bypass surgery, and/or myocardial infarction (MI).
11. Moderate or severe interstitial lung disease as characterized by a total lung capacity (TLC) of < 70% predicted, except if TLC is between 60-70% predicted and an HRCT within the 6 months prior to randomization shows only mild ILD.
12. Chronic infections including, but not limited to, HIV, tuberculosis (TB), hepatitis B (HBV) or hepatitis C (HCV), or chest X-ray (CXR) findings consistent with TB or latent fungal infection.
13. Positive serology for hepatitis B defined by positive HBV surface antigen and/or positive HBV core antibody, total.
14. Positive serology for HCV antibody.
15. A deep space infection within the past 2 years (including, but not limited to, meningitis, epiglottitis, endocarditis, septic arthritis, fasciitis, abdominal or pleural abscess, or osteomyelitis).
16. Evidence of active infection requiring IV or PO antibiotics within 2 weeks of randomization.
17. At or within 30 days of screening,
    - history of or current positive purified protein derivative tuberculin skin test (PPD) (> 5mm induration, regardless of Bacille Calmette Guerin [BCG] vaccine administration), or positive QuantiFERON®-TB Gold In-Tube Test (QuantiFERON®), or historical chest x-ray unless completion of treatment has been documented for active TB

- latent TB (a positive test, a negative chest x-ray, and no symptoms or risk factors), unless one month of prophylaxis has been completed prior to inclusion
- an indeterminate QuantiFERON® unless followed by a subsequent negative PPD or negative QuantiFERON® as well as a consultation with and clearance by local infectious disease (ID) department.

18. Significant renal insufficiency defined as:
    - Estimated creatinine clearance < 40 mL/min (using Cockcroft-Gault formula based on actual body weight); OR
    - Active, untreated SSc renal crisis at the time of enrollment.
19. Recent administration of a live vaccine (< 8 weeks) or any other immunization within 4 weeks of treatment.
20. History of anaphylaxis or IgE-mediated hypersensitivity to murine proteins or any component of rituximab.
21. Pregnancy.
22. Lactation.
23. History of malignancy within the last 5 years, except for resected basal or squamous cell carcinoma, treated cervical dysplasia, or treated in situ cervical cancer Grade I.
24. A woman of childbearing potential (not post-menopausal or surgically sterile) who is unwilling to use a medically acceptable form of birth control (including, but not limited to, a diaphragm, an intrauterine device (IUD), progesterone implants or injections, oral contraceptives, the double-barrier method, or a condom) throughout the duration of the study.
25. History of non-compliance with other medical therapies.
26. A history of alcohol or drug abuse within 1 year of randomization.
27. Receipt of any investigational drug or device within 4 weeks before the screening visit, with the exception of investigational prostanoids, endothelin receptor antagonists, PDE-5 inhibitors, and guanylate cyclase stimulators.
28. Recipient of lung transplant.
29. Laboratory parameters at the screening visit showing any of the following abnormal results:
    - Transaminases > 2x the upper limit of normal (ULN) and/or bilirubin > 2x ULN.
    - Absolute neutrophil count (ANC) < 1,500/mm^3 (or < 1.5 x 10^9/L).
    - Platelet count < 100,000/mm^3 (or < 100 x 10^9/L).
    - Hemoglobin < 9 g/dL.
30. Concurrent treatment in a clinical research study using a non-FDA approved agent, with the exception of an open-label study/study extension of investigational prostanoids, endothelin receptor antagonists, PDE-5 inhibitors, and guanylate cyclase stimulators, provided the open-label investigational drug will be available and dose will remain stable through the ASC01 primary endpoint time point of 24 weeks after randomization in ASC01.
31. Any condition or treatment, which in the opinion of the investigator, places the subject at unacceptable risk as a participant in the trial.

4.2.1 Coenrollment Guidelines

While participating in ASC01, participating in observational registries or cohorts is permitted. Subjects may not be enrolled in another clinical trial, except as noted in Exclusion Criterion #29. For any co-enrollment in studies, the combined blood volumes must not exceed the NIH policy limit nor may the objectives or procedures confound the ASC01 study endpoint assessment.

5 TREATMENT OF SUBJECTS

5.1 Description of Rituximab

5.1.1 Product Description

Rituxan® (rituximab) is a genetically engineered IgG1 kappa chimeric murine/human monoclonal antibody containing murine light- and heavy-chain variable region sequences and human constant region sequences. The chimeric antibody is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. Rituximab is composed of 1,328 amino acids, and has an approximate molecular weight of 145 kilo Dalton (kD) [116]. The antibody reacts specifically with the CD20 antigen found on the surface of malignant and normal B cells, and established B cell lines. Studies have shown that rituximab binds via its Fc domain to human complement and lyses lymphoid B cell lines by complement-dependent cytotoxicity through the induction of apoptosis and via antibody-dependent cell-mediated cytotoxicity [118].

The drug is manufactured by Genentech, Inc. and by Biogen IDEC, Inc. Rituximab is supplied as a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials, which must be diluted before administration [116].

5.1.2 Packaging and Labeling of Study Product

Rituximab labeled for investigational use will be provided without charge by Genentech, Inc. or IDEC Pharmaceuticals. The sponsor of the trial will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all trial drugs in accordance with Title 21 CFR Parts 312.57 and 312.62 and with Genentech/IDEC requirements.

Placebo will be supplied in packaging identical to rituximab.

5.1.3 Storage and Handling of Study Product

Rituximab vials will be stored in a refrigerator at 2° to 8°C (36° to 46°F). Rituximab will not be used beyond the expiration date stamped on the carton and printed on the vial. Rituximab vials will be protected from direct sunlight. The product will not be frozen or shaken.

After dilution to 1-4 mg/mL in normal saline, rituximab is physically and chemically stable at 2° to 8°C (36° to 46°F) for 24 hours, and subsequently for 24 hours at room temperature provided that dilution has taken place in controlled and validated aseptic conditions.
It is strongly recommended that higher concentrations of rituximab (4 mg/mL) be used for this patient population in order to minimize fluid administration.

Placebo will be handled in the same fashion as described above.

5.2 **Dosage Regimen**

Rituximab (1000 mg) or placebo will be administered as 2 infusions given 2 weeks apart.

5.3 **Administration of Rituximab**

The following drugs, safety equipment, and supplies must be available during the rituximab infusions:

- Oxygen
- Oral and endotracheal airways and intubation equipment
- Epinephrine 1:1000 solution for IV or endotracheal injection
- Antihistamines
- Corticosteroids
- IV infusion solutions, tubing, catheters, and tape
- Defibrillator with electrocardiogram (ECG) monitor
- Dopamine
- Atropine
- Bronchodilators

Patients will be closely monitored during the infusions. Guidance for monitoring for infusion reactions includes continuous heart rate monitoring during the infusions, with capability to detect early bradycardia. Vital signs should be monitored frequently as suggested below. During the first infusion, vital signs should be checked every 10 minutes during infusion for the first hour, and then every 15 minutes until the subject is discharged 1 hour after the infusion, or as clinically indicated. For the second infusion, vital signs should be checked every 15 minutes during infusion for the first hour, and then every 30 minutes until the subject is discharged 30 minutes after the infusion, or as clinically indicated. Systolic and diastolic pressures should be should be monitored, with mean arterial pressures (MAP) monitored as clinically indicated. Oxygen saturations should be measured at baseline and then every 30 minutes or as clinically indicated. Study centers may defer to local institutional standards of practice for monitoring hemodynamic and pulmonary status.

Patients with significant PAH typically do not have systemic hypertension, but rather have low blood pressures. Because transient hypotension may occur during rituximab infusion, it is necessary to anticipate an exacerbation of baseline hypotension. Persistent hypotension with a SBP < 90 mmHg is an exclusion criterion for this study. If after randomization on the

day of rituximab administration prior to beginning the infusion the subject's systolic BP is < 90 mmHg or, if measured/calculated, the MAP is < 55 mmHg, then the guidance in **Figure 6**'s algorithm below is recommended. To summarize, for a SBP < 90 mmHg or MAP < 55 mmHg prior to starting the infusion a fluid bolus of up to 500 cc normal saline (NS) may be administered. If the pressures remain below these parameters and the physician is worried about hypotension then the infusion should not be given and the subject should be sent home. Another attempt to infuse the study drug may be made within 3 days. If on the second attempt the subject's BP remains too low in the opinion of the investigator, then he/she will be withdrawn from the study. PAH specific medications should not be withheld to increase the blood pressure. In summary, study centers may defer to local institutional standards of practice for subject monitoring and clinical management during study drug infusions.

Patients with Class IV or advanced Class III symptoms should receive close nursing attention (preferably 1:1 care) during the infusion. These patients are at high risk for hemodynamic compromise should they suffer a hypersensitivity reaction. Therefore, each participating institution will have an established algorithm for acceleration of care should a patient hemodynamically decompensate. It is essential that a code team can rapidly and directly respond to the patient's hemodynamic deterioration and immediately transfer the patient to an inpatient unit for accelerated care (i.e. an off-site infusion center would not be appropriate for these patients).

Research coordinators will contact subjects by phone within 24 hours after discharge from the infusion center to assess for adverse events.

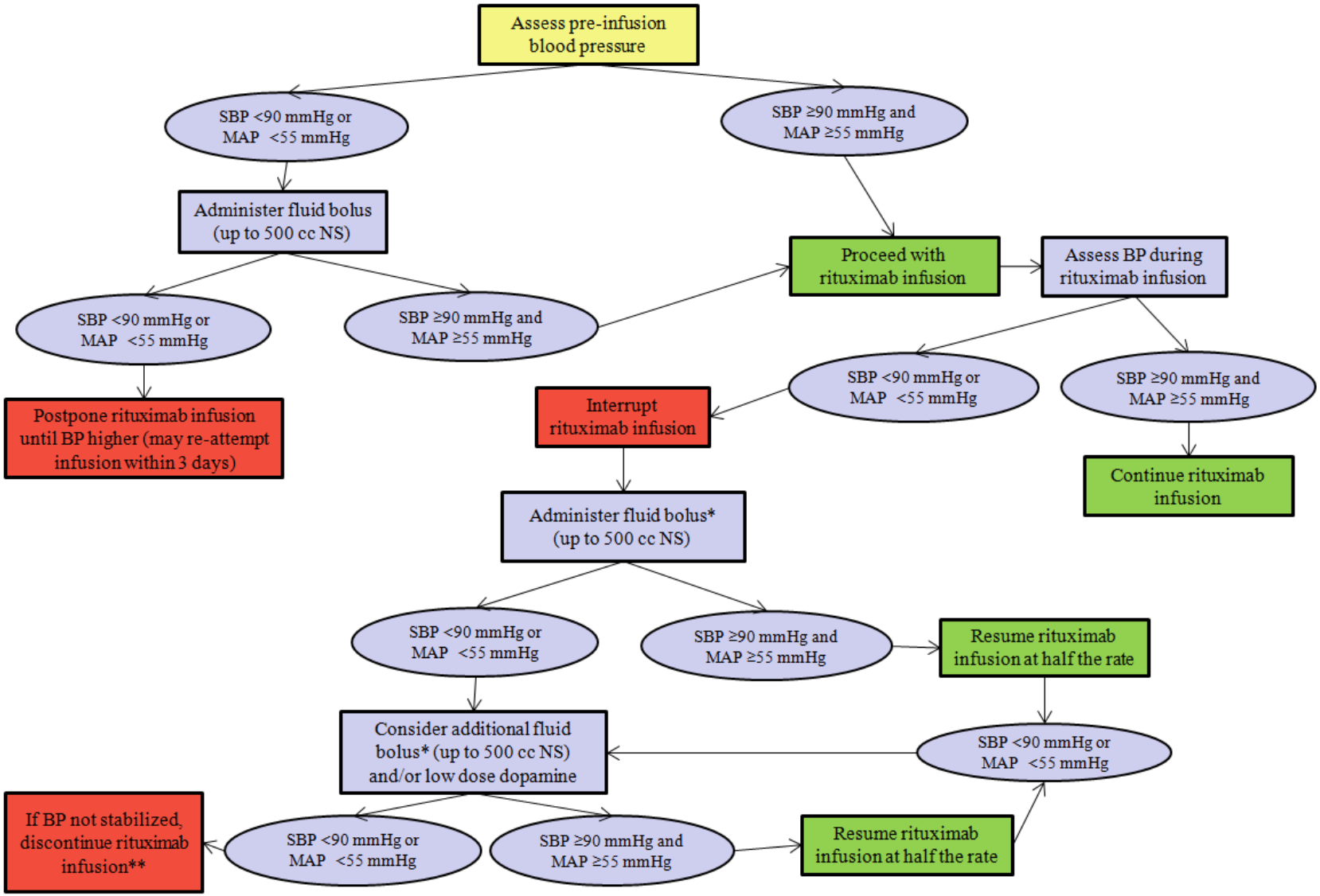


Figure 6. Guidance algorithm for managing hypotension during rituximab infusion.

5.3.1 **Medications to Hold Prior to Study Drug Administration**

Since transient hypotension may occur during the rituximab infusion, all subjects taking antihypertensive(s) will be instructed to hold their medication(s) for 12 hours before each infusion in order to decrease the risk of hypotensive events. If a subject has a systolic blood pressure > 180 mm mercury (Hg) or diastolic blood pressure > 100 mmHg at screening, then the antihypertensive(s) will **not** be withheld prior to the infusion. However, it is uncommon for patients with PAH to be hypertensive. Rather, systemic hypotension is more commonly found in this patient group. For hypotensive patients, management will be conducted as outlined in Section 5.4.3.2, *Cardiovascular* and in **Figure 6.**

5.3.2 **Preparation for Administration**

Appropriate aseptic technique will be used. The necessary amount of rituximab will be withdrawn and diluted to a final concentration of 1 to 4 mg/mL into an infusion bag containing 0.9% Sodium Chloride USP. It is strongly recommended that higher concentrations of rituximab (4 mg/mL) be used for this patient population in order to minimize fluid administration. The bag will be gently inverted to mix the solution, and any unused portion left in the vial will be discarded. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration and should not be used if either is present. No incompatibilities between rituximab and polyvinyl chloride or polyethylene bags have been observed.

5.3.3 **Pre-medication for Rituximab Infusion**

All subjects will receive the following pre-medication prior to each rituximab infusion to prevent hypersensitivity reactions and to reduce the frequency of infusion-related reactions:

- Night before and morning of each study drug infusion:
  - Prednisone 40mg PO (or equivalent)
- Approximately thirty minutes prior to each study drug infusion:
  - Methylprednisolone 100 mg IV (or equivalent).
- Approximately thirty to sixty minutes prior to each study drug infusion:
  - Diphenhydramine 50 mg PO (or equivalent). Dose may be repeated every four hours, as needed.
    - Note: Subjects should be advised that this pre-medication given for the prevention and treatment of infusion-related reactions may cause drowsiness and impairment of driving ability prior to discharge.
  - Acetaminophen 650 mg PO (or equivalent). Dose may be repeated every four hours, as needed.

5.3.4 **Administration**

RITUXIMAB MUST NOT BE ADMINISTERED AS AN IV PUSH OR BOLUS.

The following administration guidelines should be adhered to:

- Attach the IV administration tubing to the IV bag of prepared rituximab by piercing the bag with the spike end of the tubing.
- Attach the precision regulator extension set to the primary IV administration set.
- Purge all tubing of air by flushing with the prepared solution, allowing only a small amount of solution to be discarded.
- Identify the site for IV access. Prepare for venipuncture per institution standards of practice.
- Introduce the angiocatheter into the vein, and attach to the male port of the precision regulator extension set.

5.3.4.1 Guidance for NYHA Class II or III Subjects

- Open the roller clamp of the IV tubing, and set the precision regulator drip rate to an initial rate of 50 mg/hour.
- Do not mix or dilute rituximab with other drugs.
- If hypersensitivity or infusion-related events do **not** occur, escalate the infusion rate in 50 mg/hour increments every 30 minutes, to a maximum of 400 mg/hour. Subsequent infusions of rituximab can be administered at an initial rate of 100 mg/hour, and increased by 100 mg/hour increments at 30-minute intervals, to a maximum rate of 400 mg/hour as tolerated.

Table 5.1 Rituximab Infusion Rate for First Infusion*

Time (minutes)	Drug per hour (mg/h)	Infusion Rate (mL/h)
0-30	50	12.5
31-60	100	25
61-90	150	37.5
91-120	200	50
121-150	250	62.5
151-180	300	75
181-210	350	87.5
211-240	400	100
241-255	400	100

Note: Advance infusion rate as tolerated

*Based on dilution of rituximab to 4 mg/mL in normal saline

Table 5.2 Rituximab Infusion Rate for Second Infusion*

Time (minutes)	Drug per hour (mg/h)	Infusion Rate (mL/h)
0-30	100	25
31-60	200	50
61-90	300	75
91-120	400	100
121-150	400	100
151-180	400	100
181-210	400	100

Note: Advance infusion rate as tolerated

* Based on dilution of rituximab to 4 mg/mL in normal saline

- Check for extravasation at the infusion site.
- Allow the study medication to infuse until the line of fluid is at the needle insertion site, ensuring that all of the medication is infused.

5.3.4.2 Guidance for NYHA Class IV Subjects

Given the greater concern for potential cardiovascular events related to rituximab administration in this sub-group of subjects, the starting infusion rate and algorithm for titration of rate should be reduced to half of the standard rate outlined in Section 5.3.4.1, *Guidance for NYHA Class II or III Subjects*. Strict adherence to the 30 minute intervals of rate titration will be dependent upon subject tolerability of the infusion.

5.3.5 Criteria for Permanent Discontinuation of Rituximab Infusion

If a subject experiences any of the events listed below, rituximab infusions will be discontinued, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for all Treated Subjects*):

- Syndrome resembling tumor lysis syndrome
- NCI-CTCAE Grade 3 (severe) hypersensitivity reaction
- NCI-CTCAE Grade 3 or higher (severe/life-threatening) cytokine release acute infusion reaction
- NCI-CTCAE Grade 3 or higher (severe/life-threatening) cardiac arrhythmias. In addition, see Protocol Section 3.3.2, *Discontinuation of Protocol-Specified Treatment Requirements* for significant cardio-pulmonary disease progression events warranting discontinuation of protocol-specified treatment requirements.
- NCI-CTCAE Grade 3 or higher (severe/life-threatening) mucocutaneous reactions

- Significant hypotension during the infusion not responsive to standard medical intervention
- Events listed in Section 3.3.2, *Discontinuation of Protocol-Specified Treatment Requirements*

5.3.6 Criteria for Withholding Rituximab Infusion

Rituximab infusions are to be withheld if the subject is experiencing any of the following:

- Fever > 38° C
- Symptoms of an upper respiratory infection
- Acute diarrhea
- Signs or symptoms of intercurrent infection or serum sickness such as fever, joint pain, skin rash or hives, hematuria, or hypocomplementemia
- Cytopenia as defined as ANC < 1500/mm^3 (or < 1.5 x 10^9/L), platelets < 100,000/mm^3 (or < 100 x 10^9/L), or hemoglobin < 9.0 g/dL
- Hypotension prior to initiation of infusion

The infusion may be given if a subject has been afebrile for 2 days and has clear lungs. If the subject has been started on antibiotics, the infusion may be given after 2 days, provided cultures are negative. If initiation of rituximab was withheld for hypotension, the subject may return within 3 days for rituximab administration, as long as the return visit is within the windows specified below.

If a subject has a rituximab infusion withheld, the following time windows for infusion will be applicable:

- First Infusion: The first infusion must be given within 7 days of randomization.
- Second Infusion: The infusion must be given 2 – 4 weeks after the first infusion; otherwise the subject will not receive the second infusion.

5.3.7 Criteria for Temporarily Interrupting and Restarting Rituximab Infusion

The rituximab infusion should be interrupted if the patient develops worsened hypotension or symptomatic bradycardia. SSc-PAH patients may have baseline low blood pressure which the rituximab infusion may exacerbate. Please refer to Section 5.4.3.2, *Cardiovascular* and the Algorithm in **Figure 6** for the suggested treatment of hypotension during rituximab infusions. Sinus bradycardia during rituximab infusion should be quickly responded to as described in Section 5.4.3.2, *Cardiovascular*. If additional measures seem safe and appropriate in order to continue the rituximab infusion, they may be implemented by the clinical site. In all cases, clinical judgment regarding the safety of the individual subject should prevail. If there is adequate response of the bradycardia and/or hypotension and the subject is hemodynamically stable, the infusion may be restarted The rituximab infusion should be interrupted for NCI-CTCAE Grade 2 or higher cytokine release reactions or Grade 2 hypersensitivity reactions. In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hour to 50 mg/hour) when symptoms have completely resolved. Additional treatment with bronchodilators or IV saline may be indicated. Most subjects who

have experienced NCI-CTCAE Grade 2 or less hypersensitivity infusion-related reactions have been able to complete the full course of rituximab therapy. Epinephrine, antihistamines, and corticosteroids should be available for immediate use in the event of an infusion reaction to rituximab (e.g., anaphylaxis). Rituximab therapy should be discontinued in the event of an NCI-CTCAE Grade 3 hypersensitivity or anaphylactic allergic reaction or in the event of an NCI-CTCAE Grade 3 cytokine release acute infusion reaction. Please refer to Section 5.4.3, *Management of Infusion Reactions/Known Toxicities to Rituximab*, for treatment guidelines.

5.4 Toxicity Management Plan for Rituximab

5.4.1 Known Toxicities to Rituximab

Refer to Protocol Section 1.4, *Known and Potential Risks of Rituximab Treatment*.

5.4.2 Prevention of Known Toxicities to Rituximab

1. In the absence of known allergies, all subjects will be pre-medicated orally approximately 30 – 60 minutes prior to infusion of rituximab with acetaminophen 650 mg and diphenhydramine 50 mg. The pre-medication will be repeated every 4 hours, as needed. Subjects administered an antihistamine for the treatment or prevention of infusion-related reactions should be given appropriate warnings about drowsiness and impairment of driving ability prior to discharge.

In order to reduce the frequency of infusion-related reactions, subjects will also be asked to take prednisone 40 mg PO (or equivalent) the night before and morning of each infusion. Additionally, they will be given 100 mg IV methylprednisolone (or equivalent) approximately thirty minutes prior to each rituximab infusion. Procedures for the management of infusion reactions are discussed in Section 5.4.3.1, *Infusion Reactions*. Additionally a member of the research staff will follow up with subjects within 24 hours after discharged from the infusion center to assess their condition.

2. Although **neutropenia and thrombocytopenia** have been associated with rituximab, cases are generally transient and do not cause clinically significant problems. Late onset neutropenia with rituximab has been described in patients treated with aggressive chemotherapy for hematological malignancies but no such cases have been reported in patients with autoimmune disease. Nevertheless, to minimize the risk of potential cytopenias, a complete blood count will be performed and adequacy of the differential and platelet count will be checked prior to starting the second rituximab infusion. Counts will be re-checked as required in the Schedule of Events and whenever the subject develops any infection. Procedures for the management of cytopenias will be according to institutional practice.

3. Most subjects at high risk for **infections or viral reactivation** will have been excluded from participation in this study. However, the following additional precautions will be taken to minimize the potential for significant infections and/or viral reactivation in this study [153-156].

a. Subjects will be informed about the potential effects of rituximab on immunocompetency including:
   - decreased response to immunization;
   - possible increased rate of infection;
   - possible neutropenia and hypogammaglobulinemia, and
   - possible reactivation of viral infections (including HBV and JC virus).
b. Subjects and study physicians will be given the following information regarding immunizations:
   - Live virus vaccines are contraindicated within 8 weeks prior to beginning rituximab therapy.
   - All other immunizations must be completed at least 4 weeks prior to starting rituximab. This includes the pneumococcal vaccine, unless subject was vaccinated within 5 years of study entry. If vaccination occurred greater than 5 years prior to study entry, the subject must be revaccinated at least 4 weeks prior to initiation of therapy.
   - After rituximab, recommended vaccines, including yearly influenza vaccine, are to be administered as late as possible after the last dose of rituximab. If possible, influenza vaccine should be administered at least 4 weeks prior to dosing with rituximab.
c. Subjects will be educated about the need to recognize and report the symptoms of infection. Specifically, subjects will be:
   - educated about the importance of early recognition of infections and instructed to call the study coordinator or their health care provider if they have questions or if they are ill;
   - instructed to be seen by their study physician and/or local physician if they are having shaking chills, fever greater than 101° F, respiratory illness other than a mild cold, symptoms of a urinary infection, severe or prolonged diarrhea or abdominal pain, or if they think they may be developing the flu;
   - educated about symptoms of hepatitis (nausea, loss of appetite, dark urine, light stools), herpes zoster (new, severe, unexplained pain on one side of the body especially if there is a rash), and PML (confusion, lethargy, dizziness, difficulty talking or walking, and vision disturbances).
d. Study physicians will administer appropriate prophylaxis for infections such as anti-viral chemotherapy for influenza during flu outbreaks according to individual institutional practice.

5.4.3 **Management of Known Toxicities to Rituximab**

5.4.3.1 **Infusion Reactions (Cytokine release syndrome/acute infusion reaction and Hypersensitivity/Allergic Reaction)**

Infusion reactions (cytokine release and/or hypersensitivity reactions) may respond to adjustments in the infusion rate and to medical management. If a general infusion-related

reaction (e.g., fever, chills, myalgia, nausea, headache, rash, mild or moderate mucocutaneous reactions, or symptomatic hypotension) develops, the symptoms will be treated, and the infusion slowed or stopped as warranted. The infusion may be continued at one-half the previous rate once the symptoms have resolved. Hemodynamic fluctuations can have significance for patients with PAH and physicians trained in the care of PAH patients should be available in the event of an infusion reaction. For this reason, patients in this protocol will receive study drug with hemodynamic monitoring as described under Section 5.3, *Administration of Rituximab*. For subjects who experience asymptomatic hypotension, the infusion rate will be temporarily slowed per the investigator's discretion until symptoms resolve.

The rituximab infusion should be interrupted for NCI-CTCAE Grade 2 cytokine release or Grade 2 hypersensitivity reactions. In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hour to 50 mg/hour) when symptoms have completely resolved. Treatment of infusion-related symptoms with diphenhydramine and acetaminophen is recommended. Additional treatment with bronchodilators or IV saline may be indicated. Most subjects who have experienced non-life-threatening (NCI-CTCAE Grade 2 or less) hypersensitivity infusion reactions or (NCI-CTCAE Grade 2 or less) cytokine release reactions have been able to complete the full course of rituximab therapy.

In the event of a NCI-CTCAE Grade 3 (severe) or higher hypersensitivity reaction, or a NCI-CTCAE Grade 3 (severe) or higher cytokine release acute infusion reaction, the infusion should be permanently discontinued and the subject should receive medical treatment. Epinephrine, antihistamines, corticosteroids, albuterol, oxygen, and emergency resuscitation equipment should be kept at the bedside during the infusion of rituximab for immediate use in the event of a hypersensitivity reaction to rituximab (e.g., anaphylaxis). Subjects who experience an NCI-CTCAE Grade 3 (severe) or higher hypersensitivity reaction, or an NCI-CTCAE Grade 3 (severe) or higher cytokine release acute infusion reaction will receive no additional rituximab, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*). If it is medically indicated, a subject will be hospitalized for infusion-related reactions.

5.4.3.2 **Cardiovascular**

Infusions should be discontinued in the event of an NCI-CTCAE Grade 3 (serious) or Grade 4 (life-threatening) cardiac arrhythmia. For subjects who develop an NCI-CTCAE Grade 3 or higher arrhythmias, rituximab will be permanently discontinued, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*). In addition, see Protocol Section 3.3.2, *Discontinuation of Protocol-Specified Treatment Requirements* for significant cardio-pulmonary disease progression events warranting discontinuation of protocol-specified treatment requirements.

Sinus bradycardia can be dangerous in PAH patients whose cardiac output can be heart rate-dependent. A vasovagal response to rituximab infusion can result in sinus bradycardia and

should be immediately be responded to. If a patient develops symptomatic bradycardia evidenced by lightheadedness, change in consciousness, worsening hypotension or other symptoms, then the subject should receive 0.5 mg of atropine IV with repeat dosing not to exceed 3 mg. If patient develops symptomatic bradycardia, that is unresponsive to atropine, then the infusion should not be resumed. During a period of bradycardia the infusion should be suspended. If the bradycardia resolves the infusion may be re-started at 50% the original infusion rate.

If during the rituximab infusion the SBP drops below 90 mmHg or MAP drops below 55 mmHg, then the guidance in algorithm in **Figure 6** may be followed per the investigator's clinical judgment. Different interventions may be utilized depending on the individual investigator's clinical judgment. To summarize the algorithm, the infusion may be interrupted and a fluid bolus of up to 500 cc normal saline administered. If the SBP rises to $\geq$ 90 mmHg and MAP rises to $\geq$ 55 mmHg the infusion may be resumed at half the infusion rate. If the SBP does not rise to $\geq$ 90 mmHg and MAP to $\geq$ 55 mmHg, another fluid bolus of up to 500 cc, and/or low dose dopamine may be considered. If the BP does not stabilize, clinical judgment should prevail as to when to discontinue study drug infusion. At any time if clinically indicated, the subject should be stabilized and the infusion withheld, slowed, or discontinued as appropriate per the guidance provided. Consideration must be given to administration of multiple fluid boluses ($\geq$ 1 Liter) dependent on subject's cardiopulmonary status. Procedures for follow-up per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects* should be followed.

In addition to hypotension, subjects with SSc-PAH may be sensitive to fluid overload. Investigators need to be cognizant of the total fluid load when administering the rituximab infusion. It is strongly recommended that higher concentrations of rituximab (4 mg/mL) be used to minimize fluid administration.

As stated in Section 5.1.3, *Storage and Handling of Study Product*, rituximab is physically and chemically stable at 2° to 8°C (36° to 46°F) for 24 hours after dilution and subsequently for 24 hours at room temperature after dilution provided that dilution has taken place in controlled and validated aseptic conditions. The interventions listed above and in the Algorithm in **Figure 6** to manage cardiovascular complications once the infusion has begun may take place over a 24 hour period. Subjects should be continuously monitored until they have completed the infusion or until it is determined that the infusion must be discontinued for toxicity.

5.4.3.3 **Severe Mucocutaneous Reactions**

Subjects experiencing an NCI-CTCAE Grade 3 (severe) or higher mucocutaneous reaction (to be reported under such categories as Dermatology/Skin, Gastrointestinal, or Renal/Genitourinary in the NCI-CTCAE dependent upon manifestations) should not receive additional rituximab and should seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and to guide subsequent treatment. Severe mucocutaneous reactions may require topical or systemic corticosteroid therapy. The safety of re-administration of study drug to subjects with mucocutaneous reactions has not

been determined. If a subject develops an NCI-CTCAE Grade 3 (severe) or higher severe mucocutaneous reaction, rituximab will be discontinued, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*).

5.4.3.4 **Hepatitis**

If an enrolled subject develops hepatitis B, rituximab will be discontinued, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*). Patients may have elevated transaminases secondary to endothelin antagonists. Hepatitis attributed to this class of agents should be responded to according to manufacturers' suggestions.

5.4.3.5 **Hematologic Malignancy**

If an enrolled subject develops a hematologic malignancy, rituximab will be discontinued, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*).

5.4.3.6 **Serum Sickness**

Should serum sickness occur, the infusion will be stopped permanently, and procedures for discontinuation of protocol-specified treatments and follow-up will be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*). The subject will be given, if judged clinically appropriate, antihistamines for symptomatic relief of urticaria and nonsteroidal anti-inflammatory drugs for relief of pain. In some cases, a short course of moderate dose corticosteroids may be required for relief of joint pain or fever. If a serum sickness-like reaction occurs, then additional studies will be performed at the time of clinical presentation to evaluate its possible mechanisms. These studies may include measurement of serum cytokines, C3 and C4 levels, a urinalysis, and a skin biopsy with indirect immunofluorescence (if appropriate).

5.4.3.7 **Tumor Lysis Syndrome**

In the event a syndrome resembling tumor lysis syndrome, the rituximab infusion should be discontinued. The subject should not be retreated with rituximab, and procedures for discontinuation of protocol-specified treatments and follow-up should be implemented (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*).

5.4.3.8 **Infection**

Subjects will be instructed to contact study personnel and be seen by their study physician and/or local physician if they have a fever $\geq 101^{\circ}$ F or other signs and symptoms of an infection. For subjects who develop severe or frequent infections, the need for IVIG will be evaluated by each investigator (see Section 5.6, *Prohibited Medications and Treatments*). Local institutional policies for the use of IVIG will apply. Physicians will use their discretion to treat infections according to standard of care.

5.5 Prior Medications and Therapy

Entry criteria with medications and therapy are described in Section 3.1, *Description of Study Design*, Section 4.1, *Inclusion Criteria*, and Section 4.2, *Exclusion Criteria*.

5.6 Prohibited Medications and Treatments

All subjects are to have access to any care deemed medically necessary, but administration of the following medications, for the purposes of this study, are prohibited and will be considered either protocol deviations or reasons for early endpoint unless otherwise noted below or in Section 5.7, *Concurrent Medications and Therapy*:

- Addition of new medical therapies for PAH or a change in the dose of background PAH therapies (prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators) unless the change in therapy is due to toxicity of prior medications prior to week 24 except for an increase of up to 10% in prostacyclin therapy as described in Section 5.7, *Concurrent Medications and Therapy*
- Surgical therapy for PAH
- Any systemic corticosteroids, except for
  - prednisone (or equivalent) detailed in Section 5.7.2.1, *Corticosteroids*, or
  - corticosteroid premedications as specified in Section 5.3.3, *Pre-medication for Rituximab Infusion*, or
  - the methylprednisone (or equivalent) administered for the treatment of severe mucocutaneous reactions as per Section 5.4.3.3, *Severe Mucocutaneous Reactions* and of serum sickness manifestations as per Section 5.4.3.6, *Serum Sickness.*

  *Corticosteroid doses may be adjusted within the 0-10 mg daily range, as clinically indicated, as long as they are stable for 4 weeks prior to Weeks 24 and 48 endpoint assessments.
- Any biologic immunosuppressive agent, including rituximab or other CD20 depleting agents.
- IVIG between 4 weeks prior to Day 0 and 4 weeks after Week 2
- New non-biologic immunosuppressive or immunomodulating drug therapy prior to week 24 or changes in concurrent non-biologic immunosuppressive or immunomodulating drug therapy prior to week 24 except for the management of toxicity
- Any investigational agent other than investigational prostanoids, endothelin receptor antagonists, PDE-5 inhibitors, and guanylate cyclase stimulators. The latter investigational agents are those drugs in one of these four classes that are already being used for the treatment of PAH, but are not currently licensed for this use by the FDA.

5.7 Concurrent Medications and Therapy

5.7.1 Background PAH Therapy

From the time of study entry, subjects are to remain on background PAH medical therapy with either a single agent or a combination of prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators as per the entry criteria. Doses must remain stable through the week 24 primary endpoint visit.

Investigational prostanoids, endothelin receptor antagonists and PDE-5 inhibitors are permitted if the drugs are already in use for the treatment of PAH but are not currently licensed for this use by the FDA.

Permitted Dose modifications:

- Toxicity management for any of the four classes of drugs
- Increase in prostacyclin dose of up to 10% for worsening disease independent of route of administration.

Other PAH medications, such as diuretics and anti-coagulation, should be managed by the treating physician according to institutional practice. Changes in diuretics and anti-coagulants are permitted throughout the duration of the study.

Stabilization/clinical improvement in PAH should result in no change to background therapy for the duration of the subject's participation in the study.

If a change in medical (beyond the 10% increase in prostacyclin dose) or surgical therapy is necessary for the treatment of progressive PAH before the week 24 primary endpoint visit then the subject will complete an Early Endpoint visit (see Section 6.5.6) and procedures in Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects* numbers 2 and 3 will be followed.

After the week 24 endpoint visit, the doses of background therapies for PAH may be increased and other agents may be added as needed to treat worsening PAH if the subject experiences any one of the following:

- a deterioration in functional class and a decrease in 6MWD by > 20%,
  or
- the development of signs and symptoms of clinical right heart failure, such as worsening edema, ascites, and/or weight gain,
  or
- other objective data suggesting clinical deterioration, such as worsening cardiopulmonary hemodynamics as evidenced by a ≥ 20% decrease in cardiac output, a ≥ 20% increase in mPAP, or a ≥ 20% increase in PVR.

5.7.2 Immunosuppressive or Immunomodulating Drug Treatment

Concurrent therapy with non-biologic immunosuppressive or immunomodulating drugs (including, but not limited to, methotrexate, hydroxychloroquine, penicillamine, mycophenolate, azathioprine or cyclosporine) is permitted. Doses must remain stable for at least the four weeks prior to randomization through at least the 24 week primary endpoint visit unless adjustment is needed for the management of toxicity.

Doses of concurrent non-biologic immunosuppressive or immunomodulating drugs may be increased or new non-biologic immunosuppressive or immunomodulating drugs may be initiated for worsening SSc disease manifestations after the week 24 endpoint visit.

5.7.2.1 Corticosteroids

Concurrent therapy prednisone (or equivalent corticosteroid) at ≤ 10mg daily is permitted.

- Corticosteroid doses may be adjusted with within the 0-10 mg daily range, as clinically indicated, as long as they are stable for 4 weeks prior to the weeks 24 and 48 endpoint assessments.
- Intra-articular corticosteroid injections may be administered.
- To accommodate the occasional use of extra corticosteroids by patients for reasons not associated with SSc, increases of up to 60 mg/day that are decreased back to the baseline dose within 2 weeks are permitted. Only 2 such increases are permitted during the study. Subjects must be back to baseline doses of corticosteroid by 4 weeks prior to the week 24 and week 48 visits.

5.8 Toxicity Management Plan for Concurrent Therapy:

If discontinuation of an agent is required due to toxicity, the study physician will exercise clinical judgment in terms of whether to prescribe another agent within the same therapeutic class, add an agent from another therapeutic class, or continue with other background therapies already in place.

5.8.1 Known Toxicities of Concurrent Therapy

Endothelin receptor antagonists have the potential to induce severe liver injury and damage to the fetus. Elevations in serum aminotransferases and bilirubin are markers for potential serious liver injury. It is suggested that these laboratory tests be measured prior to the initiation of any of the endothelin receptor antagonists. ERAs are classified by the FDA as Category X drugs; pregnancy must be excluded prior to treatment initiation and prevented once treatment has begun. Endothelin receptor antagonists cause a dose dependent decrease in hemoglobin. In individuals with severe CHF, endothelin receptor antagonists have been associated with increased fluid retention and hospitalization for exacerbation of CHF.

Phosphodiesterase inhibitors potentiate the hypotensive effects of nitrates and therefore are contraindicated in patients taking organic nitrates either regularly or intermittently. This class of medication has vasodilator properties and may result in transient decreases in blood pressure and nose bleeds. Caution should be exercised in prescribing PDE-5 inhibitors in

individuals with a history of coronary artery disease, myocardial infarction, or retinitis pigmentosa.

Epoprostenol is a potent vasodilator and additional reductions in blood pressure may be seen when an individual receives concurrent antihypertensives, diuretics or other vasodilators. There is the potential for epoprostenol to increase bleeding in individuals on anti-platelet agents or anticoagulants. Most of the adverse events experienced by subjects using epoprostenol are related to its vasodilatory effect and include nausea, vomiting, headache, hypotension, foot/bone pain, and flushing.

Riociguat is a guanylate cyclase stimulator indicated for the treatment of adults with PAH (WHO Group 1) to improve exercise capacity, clinical worsening and WHO functional class. Riociguat is a pregnancy Category X drug and as such is contraindicated for during pregnancy as it may cause fetal harm. Females of reproductive capacity must be enrolled in the Adempas REMS program. Pregnancy testing should be performed monthly during treatment and female patients must agree to acceptable methods of contraception while taking this medication. Co-administration of riociguat with nitrates or nitric oxide donors is contraindicated as is concomitant administration of riociguat with PDE inhibitors, either specific PDE-5 inhibitors or nonspecific PDE inhibiters. Riociguat may cause hypotension. Serious bleeding was observed in clinical trials in individuals receiving riociguat compared to placebo including serious hemoptysis. Smoking, strong CYP and P-gp/BCRP inhibitors have the potential to change the exposure to riociguat.

5.8.2 **Prevention and Management of Known Toxicities to Concurrent Therapy**

It is suggested that subjects receiving endothelin receptor antagonists have liver enzymes and a bilirubin tested monthly and a CBC checked every 3 months for anemia. If a subject is found to have elevated liver enzymes or falling hemoglobin, the subject will be managed in accordance with common clinical practice. Guidelines may be found in the package inserts of the relevant medications. The study physician will exercise clinical judgment in determining whether it is necessary to discontinue a medication for toxicity. Study physicians should be aware and subjects should be educated on the drug interactions with endothelin receptor antagonists including hormonal contraceptives, ketoconazole, warfarin, rifampicin, and statins. If subjects become pregnant during the course of the trial they will be withdrawn. Procedures for withdrawal as detailed in Sections 3.3.3, *Subject Withdrawal from the Study* and 6.5.5, *Early Endpoint Visit* will be followed. The pregnancy will be reported as detailed in Section 7.6, *Pregnancy Reporting*.

Female subjects receiving riociguat therapy should have monthly pregnancy testing and agree to acceptable methods of contraception. If a subject becomes pregnant during the course of the trial, they will be withdrawn. Procedures for withdrawal as detailed in Sections 3.3.3, *Subject Withdrawal from the Study* and 6.5.5, *Early Endpoint Visit* will be followed. The pregnancy will be reported as detailed in Section 7.6, *Pregnancy Reporting*. Study physicians should be aware and subjects should be educated on the drug interactions with riociguat. Co-administration of nitrates, nitric oxide donors and phophodiesterase inhibitors are contraindicated. Blood pressure should be monitored and doses adjusted as needed if

subjects are taking concurrent strong cytochrome CYP inhibitors and P-gp/BCRP inhibitors such as azole anti-mycotics or HIV protease inhibitors. Subjects should be educated on the possibility of serious bleeding.

5.9 Right Heart Catheterization Risks and Management Guidelines

Most published reports of adverse events related to RHC have been based on single-center experiences with relatively small sample sizes. In the 1980s, Fuster and colleagues [157] conducted a long-term, retrospective, follow-up study on 120 patients, and reported a 4.2% fatality rate associated with RHC in patients with pulmonary arterial hypertension (PAH). In the same decade, a national registry of 187 patients with PAH from 32 centers reported a complication rate of 5.3%, with no deaths or sustained morbid events related to RHC diagnostic procedures [158].

Two decades later, a study was conducted to investigate the complication rates from RHC in patients with PAH from 15 experienced PAH centers, in both the United States and Europe [159]. This 5-year retrospective and a 6-month prospective evaluation of serious adverse events related to RHC procedures in patients with PAH, defined as mean pulmonary artery pressure > 25 mmHg at rest, revealed that the safety of the procedure has improved significantly. A total of 7218 RHC procedures were performed (21% prospective). The overall number of serious adverse events was 76 (1.1%). There were 4 fatalities reported associated with the procedure (0.055%). The remaining 74 nonfatal incidents were classified as mild or moderate in severity and resolved either spontaneously or with minor interventions. The most frequent complications were hematoma at venipuncture sites, pneumothoraces, arrhythmias, vasovagal episodes, and hypotensive episodes. The most frequent complications were related to venous access (n = 29, 38%). Internal jugular (IJ) was reported to be the preferred access site (73%) that was associated with a low rate of complications (0.3%).

Measures to minimize the risks associated with RHC include the use of the IJ approach whenever feasible, with ultrasound guidance for direct visualization of the vein. The use of fluoroscopy should also be implemented whenever feasible. Direct visualization under fluoroscopy can minimize arrhythmic events, which are more prevalent in patients with an enlarged right atrium or ventricle, including those with PAH. Measurement of the pulmonary capillary wedge pressure is safer if performed using fluoroscopy for direct visualization. Fluoroscopy does involve exposure to radiation. Although the radiation exposure can vary from person to person, the whole-body radiation will be about 200 mrem during each RHC.

5.10 Other Protocol Standard of Care Recommendations

Supplemental oxygen should be provided to subjects in order to keep their oxygen saturation at or above 90% at all times.

Subjects should receive influenza vaccines annually per institutional practice preferably not within 4 weeks of study drug infusion.

5.11 Procedures for Monitoring Subject Compliance

Since the medication is administered as an IV infusion, study personnel will assure compliance with treatment. Personnel who administer the drug will document its administration. Study personnel will also monitor patients for compliance with baseline PAH stable dose medical therapy.

6 STUDY ASSESSMENTS

6.1 Assessments of Safety

To assess safety in this population, peripheral blood cell counts, liver function, and serum creatinine will be monitored before enrollment and at frequent intervals thereafter. Physical examinations will be conducted before enrollment and at frequent intervals thereafter. The information from these assessments and subject interviews will be used to characterize the frequency of all adverse events Grade 2 or above, all serious adverse events, granulocytopenias, and treatment-related adverse events of NCI-CTCAE Grade 3 or higher (see Section 7, *Safety Monitoring and Reporting*).

Recovery of B cells will be monitored by assessing B cell levels at baseline and serially post-treatment until reconstitution is documented. In addition, quantitative immunoglobulin levels, including IgG subclasses, will be serially monitored. Urine or serum pregnancy tests per institutional policy will be performed for all women of child-bearing potential at screening and prior to each rituximab infusion.

6.2 Assessments of Disease Activity and Clinical Response

Change in 6MWD as a measure of exercise capacity is the primary efficacy assessment in this trial. Disease activity will be monitored by checking autoantibodies and evaluating clinical outcomes including change in PVR measured by right heart catheterization and measuring room air oxygen saturation by pulse oximetry at screening and serially throughout the trial. Changes in severity of Raynaud phenomenon as measured by the VAS scale of the SHAQ, and change in digital ulcer count will be evaluated. Change in DLCO, a measure of disease activity as well as safety, will be followed. BNP/NT-proBNP and serum Na^+ will be followed as non-invasive means of assessing heart failure. Change in quality of life will be determined by serial administration of the SF-36 and the SHAQ.

6.3 Assessments of Mechanistic Endpoints

The CD19+ B cell assays will be performed by the Human Immune Monitoring Center (HIMC) at Stanford University. B cell panels may include, but are not limited to IgD FITC, CD24 PE, CD19 PerCP-Cy5.5, CD28 PE-Cy7, CD27 APC, CD20 APC-H7, CD3 V450, CD45 Pacific Orange.

With a fraction of the PBMCs analyzed by flow cytometry for the above B cell panels, a sub-study will measure and monitor B cell clonality utilizing massively parallel V-D-J pyrosequencing.

IL-1 and IL-6 concentrations, as well as other cytokines, will be measured using multiplex assays [40].

Quantitative immunoglobulin levels, including IgG subclasses, will be measured using a Luminex kit on the same instrument. Viable cells will be assayed by flow cytometry to assess the number of CD19+ cells in the phenotyping panel using a LSRII platform.

The autoantibody biomarkers and RF will be analyzed by the Immunoassay Core Laboratory at the University of Pittsburgh.

Subjects will be consented for permission to store any leftover blood samples to be used for future IRB-approved research to study the disease SSc-PAH, the immune system, and the effect of treatment on this disease.

6.4 Other Assessments

Rituximab serum levels (PK) and HACA levels will be collected at baseline, Week 24, and Week 36, and analyzed by Genentech.

6.5 Evaluations by Study Visit

The visit schedule and evaluation procedures for the screening, baseline, treatment, and follow-up period are found in the Schedule of Events, Table 6.1.

Note: all subjective assessments completed by the subject (SHAQ, SF-36) **must** be done at the beginning of a visit, prior to **any** other study related procedures.

6.5.1 Screening Period (Visit 1)

Unless otherwise specified below, the screening evaluations must be performed within 28 days of the first treatment visit (Day 0), with the exception of the 6MWT which must be completed within 14 days of the first treatment visit (Day 0). This study will be explained in lay language to each potential participant. Each participant will sign an informed consent before committing to study screening procedures.

Note: if the Screening physical exam, oxygen saturation, chemistry panel, and pregnancy test are performed within 7 days of Day 0, they do not need to be repeated during the Randomization/Baseline or Day 0 visits unless clinically indicated.

Assessments:

- Obtain written informed consent from subject, and provide the subject a copy of the signed consent form prior to any screening procedures being performed

- Medical history, including Family History of Autoimmune Disease
- Demographics
- Record all concomitant medications
- Full physical exam including vital signs
- Weight and height
- NYHA Functional Class Assessment
- Right heart catheterization
- 12-lead ECG (unless done concomitantly with RHC)
- Pulmonary Function Tests: Spirometry, DLCO, TLC
- Chest X-Ray: posteroanterior (PA) and lateral
- 6-minute walk test (6MWT)
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein
- Infectious Disease Screen: HIV antibody; hepatitis B core antibody total, hepatitis B surface antibody, hepatitis B surface antigen; hepatitis C antibody with a hepatitis C virus (HCV) ribonucleic acid (RNA) (polymerase chain reaction [PCR]) if antibody positive
- TB testing (PPD or QuantiFERON®-TB Gold In-Tube Test [QFT-G_IT]), unless performed within 30 days prior to screening and documented as negative in the subject's records, or unless subject is known to have a positive or indeterminate test and has documentation of appropriate therapy.
- Urinalysis
- Pregnancy test (urine or serum per institutional policy): only if a subject is a female of childbearing potential
- Vaccinations: All immunizations must be completed at least 4 weeks prior to starting rituximab/placebo. The subject must be vaccinated with the pneumococcal vaccine at least 4 weeks prior to initiation of therapy, unless the subject was vaccinated within 5 years of study entry. If vaccination occurred greater than 5 years prior to study entry, the subject must be revaccinated at least 4 weeks prior to initiation of therapy. Depending on the season, influenza vaccine should be administered at least 4 weeks prior to dosing with rituximab/placebo. Otherwise, it should be administered as late as possible in the flu season to still be effective after the last dose of rituximab/placebo.
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.

6.5.2 **Randomization/Baseline Visit (Visit 2)**

The randomization/baseline visit evaluations may be combined with the Day 0 visit, or done within the 7 days prior to the first rituximab infusion. They are not required for eligibility verification, and therefore, they may be done after randomization.

Note: if the Screening chemistry panel was drawn within 7 days of Day 0, it does not need to be repeated during this Randomization/Baseline visit unless clinically indicated.

Assessments:

- SF-36
- SHAQ
- Digital Ulcer Count
- Concomitant Medication Assessment
- Adverse Event Assessment
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils), CD19+ B cells
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies: blood tested locally *and* sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center
- Autoantibodies: anti-U1 RNP, anti-U3 RNP, anti-B23, anti-cardiolipin, anti-Th/To, anti-CENP-B, RF, and any other autoantibodies: frozen serum sent to University of Pittsburgh Immunoassay Core Laboratory.
- Serum Immunoglobulins: quantitative immunoglobulin levels including IgG subclasses: frozen serum sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center
- IL-1, IL-6, and other cytokine assays: frozen serum sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center
- HACA and rituximab serum level (PK): batched and sent to Covance Laboratories.

6.5.3 **Treatment Period**

6.5.3.1 **Day 0 (Visit 3)**

This visit is to occur within 7 days of the randomization/baseline visit, and assessments/procedures listed under the randomization/baseline visit may be done on Day 0 or within the 7 day allowed window as scheduling allows. **All assessments listed under the Randomization/Baseline and Day 0 Visits must be completed prior to infusion of the first dose of rituximab or placebo.**

Note: if the Screening physical exam, oxygen saturation, and pregnancy test were performed within 7 days of Day 0, they do not need to be repeated during this Day 0 visit unless clinically indicated.

Assessments:

- Abbreviated Physical Examination with vital signs
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- Pregnancy test (urine or serum per institutional policy): only if a subject is a female of childbearing potential
- Concomitant Medication Assessment
- Adverse Event Assessment

Study Drug Infusion Procedures:
The pharmacy is to store, handle, and prepare the study drug (rituximab or placebo) according to the recommendations listed in Sections 5.1.3, *Storage and Handling of Study Product*, 5.2, *Dosage Regimen*, and 5.3.2, *Preparation for Administration*, and per the prescribing information.

In addition, sites will verify that they have the appropriate safety equipment and supplies in the infusion area available per Section 5.3, *Administration of Rituximab*.

The first rituximab or placebo infusion will be administered at the Day 0 visit. **The infusion should be performed after all other clinical assessments for this visit.** Please refer to Section 5.3.1 for medications to hold prior to infusion, Section 5.3.3 for pre-medications and Section 5.3.4 for study drug administration guidelines.

After the completion of the first infusion of study drug, the subject will be reassessed and vital signs repeated every 15 minutes until 1 hour after the infusion. If the subject is stable, the subject will be discharged home.

- Telephone Assessment: A member of the research staff will contact the subject within 24 hours after discharge from the infusion unit to assess their condition.

6.5.3.2 **Week 2/Day 14 ±4 days (Visit 4)**

This is the second rituximab/placebo infusion visit, to occur within 14 ± 4 days of Day 0. Please refer to Section 5.3.1 for medications to hold prior to infusion, Section 5.3.3 for pre-medications and Section 5.3.4 for study drug administration guidelines.
All visit assessments must be performed prior to study drug infusion.

Assessments:

- Abbreviated Physical Examination with Vital Signs
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
    - *Note*: hemoglobin, platelets, lymphocytes, neutrophils, and bands results from a hematology specimen drawn within 24 hours of the infusion must be reviewed prior to initiating the study drug infusion (see Section 5.3.6, *Criteria for Withholding Rituximab Infusion*).
- Pregnancy test (urine or serum per institutional policy): only if a subject is a female of childbearing potential.
- CD19+ B cell studies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- Concomitant Medication Assessment
- Adverse Event Assessment

Refer to **Study Drug Infusion Procedures in Protocol Section 5.3, *Administration of Rituximab*.** After the completion of the second infusion of study drug, the subject will have vital signs repeated. The subject will be re-assessed at 30 minutes post-infusion. If the subject is stable, the subject will be discharged home.

- Telephone Assessment: A member of the research staff will contact the subject within 24 hours after discharge from the infusion unit to assess their condition.

6.5.4 Follow Up Visits

6.5.4.1 Week 4 ±7 days (Visit 5)

Assessments:

- Abbreviated Physical Examination with Vital Signs
- NYHA Functional Class Assessment
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- SHAQ
- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- Digital Ulcer Count

- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- Concomitant Medication Assessment
    - Adverse Event Assessment

6.5.4.2 **Week 8 ± 7 days (Assessment 6)**

The Week 8 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.3 **Week 12 ± 7 days (Visit 7)**

Assessments:

- Abbreviated Physical Examination with Vital Signs
- NYHA Functional Class Assessment
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- SHAQ
- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- Digital Ulcer Count
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies
- Autoantibodies

- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.4 **Week 16 ± 7 days (Assessment 8)**

The Week 16 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.5 **Week 20 ± 7 days (Assessment 9)**

The Week 20 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.6 **Week 24 ± 7 days (Visit 10)**

Assessments:

- Full Physical Examination with Vital Signs
- NYHA Functional Class Assessment
- Right heart catheterization: The subject should be on the same amount of supplemental oxygen used during the Screening RHC unless safety will be impacted. The methods of calculating pulmonary capillary wedge mean pressure and cardiac output should be the same as that used for the Screening RHC and, if feasible, the procedure should be scheduled at approximately the same time of day as the Screening RHC.
- Pulmonary Function Tests: DLCO only
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- SF-36
- SHAQ

- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- Digital Ulcer Count
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies
- Autoantibodies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- HACA and rituximab serum level (PK). Batched and sent to Covance Laboratories.
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.7 **Week 28 ± 7 days (Assessment 11)**

The Week 28 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.8 **Week 32 ± 7 days (Assessment 12)**

The Week 32 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.9 **Week 36 ± 7 days (Visit 13)**

Assessments:

- Abbreviated Physical Examination with Vital Signs
- NYHA Functional Class Assessment

- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- SHAQ
- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete Screening the 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- Digital Ulcer Count
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- HACA and rituximab serum level (PK). Batched and sent to Covance Laboratories.
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.10 **Week 40 ± 7 days (Assessment 14)**

The Week 40 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.11 **Week 44 ± 7 days (Assessment 15)**

The Week 44 assessment will be conducted via telephone. If safety concerns arise, the subject should be asked to come to the site for an unscheduled visit.

Assessments:

- Digital Ulcer Count Assessment
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.12 **Week 48 ± 7 days (Visit 16)**

Assessments:

- Full Physical Examination with Vital Signs
- NYHA Functional Class Assessment
- Pulmonary Function Tests: DLCO only
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- SF-36
- SHAQ
- Digital Ulcer Count
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils), CD19+ B cells
- Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP
- CD19+ B cell studies
- Autoantibodies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays
- Concomitant Medication Assessment
- Adverse Event Assessment

6.5.4.13 **Week 104 (Visit 17)**

An assessment of vital status approximately two years post randomization will be conducted via telephone or review of public records or health registries (to which the subject has consented to share this data).

For subjects who completed their participation prior to the approval of protocol version 8.0, every possible effort to contact these subjects to consent them for this assessment should be made.

Assessments:

- Vital status
- Treatment Satisfaction assessment

6.5.5 **Quarterly Monitoring Visits**

Subjects who complete the Week 48 visit or who reach endpoint early after receiving **any** study drug, but whose B cell numbers have not recovered, will be followed at least quarterly to monitor recovery. Quarterly monitoring visits (12 weeks ± 14 days from the last visit) will consist of obtaining a B cell count (at both the central lab *and* at Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center) and CBC (at the central lab), and will continue until recovery or for 2 years after initial treatment (whichever occurs first). Recovery is defined as the time point at which B cells recover to within 10% of baseline levels; if a baseline value is not available then recovery is defined as a B cell level of at least 90% of the lower limit of normal. During this monitoring period, AEs and SAEs will be assessed, providing the subject has not withdrawn consent, only to capture any infectious event ≥ Grade 3 using the NCI-CTCAE. No additional study-related data will be collected.

6.5.6 **Early Endpoint Visit**

Subjects who withdraw early from the Baseline to Week 48 portion of the study will be asked to complete an Early Endpoint Visit. All scheduled exams, procedures, and laboratory tests scheduled for Week 24 will be performed at this visit, unless the early withdrawal is after that time, in which case all scheduled exams, procedures, and laboratory tests scheduled for Week 48 will be performed. If prohibited medications or treatments as defined in Section 5.6, *Prohibited Medications and Treatments* are required for treatment of progressive PAH, the Early Endpoint Visit, including the repeat right heart catheterization if withdrawal occurs prior to 24 weeks, should occur prior to the change in medication or therapy.

If the subject received **any** study drug prior to early endpoint and his/her B cell numbers have not recovered, that subject requires quarterly monitoring as described in Section 6.5.5, *Quarterly Monitoring Visits.*

Data from subjects who initiate rituximab therapy, but do not complete all study visits, will still be included in the Intent-to-Treat (ITT) and safety analyses.

6.5.7 **Visit Windows**

The screening visit has a window of 28 days between signing of the informed consent document and initiation of study treatment. This visit may be divided into several visits as scheduling dictates to complete all assessments and procedures. The baseline/randomization visit must be performed within 7 days of start of study treatment (Day 0). These assessments are not required for eligibility evaluation and so may be completed after randomization. Assessments and procedures listed under the baseline/randomization visit may be done on Day 0 as scheduling allows, so long as all assessments are completed prior to the first dose of rituximab treatment. The Week 2 visit (second infusion of rituximab) must be completed within 14 ± 4 days of Day 0. All monthly follow-up visits and phone assessments have a visit window of ± 7 days.

Whenever possible, a rescheduled visit should remain within the designated visit window. The coordinating center should be notified if the study procedures for any scheduled visit cannot be performed within the designated window.

6.5.8 **Unscheduled Visits**

If disease activity increases or other concerns arise between regularly scheduled visits, subjects should be instructed to contact study personnel to determine whether an "unscheduled" visit should be completed. The following evaluations will be performed at each unscheduled visit:

- Adverse Event Assessment
- Concomitant medication assessment
- Physical Examination
- Oxygen Saturation (forehead or ear probe must be used for saturations < 95% by finger probe). Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
- Other evaluations may be performed at the investigator's discretion

If the unscheduled visit is **due to an increase in SSc-PAH disease activity**, these additional evaluations should also be performed:

- NYHA Functional Class Assessment
- 6MWT: The subject should complete the assessment with the same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD *decreased* from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day)
- SF-36
- SHAQ
- Digital Ulcer Count
- Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelets, WBC, bands, eosinophils, lymphocytes, monocytes, neutrophils)
- CD19+ B cell studies
- Autoantibodies
- Serum Immunoglobulins
- IL-1, IL-6, and other cytokine assays

Table 6.1, Schedule of Evaluations

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Description	Screen	Base-line[1]	Day 0[1]	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 104	Quar-terly	Un-sched
Visit window	-28 days	-7 days		±4 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days		±14 days	-uled
Type of Visit	Clinic	Clinic	Clinic	Clinic	Clinic	Phone	Clinic	Phone	Phone	Clinic	Phone	Phone	Clinic	Phone	Phone	Clinic	Phone	Clinic	Clinic
Clinical Draw (mL)	21.5	11	2	8	11	0	11	0	0	11	0	0	11	0	0	13	0	3	3
Research Draw (mL)	0	41	0	29	29	0	33	0	0	38	0	0	34	0	0	33	0	24	33
Visit Draw Total (mL)	21.5	52	2	37	40	0	44	0	0	49	0	0	45	0	0	46	0	27	36
General Assessments																			
Informed Consent	X																		
Demographics	X																		
Medical History	X																		
Physical Examination& Vitals[2]	X		X[22]	X	X		X			X			X			X			X
Vaccinations[17]	X																		
NYHA Functional Class Assessment	X				X		X			X			X			X			X[24]
Right Heart Catheterization[20]	X									X									
Pulmonary Function Tests[3]	X									X						X			
Oxygen Saturation[4]	X		X[22]		X		X			X			X			X			X
ECG[5]	X																		
Chest X-Ray	X																		
TB testing[21]	X																		
6MWT [6,18]	X[18]				X		X			X			X			X			X[24]
SF-36		X								X						X			X[24]
SHAQ		X			X		X			X			X			X			X[24]
Digital Ulcer Count		X			X	X	X	X	X	X	X	X	X	X	X	X			X[24]
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Adverse Event Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X[23]	X
Post-infusion Phone Assessment[7]			X	X															
Assessment of Vital Status[26]																	X		
Treatment Satisfaction[26]																	X		
Laboratory Assessments																			
Hematology[8]	X	X		X[25]	X		X			X			X			X		X	X[24]
CD19+ B cell count (local)		X																	
Chemistry[9]	X[9]	X[22]			X		X			X			X			X			
Infectious Disease Screen[10]	X																		
Urinalysis	X																		
Pregnancy Test (if applicable)[11]	X		X[22]	X															
CD19+ B Cell Studies[12] (24mL)		X		X	X		X			X			X			X		X	X[24]
Autoantibodies[13] (4mL)		X					X			X						X			X[24]
Serum Igs[14] & IL-1, IL-6,& Other Cytokine Assays[15] (5mL)		X		X	X		X			X			X			X			X[24]
HACA and PK Assays[19] (3-5mL)		X								X			X						
Study Medication																			
Rituximab/Placebo[16]			X	X															

[1] Baseline and Day 0 assessments may be combined into one visit as scheduling dictates so long as they are completed within 7 days of the first rituximab infusion.
[2] Physical Examination: Full PE (including height, weight, vital signs, general appearance, skin, head/eyes/ears/neck/throat, respiratory/chest, cardiovascular, abdominal, neurological, lymph nodes, musculoskeletal/extremities) at Screen, Week 24, and Week 48. Abbreviated PE (including weight, vital signs, skin, respiratory/chest, cardiovascular, abdominal, lower extremity edema) at all other clinic visits.
[3] Pulmonary Function Tests: Height must be measured at time of PFTs. Full PFTs with spirometry, DLCO, and TLC at Screening. DLCO only at Weeks 24 and 48. Sites to send absolute values to Rho to convert for Hgb and altitude adjustments.
[4] Oxygen Saturation: forehead or ear probe must be used for saturations < 95% by finger probe at rest. Measure first at room air, then add/titrate oxygen if needed to maintain O_2 saturation ≥ 90%. The room air oxygen saturation measurement will be conducted at the discretion of the study physician. The measurement should not be performed if subject safety will be compromised.
[5] ECG: If not done with RHC (center-specific).
[6] 6MWT: Subject should complete assessment with same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted (if the 6MWD decreased from the Screening 6MWD by more than 20%, repeat the 6MWT within 7 days, but not on the same day).
[7] A member of the research staff will contact the subject within 24 hours after discharge from the infusion to assess for adverse events
[8] Hematology: CBC with differential count and platelet count (hematocrit, hemoglobin, platelet count, WBC, bands, eosinophils, lymphocytes, monocytes, and neutrophils). At Baseline, Week 48, and during Quarterly monitoring, CD19+ cells will also be enumerated.
[9] Chemistry: albumin, alkaline phosphatase (ALP), ALT/SGPT, AST/SGOT, BUN, creatinine, potassium, sodium, total and indirect bilirubin, total CO2, total protein, BNP/NT-proBNP. Note: BNP/NT-proBNP is not collected at screening.
[10] Infectious Disease Screen: HIV antibody, hepatitis B surface antibody and surface antigen, HCV antibody with HCV RNA (PCR) if antibody positive.
[11] Pregnancy Test: For women of child-bearing potential a pregnancy test (urine or serum per institutional policy) must be obtained at screening and within 72 hours of each rituximab infusion.
[12] CD19+ B Cell Studies: Blood sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center.
[13] Autoantibodies: will include but are not limited to Anti-U1 RNP, anti-U3 RNP, anti-B23, anti-cardiolipin, anti-Th/To, anti-CENP-B, and RF. Frozen serum sent to University of Pittsburgh Immunoassay Core Laboratory.
[14] Serum Immunoglobulins: Quantitative Immunoglobulin Levels including IgG subclasses (frozen serum sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center).
[15] IL-1, IL-6, and Other Cytokine Assays: Frozen serum sent to Stanford Human Immune Processing Center for the Stanford Human Immune Monitoring Center.
[16] Rituximab: Per Protocol Section 5, *Treatment of Subjects*.
[17] Vaccinations: All immunizations must be completed at least 4 weeks prior to starting rituximab/placebo. Subject must be vaccinated with the pneumococcal vaccine at least 4 weeks prior to initiation of therapy, unless subject was vaccinated within 5 years of study entry. If vaccination occurred greater than 5 years prior to study entry, the subject must be revaccinated at least 4 weeks prior to initiation of therapy. Depending on the season, influenza vaccine should be administered at least 4 weeks prior to dosing with rituximab/placebo. Otherwise, it should be administered as late as possible in the flu season to still be effective after the last dose of rituximab/placebo.
[18] The 6MWT must be completed within 14 days of the first treatment visit (Day 0).
[19] HACA and PK Assays: HACA and rituximab serum level (PK). Batched and sent to Covance Laboratories.
[20] When completing the repeat RHC, the subject should be on the same amount of supplemental oxygen used during the Screening RHC unless safety will be impacted. The method of calculating cardiac output should be consistent with that used at the Screening RHC.
[21] TB testing (PPD or QuantiFERON®-TB Gold In-Tube Test [QFT-G_IT]): unless performed within 30 days prior to screening and documented as negative in the subject's records or unless subject is known to have a positive test and has documentation of appropriate therapy.
[22] If the Screening physical exam, oxygen saturation, and chemistry panel are performed within 7 days of Day 0, they do not need to be repeated during the Randomization/Baseline or Day 0 visits unless clinically indicated.
[23] Only infectious events ≥ NCI-CTCAE grade 3 will be reported.
[24] Required only if the unscheduled visit is due to an increase in SSc-PAH disease activity; see Section 6.5.8, *Unscheduled Visit* for further details.
[25] Hemoglobin, platelets, lymphocytes, neutrophils, and bands results from a hematology specimen drawn within 24 hours of the infusion must be reviewed prior to initiating the study drug infusion (see Section 5.3.6, *Criteria for Withholding Rituximab Infusion*).
[26] A member of the research staff will obtain verbal consent to conduct an assessment of vital status and treatment satisfaction approximately two years post randomization for each subject. If the subject cannot be reached, a review of public records or health registries should be completed.

7 SAFETY MONITORING AND REPORTING

7.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting that data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 7.5, *Reporting of Adverse Events*) and appropriately to the investigational new drug application (IND) sponsor (DAIT/NIAID), principal investigators in the trial, Institutional Review Boards (IRBs), and health authorities. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice,* and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0*: http://ctep.cancer.gov/reporting/ctc.html.

7.2 Definitions

7.2.1 Adverse Event (or Adverse Experience)

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice)." [From OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" < http://www.hhs.gov/ohrp/policy/advevntguid.pdf>

7.2.2 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

7.2.3 Unexpected Adverse Reaction

A SAR is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

7.2.4 **Serious Adverse Event**

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

7.3 **Collection and Recording of Adverse Events**

7.3.1 **Investigational Product**

The primary investigational product in this protocol is rituximab/placebo. In addition, subjects in this protocol are required to receive background medical therapy for PAH (prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators [riociguat (Adempas)]). For purposes of reporting safety information on the MedWatch form, these drugs will be considered concurrent study mandated therapy.

7.3.2 **Collection Period**

Adverse events NCI-CTCAE grade 2 and above will be collected from the time the subject signs the informed consent until he/she initiates study intervention or until he/she is determined to be ineligible to receive study intervention, if the investigator determines that the adverse event is related to a study-mandated procedure, treatment, or change in treatment.

For all participants, adverse events NCI-CTCAE grade 2 and above will be collected from the time of initiation of study intervention (i.e., the administration of the first dose of study drug/study drug as defined in Section 6.5.3.1, *Visit 3 [Day 0]*) until he/she completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

7.3.3 **Collection of Adverse Events**

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the participant.

- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.
- In addition, an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event, as defined in Section 7.4, *Grading and Attribution of Adverse Events.*

7.3.4 **Recording Adverse Events**

Throughout the study, the investigator will record and grade adverse events NCI-CTCAE grade 2 and above on the appropriate AE electronic case report form (AE eCRF) regardless of their severity or relation to study medication or study procedure.

Once recorded, an AE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

7.3.5 **Recording Serious Adverse Events**

Serious AEs will be recorded on the appropriate AE eCRF and on the SAE eCRF. All requested information on the AE eCRF and SAE eCRF should be provided, if available, for submission to the Statistical and Clinical Coordinating Center (SACCC) and DAIT/NIAID.

New SAEs will be recorded until end of study participation OR for 30 days after premature withdrawal OR as in Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects* for subjects for whom B cells have not recovered.

Once recorded, an SAE will be followed until it resolves with or without sequelae OR until 30 days after the end of study participation UNLESS it is a Grade 3 or higher infection in an individual for whom B cells have not recovered (as per Section 3.3.2.1, *Follow-up Requirements for All Treated Subjects*), in which case it will be followed until resolution.

7.4 **Grading and Attribution of Adverse Events**

7.4.1 **Grading Criteria**

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version (CTCAE) 4.0.* This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the Protocol Chair(s) and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

If NCI-CTCAE criteria are defined for grading an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram), then a treatment-emergent adverse event is defined as an increase in grade from Baseline (Day 0) or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to Baseline (Day 0) will also be recorded as adverse events but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented.

7.4.2 **Attribution Definitions**

The relation, or attribution, of an adverse event to an investigational product will initially be determined by the site investigator. The site investigator will also record the initial determination of attribution on the appropriate AE eCRF. The relation of an adverse event to the study intervention will be determined using the descriptors and definitions provided in Table 7.4.2. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 7.4.2. NCI-CTCAE attribution of adverse events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy)
Unrelated Categories		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is unlikely related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

7.5 Reporting of Adverse Events

7.5.1 Reporting of Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report adverse events to the SACCC. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines. For this study, adverse events of NCI-CTCAE Grade 2 and higher will be reported.

Unless otherwise noted below in Section 7.5.1.1 as requiring 24 hour reporting, AEs must be recorder on the appropriate AE eCRF within five (5) days of the site learning of the event(s).

7.5.1.1 Procedure for Adverse Events Requiring 24 Hour Reporting

The adverse events that are bulleted below must be reported by site investigators to the SACCC regardless of relationship or expectedness to study intervention within a 24 hour period of discovering the adverse event:

- All SAEs per 21 CFR 312.32 definitions (see Section 7.2.4, *Serious Adverse Event*).
- All clinical events with a NCI-CTCAE Grade 3 or greater deemed possibly, probably, or definitely related to rituximab;
    - Note: clinical events include clinical signs/symptoms and diagnoses and laboratory abnormalities with clinical consequence (defined as the requirement for intervention, correction, increased monitoring, or further evaluation).
- Administration of atropine, dopamine, or other inotropic agents within 24 hours of rituximab/placebo infusion;
- Any event that the site considers Serious but is not easily categorized.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol-mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

The following process for reporting of the adverse events bulleted above ensures compliance with the ICH guidelines and the Food and Drug Administration (FDA) CFR regulations. When an investigator identifies such an adverse event, he or she must notify the SACCC within 24 hours of discovering the adverse event, and complete and submit the AE/SAE eCRF within 1 business day following initial notification. The SACCC is responsible for notifying DAIT/NIAID upon receipt of the site's notification of the adverse event and sending a SAE report form to DAIT/NIAID within 2 business days after receipt of the AE/SAE eCRF from the site.

7.5.1.2 **Procedure for Standard Adverse Event Reporting**

All other adverse events (Section 7.3.3, *Collecting Adverse Events*) must be recorded by the site on the appropriate AE eCRF within 5 business days of the site learning of the adverse event(s).

7.5.2 **DAIT/NIAID Reporting to the Health Authority**

After an adverse event requiring 24 hour reporting (per Section 7.5.1.1, *Procedure for Adverse Events Requiring 24 Hour Reporting*) is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for DAIT/NIAID to report the adverse event to the appropriate health authorities:

- **Annual IND Report.** This option applies if the adverse event is classified as one of the following:
    - Serious, expected, suspected adverse reactions (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction,* and Section 7.2.3, *Unexpected Adverse Reaction*).
    - Serious and not a suspected adverse reaction (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction*).

  Note that all adverse events (not just those requiring 24 hour reporting) will be reported in the Annual IND Report.
- **Expedited Safety Report.** This option applies if the adverse event is classified as one of the following:
    - Serious and unexpected suspected adverse reaction (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction* and Section 7.2.3, *Unexpected Adverse Reaction*).
    - Aggregate analysis of adverse events that suggests a causal relationship to rituximab.
    - Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial will be reported.

  Safety Reports must be reported by DAIT/NIAID to the appropriate health authorities within 15 calendar days; fatal or immediately life-threatening serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.
- Safety reports will be provided to Genentech, Inc. per the terms negotiated in the Clinical Trial Agreement (CTA).

7.5.3 **Reporting of Adverse Events to IRBs**

All investigators must report adverse events, including expedited reports, in a timely fashion to their respective IRBs in accordance with applicable regulations and guidelines.

All IND Safety Reports to the FDA will be distributed by the DAIT/NIAID or designee to all participating institutions for site IRB submission.

7.6 Pregnancy Reporting

This study includes pregnancy information as safety data. Information about any pregnancy should be reported promptly to the SACCC on the same timeline as an SAE (Section 7.5.1.1, *Procedure for Adverse Events Requiring 24 Hour Reporting*).

All pregnancies identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy in a study subject or a partner of a study subject. A pregnant subject should be instructed to stop taking study medication. The investigator should report to the SACCC all pregnancies within 1 business day (as described in Section 7.5.1.1, *Procedure for Adverse Events Requiring 24 Hour Reporting*) using the Pregnancy eCRF. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject should continue until the conclusion of the pregnancy, and an updated Pregnancy eCRF detailing the outcome of the pregnancy should be submitted to the SACCC. When possible, similar information should be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery will include:

- Subject's enrollment ID
- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

Should the pregnancy result in a congenital abnormality or birth defect, an SAE must be submitted to the SACCC using the SAE reporting procedures described above.

7.7 Reporting of Other Safety Information

An investigator should promptly notify the SACCC when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event.

7.8 Review of Safety Information

7.8.1 Medical Monitor Review

The DAIT Medical Monitor will receive monthly reports from the SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the sites on appropriate eCRFs.

In addition, the Medical Monitor will receive SAE and pregnancy reports for review and triage after the SACCC is made aware of these events (See Sections 7.5.1, *Reporting of Adverse Events to DAIT/NIAID*, and 7.6, *Pregnancy Reporting*).

7.8.2 **DSMB Review**

The Data and Safety Monitoring Board (DSMB) will review accumulating safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. To ensure patient safety between Data Review Meetings, the DSMB will be informed of all Expedited Safety Reports in a timely manner.

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews or emergency meetings. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, the following events will trigger both a comprehensive DSMB Emergency Safety Review and a temporary halt in enrollment:

a. Any immediately life threatening event or death that occurs in the study which is unexpected and possibly, probably, or definitely related to study intervention.
b. Any event, including those listed in Section 3.3.2, *Discontinuation of Protocol-Specified Treatment Requirements*, that results in permanent discontinuation of infusion of study intervention occurring in 2 of the first 5 subjects, 4 of the first 15 subjects, 6 of the first 30 subjects, or a total of 8 events after 30 subjects have been enrolled in the trial.
c. Any occurrence of PML.
d. Clinical Grade 3 or higher events or SAEs which are possibly, probably, or definitely related to study treatment but do not result in discontinuation of study treatment will be reviewed whenever the ratio of events to enrolled subjects is 20% (e.g. 9 events for 47 subjects, 10 events for 48-52 subjects, 11 events for 53-57 subjects, etc.).
    - Note: clinical events include clinical signs/symptoms and diagnoses and laboratory abnormalities with clinical consequence (defined as the requirement for intervention, correction, increased monitoring, or further evaluation).

The DSMB will have discretion to recommend actions regarding study conduct and continuation as a consequence of any planned or unplanned monitoring activity.

7.8.2.1 **Temporary Halt in Enrollment for Emergency Safety Review**

A temporary halt in enrollment will occur for the events listed in Section 7.8.2, *DSMB Review* with the exception of 7.8.2.d. For events falling under 7.8.2.d, a halt in enrollment will occur if the DSMB Emergency Safety Review is not completed within 2 weeks. In the event that the study temporarily halts enrollment, no new subjects will be consented or start on therapy with rituximab or placebo; and subjects already on rituximab or placebo will continue on therapy unless they are the focus of the DSMB review. Subjects in the screening phase of the study may continue to undergo minimal risk procedures (e.g. blood tests), but more than minimal risk procedures should be deferred. Randomization will not occur until the DSMB review is complete. The FDA will be notified of any halt in enrollment. After

careful review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

8 STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

8.1 Sample Size

As of May 2014, 30 subjects had enrolled in the study. The slow pace of enrollment prompted a redesign of the trial with the objective of reducing the sample size to enable completion of the study within 2 years while still retaining the potential to address a clinically relevant question. Sixty was the largest sample size felt to be attainable (down from 80).

Through version 5.0 of the protocol, the primary endpoint was the change in PVR from baseline to Week 24, defining a 20-30% improvement from baseline as clinically significant. Assuming a baseline mean of 810 dyne-s/cm^5, a drop at Week 24 equal to 251 dyne-s/cm^5 would be considered clinically significant. Power was 85% for a design with 80 subjects, assuming the baseline mean was the same for both arms (i.e. 810 dyne-s/cm^5), a standard deviation in the change from baseline of 350 dyne-s/cm5, and the percent change from baseline equals 0% and 30% for placebo and rituximab arms, respectively.

Contrary to the original assumption that the mean baseline PVR was 810 dyne-s/cm^5, review of the data revealed that the mean baseline PVR for the enrolled subjects was approximately 553 dyne-s/cm^5. Hence, relative to an assumed population baseline mean of 553 dyne-s/cm^5, the difference of 251 dyne-s/cm^5 used in the original power/sample size computation would equate to an improvement of 46%. The magnitude of the drop is much larger than the clinically meaningful effect and is overly optimistic (or completely unrealistic). This degree of change is not needed to demonstrate clinical efficacy. Further, if the baseline mean is smaller than originally anticipated, a larger (not smaller) study would be needed to detect a clinically meaningful effect.

The 6MWD has been the most utilized endpoint in PAH trials, and a 33m improvement from baseline is considered clinically significant [160]. For sample size calculations, we assume a change from baseline of 0m for the placebo arm and 33m for the rituximab arm. An effect size of this magnitude is attainable in a 24 week period, and has been seen in as little as 12 weeks in studies using vasodilators. Assuming a standard deviation of 58m (a best guess based on prior studies) and a 20% drop out rate, ASC01 would be powered at 50% to detect a treatment group difference of 33m for this phase II study. If a significant result is found for this primary efficacy endpoint and no safety signals emerge, this study could provide a definitive conclusion about this treatment. In order to achieve 80% power, the true difference between treatment groups would have to be at least 50m. While other studies evaluating other drugs have seen this magnitude of difference in similar timeframes, we have no data on rituximab to suggest the true difference is at least 50m. Furthermore, rituximab would be interesting as a therapy option if the effect size is as small as 33m.

8.2 Analysis Populations

8.2.1 Safety Population

The Safety Population (SP), which will be used for all safety analyses, will include all subjects for whom study treatment is initiated.

8.2.2 Intent-to-Treat Population

The Intent-to-Treat (ITT) population will include all randomized subjects who initiate rituximab therapy and meet entry criteria. Randomized subjects who withdraw from the trial prior to starting rituximab infusion will be excluded from the ITT analysis set and replaced. The primary efficacy analyses will be based on the ITT population, as will key secondary efficacy and mechanistic analyses. Subjects who, for whatever reason, do not complete their assigned therapy will be included in the ITT population in the groups to which they were randomized.

8.2.3 Per Protocol Population

The Per Protocol (PP) population will be defined as those subjects who comply with the assigned treatment protocol by completing two IV infusions of 1000mg of Rituximab/Placebo at Visit 3 and Visit 4 as well as completing primary endpoint evaluation (6MWD) at Visit 10 with no major protocol deviations that would affect their efficacy outcome. Subjects who are withdrawn from follow-up prior to Visit 10 will not be included in the PP population. A masked data review panel will evaluate deviations from the protocol including, for example, violations of entry criteria, departures from assigned treatment regimen, or administration of study procedures outside the specified visit windows. The panel may exclude subjects with serious protocol deviations from the PP population. Both the primary analyses and all secondary analyses will be replicated on the PP population.

8.3 Statistical Methods

In presenting data from this trial, continuous data will be summarized in tables listing the mean, standard deviation or standard error, median, and number of subjects in a group. Categorical data will be summarized in tables listing the frequency and the percentage of subjects in a group. These summaries will be presented separately for subjects on the two treatment arms.

8.3.1 Safety Analysis

All safety analyses will be performed using the Safety Population.

AEs including changes in laboratory values will be graded according to the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version (CTCAE) 4.0* (http://ctep.cancer.gov/reporting/ctc.html). The frequency of AEs will be summarized by system organ class, preferred term, severity (grade), and relationship to study treatment. For each of these summaries, subjects will be counted at most once within each organ class or

preferred term at the greatest severity. For these summaries, relationship to study treatment will be categorized as either treatment related (possibly, probably, or definitely related to study medication) or unrelated. Similar analyses will be performed for SAEs.

For all reported adverse events and SAEs, the risk of the event in each arm will be described using event rates and an analysis based on a Poisson regression model will be conducted to compare the rate of each type of event in the treatment groups. The proportion of subjects experiencing at least one event will be reported and the treatment groups compared based on Fisher's Exact Test.

Treatment-related mortality, defined as death occurring at any time after randomization and possibly, probably, or definitely resulting from study treatment, and all-cause mortality, defined as any death occurring after randomization, will be summarized. The proportion of subjects dying in each treatment group will be reported and the treatment groups compared based on Fisher's Exact Test.

Laboratory parameters will be summarized both overall and by treatment group using appropriate descriptive statistics. For quantitative safety parameters, change from the last pre-randomization assessment to the final visit assessment will be summarized overall and by treatment group. For qualitative parameters, descriptive information on shifts in pre- to post-randomization findings will be provided. In addition, abnormal and clinically significant abnormalities will be summarized and listed separately.

All safety comparisons and associated p-values are considered exploratory, not as formal tests of hypothesis. As such no adjustments will be made for multiple comparisons and all p-values must be interpreted cautiously.

Detailed listings of AEs, deaths, and safety-related laboratory values will be generated as specified in the Statistical Analysis Plan (SAP).

8.3.2 **Efficacy Analysis**

8.3.2.1 **Primary Efficacy Analysis**

The primary efficacy analysis for this study is designed to test the scientific hypothesis that rituximab has a beneficial clinical impact on SSc-PAH as measured by improvement or stabilization in 6MWD between baseline to 24 weeks in patients treated with standard of care plus rituximab than in patients treated with standard of care plus placebo. Operationally for this study, this scientific hypothesis will be examined by formally testing the following statistical hypothesis:

H_0: The mean change in 6MWD between baseline and 24 weeks does not differ between individuals treated with rituximab and those treated with placebo ($\delta_{rituximab} = \delta_{placebo}$ where δ_x = mean 6MWD at 24 weeks – mean 6MWD at baseline)

versus

H_A: The mean change in 6MWD between baseline and 24 weeks is more beneficial in those individuals treated with rituximab than in those treated with placebo
($\delta_{rituximab} < \delta_{placebo}$ where δ_x = mean 6MWD at 24 weeks – mean 6MWD at baseline)

While this hypothesis is inherently one-sided, two-sided tests will be used in the analysis. This primary hypothesis will be tested using a repeated measures random effects model to model change from baseline in 6MWD as the primary outcome and treatment as the primary predictor, controlling for visit day, limitations by musculoskeletal disease, baseline measures of 6MWD, and a treatment by visit day interaction A random slope and intercept will be fit for each subject. An unstructured covariance matrix will be assumed for the random effects. More details on this analysis and sensitivity analyses will be defined in the Statistical Analysis Plan (SAP). The primary efficacy analysis will be based on the ITT population.

8.3.2.2 **Secondary Efficacy Analysis**

All secondary analyses will be conducted in an exploratory fashion with p-values and confidence intervals presented as descriptive statistics with no adjustments for multiple comparisons. Tests will be two-sided and interval estimates will be generated at the 95% confidence level. As part of secondary analyses, appropriate contrasts will be constructed using model-based approaches. Larger models will be constructed for these comparisons with consideration of additional covariates that are found to differ between the treatment groups. Covariates may include, but are not limited to: study center, method of cardiac output, gender, disease duration and severity, and concomitant therapy use. All analyses will be repeated using the Per Protocol Population.

Change from baseline in PVR at Week 24 and percent change from baseline in PVR at Week 24 will be analyzed using an analysis of covariance, adjusting for treatment, method of cardiac output, and baseline PVR.

For the outcome time to clinical worsening, the two groups will be compared using survival approaches. Graphical summaries of time to clinical worsening will be generated using Kaplan-Meier survival curves with a log-rank test used to test for differences in the two arms. The two arms will also be compared after control of appropriate covariates using a Cox proportional hazards model.

For the proportion of subjects who changed or added PAH therapeutic agents, the treatment two arms will be compared using a logistic regression model, controlling for appropriate covariates. This will be done at the following time points: prior to week 24 and after week 24.

Longitudinal changes in continuous outcome measures including the subscales of the SF-36, the SHAQ, and DLCO will be compared across the two arms using linear mixed models. Initially the models will be used to describe changes between baseline and 24 weeks and

baseline and 48 weeks including only treatment, baseline levels of the outcome of interest, and an indicator if the measurement was affected by changed or new PAH therapeutic agents, with added models developed to assess the effect of other covariates.

Longitudinal changes in the six-minute walk distance at week 48 will be assessed using a similar model as the primary endpoint, but including all data through 48 weeks. This model will address any subject who had their 6MWD affected by new or changed PAH therapeutic agents after the 24 week time point. Additionally, the analysis will be conducted on the ITT population will additionally add limited by any musculoskeletal disease (yes/no) as a covariate. Additional models may be developed to assess the effect of other covariates.

Longitudinal change in the oxygen saturation will be compared across the two arms using generalized estimating equations. An ordinal response variable will be used to account for the subjects that cannot perform the room air assessment. Initially the model will be used to describe changes between baseline and 24 weeks and baseline and 48 weeks including only treatment, baseline levels of oxygen saturation, and an indicator if the measurement was affected by changed or new PAH therapeutic agents, with added models developed to assess the effect of other covariates. However, if all subjects can perform the room air assessment, the data will be analyzed as a continuous model, following the method described above for the six-minute walk distance.

Longitudinal change in number of new digital ulcers will be compared across the two arms using Poisson marginal models for longitudinal data. Initially the models will be used to describe changes between baseline and 24 weeks and baseline and 48 weeks including only treatment, baseline levels of the outcome of interest, and an indicator if the measurement was affected by changed or new PAH therapeutic agents , with added models developed to assess the effect of other covariates.

8.3.3 **Mechanistic/Immunological Analysis**

Because of the exploratory nature of the analyses, descriptive statistics and plots (including, but not limited to, those described subsequently) will be used to gain an understanding of the data prior to developing any statistical models. Means, medians, standard deviations, minimums and maximums will be computed for each continuous biomarker at each time point for placebo and rituximab groups and separately for treatment responders / non-responders. For dichotomous biomarkers, frequencies and percents will be computed at each time point for placebo and rituximab groups and separately for treatment responders / non-responders. Treatment response, to be defined in the statistical analysis plan (SAP), will be based on PVR, exercise capacity as measured by 6MWD, or quality of life as measured by SF-36 and SHAQ. To gain a better understanding of trends over time, summary statistics (e.g. means, medians, or percents) will be plotted versus time at the relevant time points. Plots for individual subjects may also be useful.

8.3.4 Exploratory Analysis

Agreement between a secondary endpoint (PVR calculated by site-based standards) and the standardized Fick-based calculation of PVR (PVRf) will be assessed using the method of Bland and Altman, separately for baseline and Week 24 right heart catheterization. Analyses will also be subset on method of calculation of cardiac output (thermodilution vs. Fick) if found to be a significant predictor in the ANCOVA model.

This secondary efficacy analysis will be repeated for PVRf. Change from baseline in the standardized Fick-based calculation of pulmonary artery capacitance (PACf) will also be analyzed in the same manner as the PVR efficacy analysis.

Additionally, the relationship between change in PVR and change in 6MWD at Week 24 will be analyzed using a Spearman's rank correlation coefficient. Relationships between additional clinical outcomes and 6MWD or PVR can also be provided. These clinical outcomes will be addressed in the SAP.

The analyses of Treatment Satisfaction responses will also be considered exploratory. Questions will include:

1. Do you think you benefited by being in the study?
2. Have you noticed any long term benefits from study treatment?
3. Do you think you received rituximab or placebo?

All exploratory analyses will be conducted on subjects in the ITT population with available data.

8.4 Interim Analysis

Results of interim analyses will be reported to the DSMB for planned Data Review Meetings. Reports prepared for these meetings will focus on study conduct and subject safety and may include information on enrollment, randomization, site activation status, protocol deviations, subject status and demographics, and safety analyses.

8.4.1 Interim Analysis of Efficacy Data

There will not be any interim analysis on efficacy data for this trial.

8.4.2 Interim Analysis of Safety Data

The DSMB will periodically review safety data as described in Section 7.8.2, *DSMB Review*. The safety analyses for this trial will be descriptive rather than inferential. Detailed listings and summary tabulations of adverse events and safety-related laboratory values will be generated as specified in the Statistical Analysis Plan. The safety analyses will be completed using the safety population without stratification.

No formal stopping rules for safety endpoints are specified.

8.5 Other Statistical Considerations

8.5.1 Covariates

The primary analysis will be adjusted for as stated above.

If the analyses described in Section 8.5.4, *Examination of Subgroups* yield covariates that differ significantly between treatment groups, then, as a secondary analysis, these covariates may also be considered for adjustment in the models specified for the primary analysis. Covariates that may be considered include, but are not limited to: age, study center, gender, race, duration of SSc-PAH disease, baseline and concomitant medication use, undergoing cardiopulmonary rehabilitation (yes/no), non-biologic immunosuppressive or immunomodulating drug use (yes/no), and non-biologic immunosuppressive or immunomodulating drug change after 6 months (yes/no).

8.5.2 Multi-center Studies

Due to the small sample size and large number of sites needed for this study, study center will not be included as a covariate in the analysis. However, if evidence of a study center effect exists and there are appropriate sample sizes at each site, a sensitivity analysis will be performed including study center in the efficacy analyses. All safety analyses will be based on data pooled across all centers with no adjustment or stratification by center.

8.5.3 Multiple Comparisons and Multiplicity

This study has a single primary analysis designed to assess the efficacy of rituximab in clinical response as measured by change in PVR. Consequently, no adjustments for multiplicity are needed for Type I error protection. The secondary efficacy analyses are considered to be descriptive with p-values and confidence intervals presented as descriptive measures of strength of evidence rather than formal statistical inference. Therefore, no multiplicity adjustments are needed for this study.

8.5.4 Examination of Subgroups

Secondary analyses of the primary and secondary objectives may be conducted for the following subgroups:

- Race
- Ethnicity
- Gender
- Baseline PVR
- Baseline 6MWD
- NYHA Functional Class
- Duration of SSc-PAH disease

Additionally, exploratory analyses of responder subgroups and subgroups defined through the mechanistic and immunological studies will be conducted.

8.5.5 **Missing Data**

Standard procedures will be used to ensure that data are as complete and accurate as possible. In analyses, a full accounting will be made for all data items. Because this is a Phase II trial that is underpowered, there will not be any additional handling of missing data for the primary analysis. However, sensitivity analyses will be performed to assess the robustness of the conclusions under different assumptions. For example, missing data due to death of a subject prior to Week 24 would be handled differently than missing data due to a missed visit.

As described in Section 3.3.3.1, *Follow-up Requirements for All Treated Subjects*, every effort will be made to obtain 6MWD for patients withdrawing prior to the 24 week visit. For the primary analysis, this observation will be included in the repeated measures model and will contribute to the estimated mean change from baseline at Week 24.

Secondary analyses will be performed on all available data; no imputation will be performed on missing data, recognizing that these exploratory analyses could be biased in an unknown direction by non-ignorably missing data.

If data are missing for the primary endpoint, sensitivity analyses will be conducted to assess whether conclusions are robust to alternative analytic approaches for handling these data. If data are missing for other key endpoints, sensitivity analyses could also be conducted, if deemed appropriate. Details of the sensitivity analyses will be provided in the Statistical Analysis Plan.

8.5.6 **Changes to the Statistical Analysis Plan**

A detailed description of the planned analyses will be provided in a SAP to be completed and signed off prior to the completion of the trial. Major changes from this protocol will be noted in the SAP. If there is sufficient reason to do so, revised plans may be issued during the course of the study. Changes to the SAP that are made subsequent to database lock will be documented in the clinical study report.

9 ACCESS TO SOURCE DATA AND DOCUMENTS

Each participating site will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from subjects participating in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, each site must permit authorized representatives of the IND sponsor, the SACCC, Genentech, Inc., and health authorities to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other subject data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. Participating sites will normally be notified in advance of auditing visits.

All subject records and study documentation will be kept after the protocol is completed. This will include all documentation of AEs, records of study drug receipt and dispensation, and all IRB correspondence. All study records will be kept for at least 2 years after the investigation is completed.

10 DATA COLLECTION, QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The period of record retention should be consistent with the record retention policies of the sponsoring agency or applicable regulatory agencies. However, in certain instances, documents should be retained for a longer period if required by the applicable regulatory agency or by the National Institutes of Health.

The investigator will report all protocol deviations to DAIT and the SACCC per the instructions in the ACE Manual of Operations. The SACCC will forward reports of protocol deviations to the responsible DAIT/NIAID medical officer for review as specified in the ACE Manual of Operations.

The SACCC is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency and accuracy of all documented data.

Data will be obtained from a variety of sources including, but not limited to laboratory notebooks, automated instrument output files, and clinical subject charts. Data from these source materials will be transmitted to the SACCC via one of two mechanisms. Data collected electronically at central laboratories will be transferred electronically directly from the laboratory to the SACCC using standard secure data transfer procedures. Data collected at the clinical sites will be transmitted to the SACCC using an internet-based remote data entry system. Clinical site personnel use an internet browser to key data into electronic CRFs (eCRFs); each eCRF page is submitted to the SACCC data server electronically as the page is completed. Univariate data validation tests are performed as the data are keyed. The central database, which resides on the SACCC server, is backed up nightly; backup tapes are saved in a secure, off-site location. At any time, authorized site personnel may log in to the remote data entry system, review and correct previously entered data, or key additional data. The data will be further validated per the study data validation plan via a series of computerized and manual edit checks, and all relevant data queries will be raised and resolved on an ongoing basis. Complete, clean data will be frozen to prevent further inadvertent modifications. All discrepancies will be reviewed and any resulting queries will be resolved with the investigators and amended in the database. All elements of data entry (i.e., time, date, verbatim text, and the person performing the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

The SACCC will periodically visit the participating clinical sites and audit the source documents in order to validate the data in the SACCC central database. Data will be provided using the subject's screening or enrollment number; the SACCC will not collect personally identifying information such as the subject's name or social security number. Subjects will provide demographic information such as race, ethnicity, and birth date.
Data collected by the SACCC will be held in the strictest confidence, and are protected from access that could reveal personally identifying information about any subject in the trial.

11 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

The study will be conducted according to Good Clinical Practice (GCP) guidelines, U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards.

11.1 Compliance with Good Clinical Practices

This trial will be conducted in compliance with the protocol, current GCPs recommended by the International Conference on Harmonization (ICH) and the applicable regulatory requirements for participating institutions. These include the tenets of the Declaration of Helsinki and review and approval by the appropriate ethics review committee or IRBs of participating organizations. The SACCC will assure compliance through a program of quality assurance audits performed both at participating sites and within the SACCC for data quality and adherence to protocol requirements. The SACCC is operated by Rho Federal Systems Division, Inc. (RhoFED), Chapel Hill, North Carolina under a contract from DAIT/NIAID.

11.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must be approved by the IRB and submitted to the FDA before they are placed into use. In both the United States and in other countries, only institutions holding a current Federal Wide Assurance issued by the Office of Human Research Protection (OHRP) at the Department of Health and Human Services (DHHS) may participate.

The investigator will inform the IRB of serious or unexpected AEs that might occur during the study and are likely to affect the safety of the subjects, or the conduct of the study. The investigators will comply fully with all IRB requirements for both the reporting of AEs, protocol or consent form changes, as well as any new information pertaining to the use of the study medication that might affect the conduct of the study.

11.3 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki, as well as compliance with all IRB requirements, will be implemented in the study, before any protocol-specified procedures are carried out. A standard consent form for subject participation will be provided with the protocol to each institution. Any modifications to the

standard information in the template will require review and approval by the SACCC and DAIT/NIAID. Informed consent will be obtained in accordance with 21 CFR 50.52. Information may be given to subjects in oral, written or video form by the investigator. All prospective subjects will be given ample time to read the consent form, and ask questions, before signing.

If subjects are to be enrolled who do not speak and read English, the consent materials must be translated into the language appropriate for the enrolling subject. Translated documents must be certified to contain the complete descriptions provided in the English version of the document. If an interpreter is used to provide or assist in describing the consent materials to an enrolling subject, the interpreter must also sign the consent materials certifying their involvement with the consent process.

After completion, a copy of the signed consent form will be given to the subject. The original signed consent form will be kept on file in the subject's study chart, available for inspection by regulatory authorities, both federal and institutional.

11.4 Data and Safety Monitoring Board

The responsibility for reviewing the ethical conduct of the study and for monitoring reports of evidence of adverse or beneficial effect is assigned to the DAIT Autoimmunity Data and Safety Monitoring Board (DSMB). The DSMB is an independent group composed of biomedical ethic experts, physicians, and other scientists who are responsible for continuing review of study information. The DSMB makes recommendations to DAIT/NIAID on issues affecting the course and conduct of this clinical study.

12 FINANCING AND INSURANCE

Participating institutions must comply with their institution's policies on compensation, insurance, and indemnity. Institutions must have adequate liability insurance coverage to satisfy their local and national requirements for study participation.

13 PUBLICATION POLICY

The Autoimmunity Centers of Excellence (ACE) policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ACE internet website at http://www.rhoworld.com. Study investigators are encouraged to communicate and publish study results with prior notification of DAIT/ NIAID and Genentech, Inc. The following procedure is suggested:

1. Manuscripts, abstracts, posters and other material for public distribution will be submitted to DAIT/NIAID, and Genentech, Inc. at least 30 days prior to submission for publication or public presentation.
2. DAIT/NIAID, and Genentech, Inc. will review and comment on the proposed material within 30 days.
3. DAIT/NIAID, and/or Genentech, Inc. may ask that confidential information be deleted or redacted in this case where a patent may be filed or where confidential

information is involved. Publication or presentation may be delayed up to 60 additional days in order to file a patent application.

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15 APPENDICES

Appendix A: Modified Scleroderma Health Assessment Questionnaire (SHAQ)

Appendix B: Short Form – 36 Health Survey (SF-36)

Appendix C: New York Heart Association Classification of Heart Disease

Appendix D: Subject Self-Reported Demographics Source Document

Appendix E: Exploratory Analysis: Standardized Fick-based Pulmonary Vascular Resistance and Pulmonary Artery Capacitance

Appendix F: RESTORE Sub-study Protocol

15.1 Appendix A: Modified Scleroderma Health Assessment Questionnaire (SHAQ)

Modified Scleroderma Health Assessment Questionnaire (SHAQ)

Subject ID:_____ **Date (MM/DD/YY):** ____/____/____

In the past seven (7) days,	Without any difficulty	With some difficulty	With much difficulty	Unable to do
1. Are you able to: Dress yourself, including tying shoelaces and doing buttons?	☐	☐	☐	☐
Shampoo your hair?	☐	☐	☐	☐
2. Are you able to: Stand up from an armless straight chair?	☐	☐	☐	☐
Get in and out of bed?	☐	☐	☐	☐
3. Are you able to: Cut your meat?	☐	☐	☐	☐
Lift a full cup or glass to your mouth?	☐	☐	☐	☐
Open a new carton of milk?	☐	☐	☐	☐
4. Are you able to: Walk outdoors on flat ground?	☐	☐	☐	☐
Climb up 5 steps?	☐	☐	☐	☐
5. Are you able to: Wash and dry your entire body?	☐	☐	☐	☐
Take a tub bath?	☐	☐	☐	☐
Get on and off the toilet?	☐	☐	☐	☐
6. Are you able to: Reach and get down a 5 pound object (such as a bag of sugar) from just above your head?	☐	☐	☐	☐
Bend down and pick up clothing from the floor?	☐	☐	☐	☐
7. Are you able to: Open car doors?	☐	☐	☐	☐
Open jars which have previously opened?	☐	☐	☐	☐
Turn regular taps on and off?	☐	☐	☐	☐
8. Are you able to: Run errands and shop?	☐	☐	☐	☐
Get in and out of a car?	☐	☐	☐	☐
Do chores such as vacuuming or yard work?	☐	☐	☐	☐

Please check any Aids or Devices that you usually use:

Cane	☐	Dressing Devices (button hook, long shoe horn, etc.)	☐
Walker	☐	Built up or Special Utensils	☐
Bath/tub seat	☐	Long Handled Appliances for reach	☐
Bathtub Bar	☐	Long Handled Appliances for Bathroom	☐
Jar Opener	☐	Raised Toilet seat	☐
Crutches	☐	Special or Built-up Chair	☐
Wheelchair	☐	Other (Specify)	☐

Please check any categories for which you usually need help from another person:

Hygiene	☐	Gripping and Opening Things	☐
Reach	☐	Errands and Chores	☐
Arising	☐	Dressing and Grooming	☐
Eating	☐	Walking	☐

Place a mark on the line to indicate the severity of the pain.

1. How much pain have you had because of your illness ***in the past week***?

0 — No pain ... 100 — Very severe pain

2. ***In the past week***, how much has your Raynaud's Phenomenon interfered with your activities?

Does not interfere ... Very severe limitation

3. ***In the past week***, how much has/have your finger ulcer(s) interfered with your activities?

Does not interfere ... Very severe limitation

4. ***In the past week***, how much have your intestinal problems interfered with your activities?

Does not interfere ... Very severe limitation

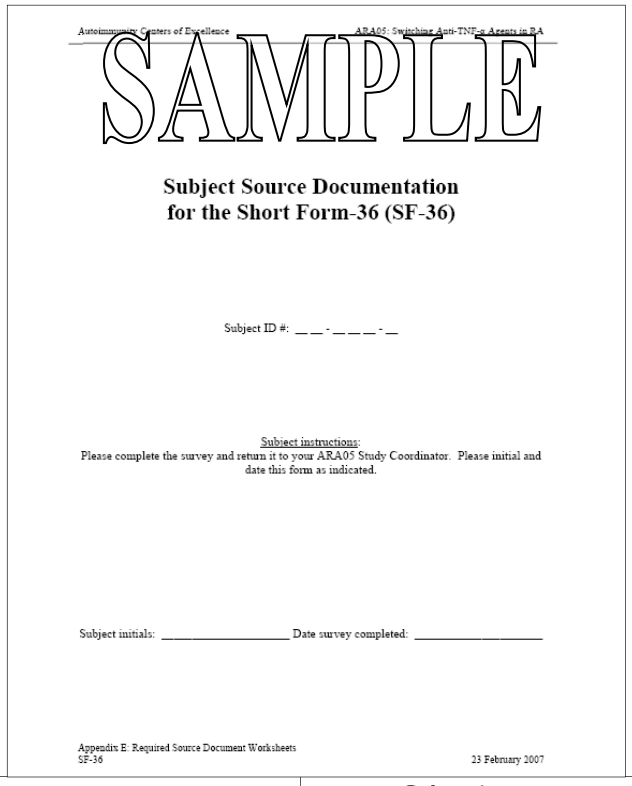
5. ***In the past week***, how much have your breathing problems interfered with your activities?

Does not interfere ... Very severe limitation

6. Overall, considering how much pain, discomfort, limitations in your daily life and other changes in your body and life, how severe would you rate your disease ***today?***

No disease ... Very severe

15.2 Appendix B: Short Form – 36 Health Survey (SF-36)



Autoimmunity Centers of Excellence ARA05: Switching Anti-TNF-α Agents in RA

SAMPLE

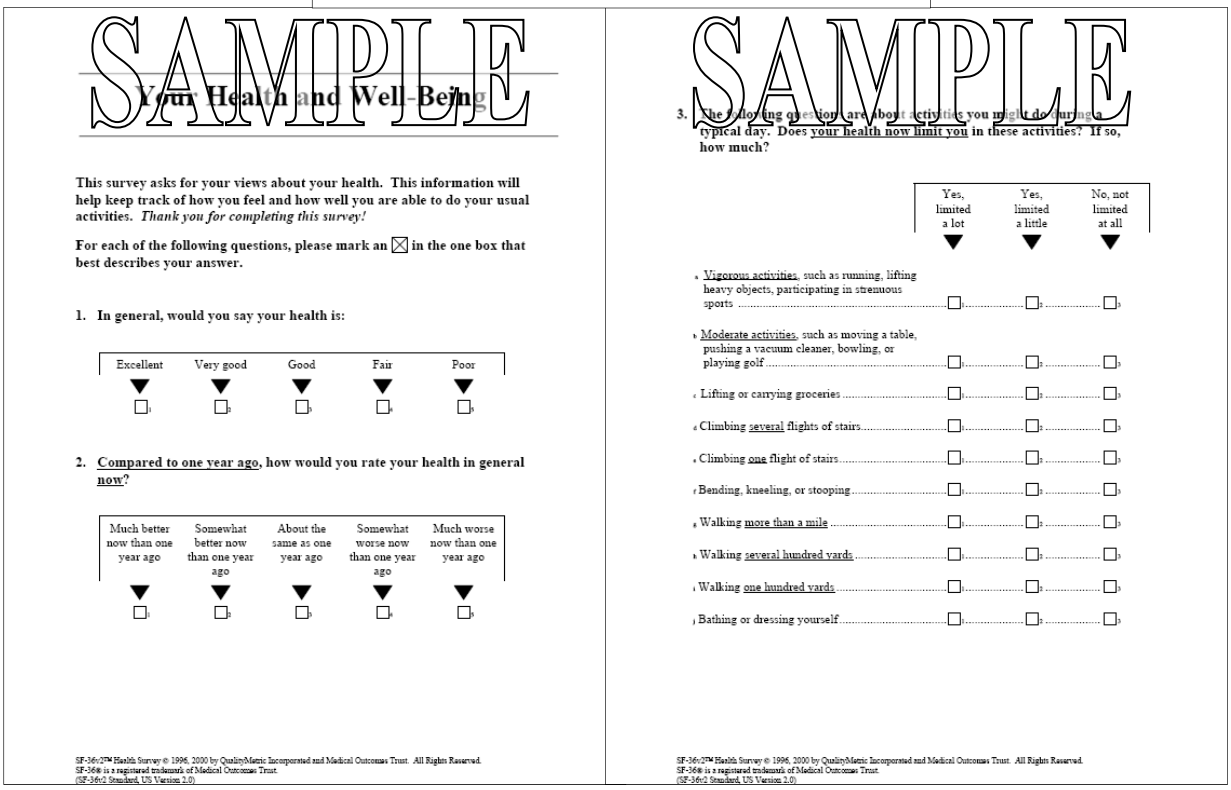
Subject Source Documentation for the Short Form-36 (SF-36)

Subject ID #: __ __ - __ __ __ - __

Subject instructions:
Please complete the survey and return it to your ARA05 Study Coordinator. Please initial and date this form as indicated.

Subject initials: _____ Date survey completed: _____

Appendix E: Required Source Document Worksheets
SF-36 23 February 2007



SAMPLE

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. ***Thank you for completing this survey!***

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
▼	▼	▼	▼	▼
☐1	☐2	☐3	☐4	☐5

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
▼	▼	▼	▼	▼
☐1	☐2	☐3	☐4	☐5

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SAMPLE

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
	▼	▼	▼
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐1	☐2	☐3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐1	☐2	☐3
c. Lifting or carrying groceries	☐1	☐2	☐3
d. Climbing several flights of stairs	☐1	☐2	☐3
e. Climbing one flight of stairs	☐1	☐2	☐3
f. Bending, kneeling, or stooping	☐1	☐2	☐3
g. Walking more than a mile	☐1	☐2	☐3
h. Walking several hundred yards	☐1	☐2	☐3
i. Walking one hundred yards	☐1	☐2	☐3
j. Bathing or dressing yourself	☐1	☐2	☐3

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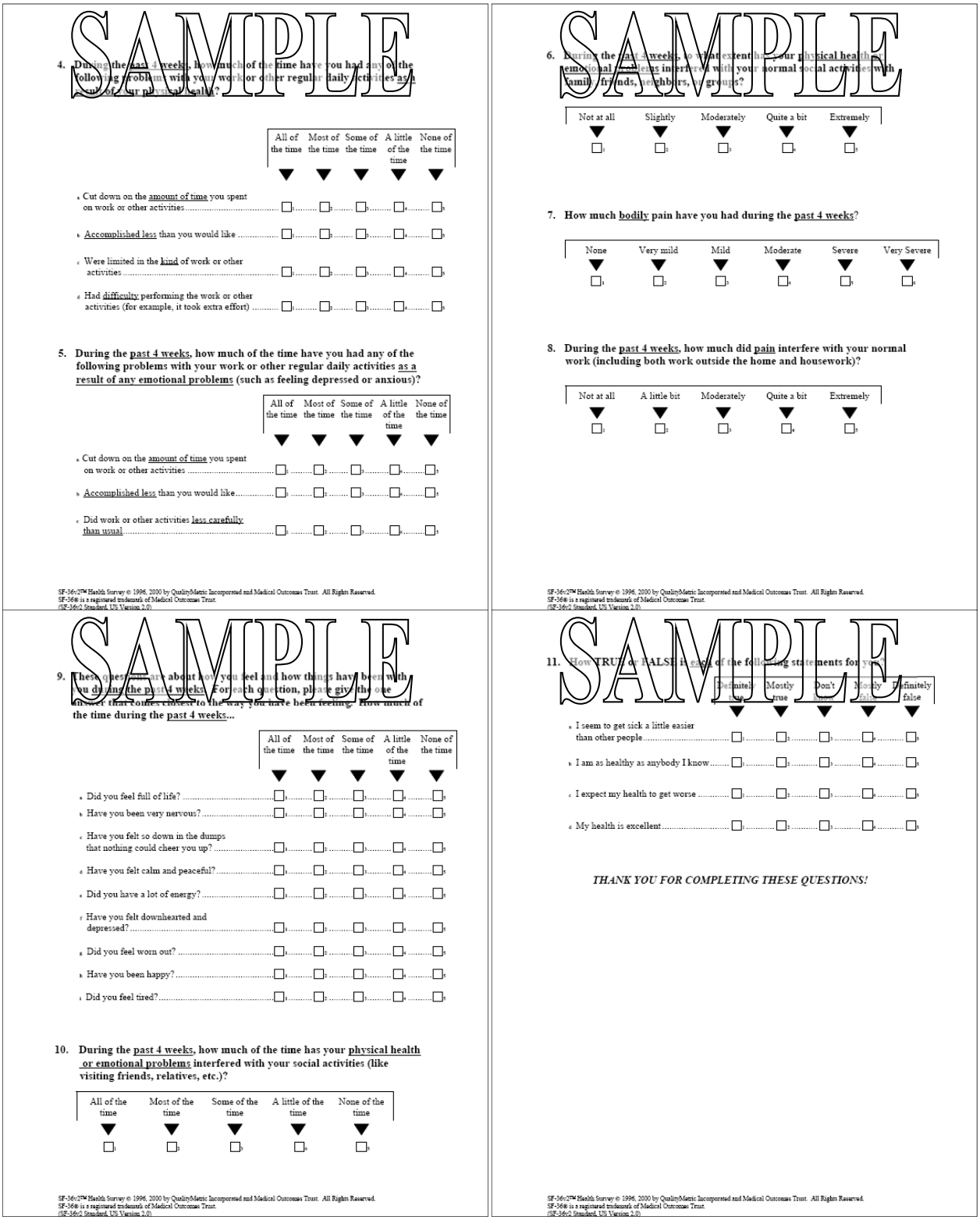
SAMPLE
4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?
All of the time
Most of the time
Some of the time
A little of the time
None of the time
a. Cut down on the amount of time you spent on work or other activities
b. Accomplished less than you would like
c. Were limited in the kind of work or other activities
d. Had difficulty performing the work or other activities (for example, it took extra effort)
5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?
All of the time
Most of the time
Some of the time
A little of the time
None of the time
a. Cut down on the amount of time you spent on work or other activities
b. Accomplished less than you would like
c. Did work or other activities less carefully than usual
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SAMPLE
6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?
Not at all
Slightly
Moderately
Quite a bit
Extremely
7. How much bodily pain have you had during the past 4 weeks?
None
Very mild
Mild
Moderate
Severe
Very Severe
8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?
Not at all
A little bit
Moderately
Quite a bit
Extremely
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SAMPLE
9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...
All of the time
Most of the time
Some of the time
A little of the time
None of the time
a. Did you feel full of life?
b. Have you been very nervous?
c. Have you felt so down in the dumps that nothing could cheer you up?
d. Have you felt calm and peaceful?
e. Did you have a lot of energy?
f. Have you felt downhearted and depressed?
g. Did you feel worn out?
h. Have you been happy?
i. Did you feel tired?
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?
All of the time
Most of the time
Some of the time
A little of the time
None of the time
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SAMPLE
11. How TRUE or FALSE is each of the following statements for you?
Definitely true
Mostly true
Don't know
Mostly false
Definitely false
a. I seem to get sick a little easier than other people
b. I am as healthy as anybody I know
c. I expect my health to get worse
d. My health is excellent
THANK YOU FOR COMPLETING THESE QUESTIONS!
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15.3 Appendix C: New York Heart Association Classification of Heart Disease

Functional Capacity	Objective Assessment
Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	A. No objective evidence of cardiovascular disease.
Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	B. Objective evidence of minimal cardiovascular disease.
Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	C. Objective evidence of moderately severe cardiovascular disease.
Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	D. Objective evidence of severe cardiovascular disease

The Criteria Committee of the New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th ed. Boston, Mass: Little, Brown & Co; 1994:253-256.

15.4 Appendix D: Subject Self-Reported Demographics Source Document

Subject Self-Reported Demographics Source Document

Subject ID #: __ __-__ __ __-__

Subject instructions: Please complete the survey by checking the box or boxes that most closely identify your race and ethnicity. Check multiple boxes if necessary. Initial and date this form as indicated and return it to your ASC01 Study Coordinator.

Date of Birth: _____ MM / DD / YYYY	**Gender:** ☐ Male ☐ Female

Ethnicity: ☐ Hispanic or Latino ☐ Not Hispanic or Latino

Race:

White:	**Asian:**
☐ White, not otherwise specified	☐ Asian, not otherwise specified
☐ Eastern European	☐ Asian Indian/South Asian
☐ European, not otherwise specified	☐ Chinese
☐ Mediterranean	☐ Filipino
☐ Middle Eastern	☐ Guamarian
☐ North Coast of Africa	☐ Korean
☐ Western European	☐ Japanese
☐ White Caribbean	☐ Vietnamese
☐ White North American	☐ Other Southeast Asian
☐ White South or Central American	
	Native Hawaiian or Other Pacific Islander:
Black or African American:	☐ Hawaiian
☐ Black, not otherwise specified	☐ Native Pacific Islander, not otherwise specified
☐ African American	☐ Samoan
☐ African Black (both parents born in Africa)	
☐ Caribbean Black	**American Indian or Alaska Native:**
☐ South or Central America Black	☐ Native American, not otherwise specified
	☐ American Indian, not otherwise specified
Other:	☐ Caribbean Indian
☐ No response	☐ Native Alaskan/Eskimo/Aleut
☐ Unknown	☐ South or Central American Indian
☐ Other, specify:	

Subject Initials:_____ **Date survey completed:**_____

15.5 Appendix E: Exploratory Analysis: Standardized Fick-based Pulmonary Vascular Resistance and Pulmonary Artery Capacitance

Standardized Fick-based pulmonary vascular resistance (PVRf, in Woods Units) and pulmonary artery capacitance (PACf, in mL/mmHg) are calculated as follows:

PVR = TPG / CO
where TPG = Transpulmonary Gradient (mmHg)
CO = Cardiac Output (L/min)

TPG = mPAP – PCWP
where mPAP = mean pulmonary artery pressure (mmHg)
PCWP = mean pulmonary capillary wedge pressure (mmHg)

Thus, the Fick-based calculation of PVR is:
PVRf = (mPAP – PCWP) / COf
where COf = Fick cardiac output (L/min)

$COf = VO_2 / ((Ca – Cv) \times cHb \times 13.6)$
where VO_2 = oxygen consumption according to LaFarge and Meittinen (mL/min)[161]
Ca = arterial oxygen saturation (%) (systemic)
Cv = venous oxygen saturation (%) (pulmonary artery)
cHb = hemoglobin concentration (g/L)

$VO_2(males) = (138.1 – 11.49\ lnAge + 0.378\ HR) \times BSA$
$VO_2(females) = (138.1 – 17.04\ lnAge + 0.378\ HR) \times BSA$
where lnAge = natural log of age in years
HR = heart rate (beats/min)
BSA = body surface area (m^2)

The DuBois and DuBois formula is used to calculate body surface area.
$BSA\ (m^2) = 0.20247 \times Height(m)^{0.725} \times Weight(kg)^{0.425}$

Pulmonary artery capacitance is calculated as follows.
PACf = SVf / PP
where SV = stroke volume (mL)
PP = pulse pressure (mmHg)

SVf = COf x 1000 / HR
PP = systolic PAP – diastolic PAP
where PAP is the pulmonary artery pressure

15.6 Appendix F: RESTORE Sub-study Protocol

Sub-study Protocol Title: Right Ventricular Response to Rituximab in Systemic Sclerosis-Associated Pulmonary Arterial Hypertension – A Magnetic Resonance Imaging Sub-study

Short Title: RESTORE Sub-Study

RESTORE Sub-study Sponsor:

VERA MOULTON WALL CENTER FOR PULMONARY VASCULAR DISEASE, STANFORD UNIVERSITY

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Sub-study Protocol Synopsis

Title of Sub-study Protocol: Right Ventricular Response to Rituximab in Systemic Sclerosis Associated Pulmonary Arterial Hypertension – A Magnetic Resonance Imaging Sub-study (RESTORE Sub-Study)

Investigators: David N Rosenthal, MD; Roham T Zamanian, MD, FCCP; Jeffrey A Feinstein, MD, MPH

Objectives:
The primary objective of the RESTORE sub-study is to evaluate the therapeutic effect of rituximab on the right ventricle (RV) in patients with SSc-PAH. Changes in right ventricular end diastolic volume index (RVEDVI) and stroke volume (SV) determined by cardiac MRI will be used as surrogates of RV function and prognosis. We hypothesize that RVEDVI and SV will reduce 24 weeks following rituximab therapy as compared to the placebo control.

Criteria for Evaluation:
All cardiac MRI (CMRI) endpoints will be assessed at baseline and at the Week 24 follow-up (or at time of discontinuation from the study) in 20 patients from each arm (80 studies total). The change in RVEDVI, RV mass, and SV will be analyzed and compared between placebo and rituximab cohorts. These sub-study endpoints will be used to augment the hemodynamic findings of the main study (ASC01). It is likely that comparison between hemodynamic response and surrogates of RV function by cardiac MRI will be valuable. Correlation of hemodynamic and CMRI parameters may allow for further determination of mechanisms of therapeutic effect of rituximab in this study.

Study Design:
Enrollment for the RESTORE sub-study will parallel that of the ASC01 protocol. The sub-study will enroll patients who have consented for the primary clinical study across all centers that participate in the RESTORE sub-study. The sub-study is designed to compare 20 rituximab with 20 placebo treated patients at baseline and at the Week 24 follow up. All ASC01 study inclusion and exclusion criteria apply. Furthermore, there are additional exclusion criteria that pertain only to the RESTORE sub-study: 1) known hypersensitivity to Gadolinium, 2) inability to tolerate or cooperate with MRI, 3) morbid obesity, and 4) presence of metallic objects or pacemakers.

Efficacy Endpoints:
The primary efficacy endpoint will be change in RVEDVI measured by cardiac MRI from baseline to 24 weeks after treatment initiation.

The secondary efficacy endpoints will be as follows:

1. Change in RV mass measured by cardiac MRI from baseline to 24 weeks after treatment initiation.
2. Change in SV measured by cardiac MRI from baseline to 24 weeks after treatment initiation.

Images and Analyses:
Cardiac MR imaging will be performed on any 1.5 T scanner (standard clinical equipment will meet these specifications) with simultaneous ECG recording. Image acquisition is based on routine MR protocols for traditional volumetric parameters supplemented with a delayed enhancement perfusion protocol (gadolinium). The detailed MRI protocol is specified in the Manual of Procedures (MOP), but will include short axis and four chamber views of the heart, and MRI angiography in the pulmonary artery and aorta. This will be followed by contrast enhanced imaging using gadolinium to determine myocardial perfusion in a four chamber view, and to evaluate for late enhancement using four chamber and short axis views.

Image analysis will be carried out off-line by investigators at Stanford University who are blinded to treatment arm, using validated MRI software tools such as QMass/QFlow (Medis Medical Imaging Systems, Raleigh, NC).

Sample Size and Statistical Analyses:
This sub-study will include 20 subjects treated with rituximab and 20 subjects treated with placebo.

The primary efficacy analysis of change in RVEDVI from baseline to 24 weeks will be performed on the intention-to-treat (ITT) population. Analysis of covariance (ANCOVA) will be used to model baseline to Week 24 change in RVEDVI as the primary outcome and treatment as the primary predictor, controlling for center and baseline value of RVEDVI.

Secondary analyses will include repeating the primary analysis for the following outcomes: change in RV mass from baseline

to 24 weeks and change in SV from baseline to 24 weeks.

1 Hypothesis

Primary Hypothesis: Right ventricular end-diastolic volume index (RVEDVI), as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.

Secondary Hypotheses:

- Right ventricular mass, as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.
- Stroke volume, as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.

Exploratory Hypotheses:

- Cardiac output, as determined by cardiac MRI, will agree with the main study assessment of cardiac output as well as the standardized Fick-based calculation of cardiac output
- Right ventricular end diastolic volume, as determined by cardiac MRI, will correlate with the standardized Fick-based calculation of pulmonary artery capacitance

2 Background and Rationale

2.1 Background

Pulmonary arterial hypertension (PAH) is a debilitating, progressive, and fatal complication of systemic sclerosis (SSc). Epidemiologic data show that patients with SSc-associated PAH (SSc-PAH) have a poor prognosis. While hemodynamic parameters such as right atrial pressure and cardiac index are predictors of survival in WHO group I PAH, they are relatively crude and indirect markers of RV performance in PAH [162, 163]. Interestingly, we have recently demonstrated that in comparison to other diseases with associated PAH, SSc-PAH is characterized by less severe hemodynamic perturbation, but also a more robust neurohormonal activation and worse 1-year survival [164]. These findings suggest that the right ventricular response to PAH in systemic sclerosis may differ from that seen in other etiologies of PAH and potentially plays an important role in shaping the natural history of this form of PAH.

Recent studies have demonstrated the utility of MRI based protocols for evaluation of right ventricular performance in PAH [165, 166]. Protocols which evaluate RV structure and function supplement the routine hemodynamic measures obtained during cardiac catheterization and add additional dimensions to the evaluation of disease and response to therapies. On the one hand, the measures from MRI are more directly related to the pathophysiology of right ventricular injury than are the less direct (but widely employed) measures of right atrial pressure and cardiac index, or the even further removed index of 6-minute walk distance (6MWD). Thus, these MRI measures, while perhaps less directly clinically applicable than 6MWD, do offer the potential for a more precise delineation of the cardiac impact of a given therapy such as rituximab. These data will be complementary to

pulmonary vascular resistance (PVR), which is a more pure vascular measure. Additionally, the reproducibility of the MRI measures of RV function is very high, so relatively small changes can be detected and thus MRI offers the hope of detecting early cardiac remodeling changes even if a full clinical impact is not yet demonstrable.

2.2 Rationale

PAH is a cardiopulmonary disease manifested by increased PVR due to obliterative arteriopathy. The increase in PVR creates increased pressure work for the right ventricle. Clinical evidence suggests that the capacity of the right ventricle to respond to this increase in afterload by remodeling may be a critical determinant of disease progression and overall prognosis [167-169]. Although right heart catheterization (RHC) is the gold standard for diagnosis of PAH, the hemodynamic measures which are obtained during RHC do not appear to provide comprehensive evaluation of RV function nor do they offer accurate prognosis for all forms of PAH [170]. For example, patients with PAH resulting from congenital heart diseases are typically characterized by very poor hemodynamics, yet these patients have among the best survival of any PAH cohort. One potential explanation for this is the long-standing and early onset of adverse hemodynamics in such patients, which may allow for more effective right ventricular remodeling.

Specifically relevant to the ASC01 study is the role of RV performance in SSc-PAH. Using a large multicenter PAH registry, we have recently demonstrated that SSc-PAH (Chung, Zamanian unpublished data) patients had the most favorable hemodynamics as demonstrated by lower mean pulmonary artery pressure, higher cardiac index and lower PVR when compared to idiopathic PAH and other autoimmune-associated PAH. Despite this apparently favorable hemodynamic profile, the 1 year survival for SSc-PAH was worse when compared with the other cohorts. This again supports the idea that it is not hemodynamic profile alone that determines prognosis, but rather the cardiac response to these hemodynamics in the form of RV remodeling.

Cardiac MRI allows for a comprehensive non-invasive evaluation of RV chamber size, myocardial mass and systolic function, providing information which is complementary to that obtained by cardiac catheterization. Evaluation of baseline RV status using cardiac MRI in SSc-PAH and the response to rituximab will help determine the role which RV remodeling plays in shaping the overall patient outcome in PAH, and may also demonstrate a mechanism by which rituximab affects disease outcome, if such effect is found.

The RESTORE sub-study will further help delineate the utility of cardiac MRI in studying treatment response in PAH. While there have been some data evaluating this, there is not yet adequate data to specify which markers of RV remodeling will be most useful in evaluating the evolution of patients with PAH. The RESTORE sub-study, by providing MRI data in a well-characterized set of patients, will help better define this question as well.

2.3 Significance

This sub-study is likely to enhance the capacity of the ASC01 study in evaluation of response of SSc-PAH to rituximab therapy by evaluating a set of outcomes which are complementary

to secondary study aims, but which are more focused on right ventricular performance. This will assist in evaluating the factors contributing to the clinical outcomes observed in the main study by providing data which help clarify the cardiac responses during therapy. These data will help clarify the contribution to outcome that arises from the vascular outcome of PVR.

3 Study Overview

3.1 Overall Study Design

The RESTORE sub-study will include all sites participating in the primary ASC01 study that have the capability to perform the required MRI studies. Enrollment within the RESTORE sub-study will consist of consecutive ASC01 study subjects at each site, provided that the subject provides consent for this separate sub-study. This should diminish any unintentional bias in patient selection at each site. Thus, both placebo and rituximab arms will be included in the RESTORE sub-study. Twenty subjects from each arm will be recruited into this sub-study. Each subject will be studied at baseline (i.e. prior to initiation of study drug) and at Week 24, or at time of discontinuation. Subjects in RESTORE will undergo comprehensive cardiac MRI evaluation. Given that the primary comparison is a pre/post match, and that the MRI interpretation is performed by individuals blinded to the treatment assignment, it is not likely to introduce bias if one or more sites cannot participate in the RESTORE sub-study. However, should this be the case, the overall study population will be compared to the sub-population captured at these sites with respect to baseline characteristics. Since the RESTORE sub-study will use the same entry criteria as the parent protocol, there should not be a problem with differential patient selection, with the exception of the additional exclusion criteria specified below that relate to the contra-indications to MRI. While it does not appear likely that these criteria will bias the sub-study population, the data analysis will again compare the baseline characteristics of study subjects for the parent protocol to that of the RESTORE sub-study.

3.2 Study Population

3.2.1 Inclusion Criteria

Each participant included in this sub-study must be a participant in and meet the inclusion criteria for the ASC01 clinical protocol. In addition, written informed consent for the RESTORE sub-study must be provided.

3.2.2 Exclusion Criteria

Each participant included in this sub-study must not meet any of the exclusion criteria for the ASC01 clinical protocol. In addition, subjects who meet any of the following are disqualified from enrollment in the sub-study:

- Known hypersensitivity to Gadolinium
- Inability to tolerate or cooperate with MRI (small spaces/claustrophobia)
- Morbid obesity
- Presence of metallic objects or pacemaker

3.3 Recruitment

Recruitment for this sub-study will be limited to subjects who have consented and elected to participate in the ASC01 clinical protocol. Replacement of subjects in this sub-study will occur in the same manner as the parent protocol. Randomized subjects who withdraw from the trial prior to starting rituximab infusion will be replaced. Subjects who discontinue study treatment or withdraw from the trial after initiating the first treatment may not be replaced.

4 Study Design

4.1 Description of Study Design

Twenty subjects from each treatment arm, distributed among all participating sites, will be recruited for this sub-study. Each subject will be studied at baseline (i.e. prior to initiation of study drug) and after 24 weeks, or at time of discontinuation. In addition to the data collection and testing specified in the ASC01 protocol, subjects in RESTORE will undergo comprehensive cardiac MRI evaluation as described in the RESTORE Manual of Procedures (MOP). In brief, this will include short axis, body axial, four-chamber and three-chamber cardiac-gated MRI sequences; pulmonary and aortic angiography in the proximal main pulmonary artery and each of the proximal branch pulmonary arteries; and the ascending aorta; measurement of myocardial perfusion using gadolinium enhanced cardiac MRI in the same views specified above, and measurement of delayed gadolinium enhancement in those views.

4.2 Endpoints

The primary efficacy endpoint will be change in RVEDVI measured by cardiac MRI from baseline to 24 weeks after treatment initiation.

The secondary efficacy endpoints will be as follows:

- Change in RV mass measured by cardiac MRI from baseline to 24 weeks after treatment initiation.
- Change in SV measured by cardiac MRI from baseline to 24 weeks after treatment initiation.

An exploratory endpoint is also defined:

- Cardiac output measured by cardiac MRI

5 Study Objectives

The primary objective of the RESTORE sub-study is to evaluate the therapeutic effect of rituximab on the right ventricle (RV) in patients with SSc-PAH. To achieve this objective, we have developed the following hypotheses:

> Primary Hypothesis: Right ventricular end-diastolic volume index, as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.

Secondary Hypotheses:

- Right ventricular mass, as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.
- Stroke volume, as determined by cardiac MRI, will reduce 24 weeks following rituximab therapy as compared to placebo control, in subjects with SSc-PAH participating in ACE Protocol ASC01.

Exploratory Hypotheses:

- Cardiac output, as determined by cardiac MRI, will agree with the main study assessment of cardiac output as well as the standardized Fick-based calculation of cardiac output
- Right ventricular end diastolic volume, as determined by cardiac MRI, will correlate with the standardized Fick-based calculation of pulmonary artery capacitance

6 Treatment of Subjects and Study Measurements

6.1 Subject Clinical Considerations

Consideration must be given to the need for rate adjustment of intravenous Flolan® during the MRI procedures for those subjects requiring this concomitant background SSc-PAH therapy. Given the requirement for IV extension tubing from the subject to infusion pump while in the MRI scanner, Flolan® flow rate adjustment (increase) will be required to compensate for the longer tubing length to allow for consistent dosing.

Further guidance on this issue may be found in the RESTORE MOP.

6.2 Imaging Process

Cardiac MR imaging will be performed using any 1.5 T scanner with simultaneous ECG recording, according to a protocol for correction of phase offset errors. All images will be obtained using breath-hold in end-expiration. Acquisition will be performed including the following sequences:

- Short-axis cine images of the heart from apex to base, covering the whole LV and RV.
- Body axial cine images of the heart, extending from the surface of the diaphragm to the superior margin of the pulmonary valve.
- Long axis cine images of the heart, extending from the surface of the diaphragm to the superior margin of the pulmonary valve.
- Angiography (flow imaging) will be obtained in the aorta from an imaging plane located perpendicular to the long axis of the ascending aorta, with the plane located just distal to the sino-tubular ridge, and distal also to the origin of the coronary arteries.
- Angiography (flow imaging) will be obtained in the main pulmonary artery from an imaging plane located perpendicular to the long axis of the main pulmonary artery, with the plane located midway between the pulmonary valve and the bifurcation of the pulmonary artery into the branch pulmonary arteries.

- Angiography (flow imaging) will be obtained in each of the branch pulmonary arteries from an imaging plane perpendicular to the long axis of the applicable artery, with the plane located midway between the origin of the artery and the origin of the first branch from that artery.
- Myocardial perfusion imaging during gadolinium infusion will be obtained using short axis views of the heart, as per the baseline cine imaging.
- Delayed imaging after injection of gadolinium will be performed using both short axis and axial views of the heart.

Full details of the procedures can be found in the RESTORE MOP.

6.3 Imaging Analyses

All cardiac MRI images obtained during the RESTORE sub-study will be provided to the Stanford laboratory for interpretation as described in additional detail in the following section. Images will be shipped on digital media (CD/DVD).

All cardiac MRI studies will be analyzed in the same fashion, using commercial software. MRI studies will be analyzed at one time after the conclusion of the study (after last patient last visit).

Studies will be analyzed by one interpreter, either one of the sub-study investigators or personnel trained directly by these investigators. A test set of images will be completed in duplicate and intra-observer variability will be calculated. If this variability is not acceptable (i.e. coefficient of variation < 15%) [171], the interpreter will review interpretation criteria and then repeat the intra-observer variability using the test set (these images will be obtained using the sub-study protocol, but may not include sub-study subjects). The interpreter of images will not have access to treatment assignment nor to the sequence of the studies, but the 2 studies for each subject will be reviewed as a pair.

For interpretation of the MRI, diastole and systole will be assigned manually using the largest and smallest volume of both the RV and LV (this may result in separate assignment of diastole and systole for the 2 ventricles). The diastolic endocardial and epicardial contours of both the RV and LV will be traced manually by one of the study interpreters, and will be processed using software to obtain RV and LV mass, and end-diastolic volume. The chamber volumes will also be manually traced at end-systole, yielding end-systolic volumes. In the LV, papillary muscles will be traced separately, and thus incorporated into the mass rather than volume calculation. Due to difficulties in obtaining consistent tracings with this method in the RV, the RV tracings will exclude papillary muscles. LV and RV volumes/mass will be determined using short axis images. Ejection fraction will be calculated by determining the difference between diastolic and systolic volume for each chamber, and dividing by the diastolic volume of the corresponding chamber. For measurement of flow, the angiography (phase contrast) images will be used, with flow measured in the ascending aorta and main pulmonary artery. For all parameters, the values will be normalized by body surface area (i.e. expressed as indexed values).

For each study, a data collection form will be completed and signed by the study interpreter at the time of the interpretation. This form will be retained at Stanford, with the information entered in an Excel spreadsheet and transferred to the SACCC electronically.

7 Data Management

7.1 Tracking Images and Quality Control Procedures

Clinical sites are responsible for collecting, labeling, tracking, and shipping the images to Stanford University as specified in the RESTORE MOP. The exception is the first cardiac MRI study conducted at each site for this sub-study, which will be sent to the SACCC to forward to Stanford without awaiting protocol completion, to allow a blinded quality control review. After an image is collected for a subject, the clinical site is responsible for entering the subject ID and accession numbers into the web-based tracking system (RhoLAB) operated by the SACCC. The RhoLAB tracking system will notify the Stanford laboratory that a CD/DVD is being shipped. The information supplied to Stanford by the SACCC will contain accession numbers, but not subject ID numbers. The only links between subject ID numbers and accession numbers will be at the clinical site and at the SACCC. These links will allow cardiac MRI results to be linked to clinical outcomes through the SACCC. As CDs/DVDs are received at Stanford, the receiving facility must log into the RhoLAB tracking system and verify that the CDs/DVDs were received and document the condition of the CDs/DVDs at the time of receipt. After analyses are completed at Stanford, the analysis results will be supplied by Stanford to the SACCC electronically using accession numbers as unique identifiers.

Upon arrival at Stanford the images will also be logged and stored in a dedicated, locked file. The actual CD/DVD will be retained at Stanford in the event that re-interpretation is required. In addition, this image may be required for quality control purposes.

The intra-observer variability will be determined at the onset of the sub-study interpretation. This will be accomplished by having the reader interpret a set of 5 studies in duplicate, allowing two weeks between the initial and repeat interpretation. For this purpose, the 2 interpretations will be sent to the SACCC for measurement of the coefficient of variation between the duplicate readings. If this does not meet the standard specified in Section 6.3, *Imaging Analysis*, then the individual will review interpretation criteria and repeat the quality control process.

7.2 Database Management

All data will be maintained in hard copy form at Stanford and transmitted to the SACCC electronically. Upon receipt of such data, the SACCC will store it in a central database on the SACCC network that provides protection of subject confidentiality and allows the cardiac MRI study data to be merged with the clinical data from the ASC01 study.

7.3 Retention of Documents

The Stanford database ensures integrity of the data through multiple levels of password protection and routine backups of all data. All data and subject material will be password

protected on the local database, which will only be accessible to the Protocol Chair and Co-Chairs. All data will be subsequently stored on the SACCC network, which will allow the RESTORE sub-study data to be merged with the clinical data from the ASC01 study.

All study-related documents will be maintained in the core facility for 6 years, or longer if required by the FDA.

8 Statistical Considerations and Analytic Plan

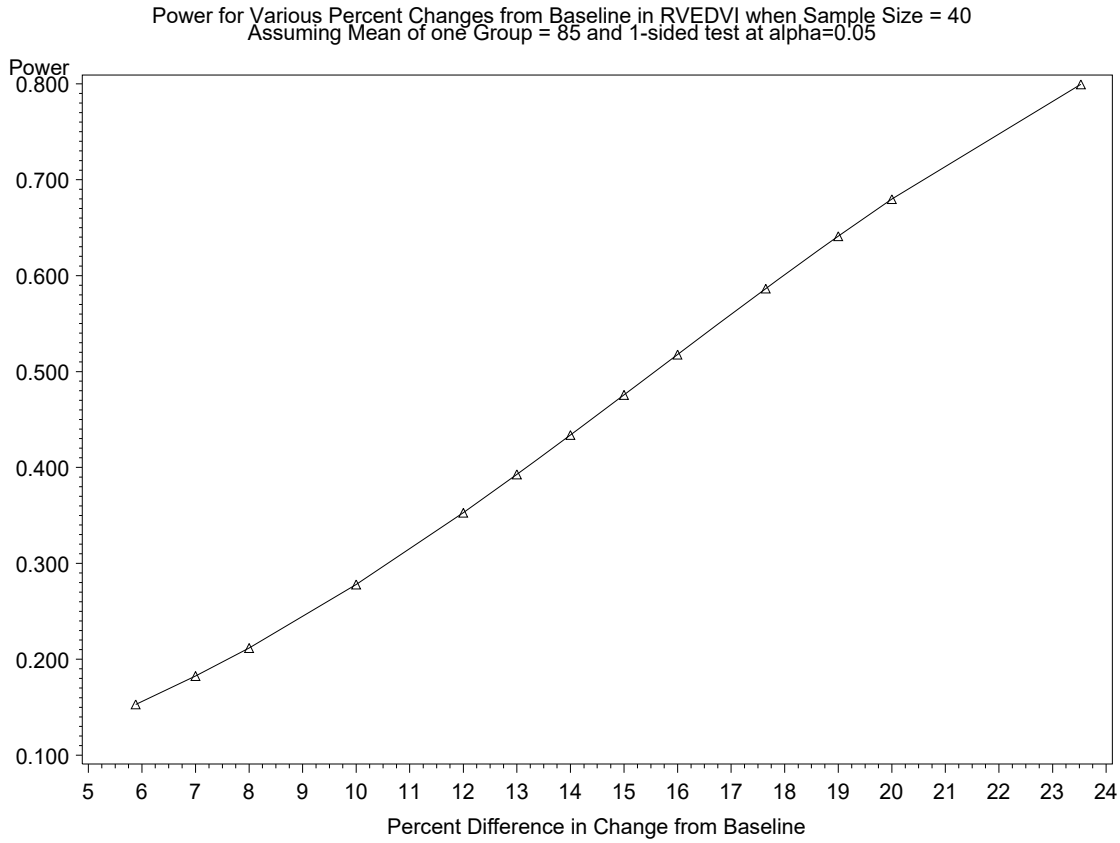
8.1 Subject Selection or Allocation

The RESTORE sub-study will include all sites participating in the primary ASC01 study that have the capability to perform the required MRI studies. Enrollment within the RESTORE sub-study will consist of consecutive patients at each site, provided that the patient provides consent for this separate sub-study. With regard to patient treatment assignment, all data will be analyzed on the basis of intention-to-treat, in the identical manner as the parent protocol. Any patient excluded from analysis in the parent protocol will also be excluded from analysis in the RESTORE sub-study.

8.2 Sample Size

The sample size for this sub-study is determined in large part by the size of the parent protocol and budget considerations. With these factors in mind, a sample size of 20 subjects in each arm (40 total subjects) is feasible.

Data from van Wolferen indicate that in subjects with pulmonary hypertension, RVEDVI determined by cardiac MRI was 85 +/- 25 ml/m2. Assuming that the standard deviation does not change during the sub-study, and that there is a 10% (8.5 ml/m^2) mean reduction in right ventricular volume index over the placebo group, one obtains the following relationship between the percent reduction and the expected power:



A mean reduction of 10% would power this sub-study at 27.8%. A recent study of resynchronization therapy for left ventricular failure demonstrated a reduction in left ventricular systolic volume index of approximately 15% of the baseline value; if this level is found, the power would increase to 47.6% [172].

Since the main purpose of the RESTORE sub-study is to get a better understanding of how rituximab affects RVEDVI for subsequent research studies, this level of power will suffice.

8.3 Statistical Analysis of Study Objectives

The primary aim of this sub-study is a comparison of the change in RVEDVI between the rituximab and the placebo arm of the parent trial, in subjects who have cardiac MRI at baseline and at Week 24, or at time of discontinuation. This comparison will be made using an ANCOVA model with change in RVEDVI as the response and treatment, center, and baseline value as covariates. It is not anticipated that non-parametric tests will be required, but the data distribution will be evaluated and suitable data transformation will be performed as needed. However, based upon experience in other studies using similar outcomes, such is not likely to be required [165, 166]. A p-value of 0.05 will be taken as the threshold for significance. For the secondary outcomes of RV mass and stroke volume, a similar analysis will be performed.

9 Safety Monitoring and Reporting

Monitoring and reporting of any adverse events associated with MRI procedures for this sub-study will be the responsibility of the clinical sites and will be reported to the IND holder using the standard mechanisms implemented as described in Section 7, *Safety Monitoring and Reporting,* of the ASC01 clinical protocol which is incorporated by reference for this sub-study.

9.1 Risk Analysis

This is an observational protocol that presents the incremental risk associated with performing 2 cardiac MRI studies in the study population. This is substantially the same as the risk of the administration of gadolinium, as the other MRI risks are minor (although claustrophobia is not uncommon, and may require termination of the protocol). For subjects with normal renal function, adverse effects from gadolinium are reported in 0.48% of subjects [173]. Most reactions were minor; there was one case of anaphylactic shock in 9528 doses of gadolinium. In patients with pre-existing renal failure, there is a risk of provoking renal toxicity. However, this risk is low at the doses of gadolinium proposed in this protocol and is extremely low in the patient population specified in the parent protocol, which excludes subjects with significant renal pathology. Of concern regarding use of gadolinium are reports of the development of nephrogenic systemic fibrosis in subjects with severe renal failure who receive gadolinium. However, this risk is again low or absent in the subjects targeted by the parent protocol, as the risk of nephrogenic systemic fibrosis emerges only when the creatinine clearance is in the 15-29 mL/min range, or in patients who are dependent upon dialysis.

10 Access to Source Data and Documents

The requirements outlined in Section 9, *Access to Source Data and Documents,* of the ASC01 clinical protocol are incorporated by reference for this sub-study.

11 Quality Assurance

The requirements outlined in Section 10, *Data Collection, Quality Control and Quality Assurance,* of the ASC01 clinical protocol are incorporated by reference for this sub-study.

12 Other Administrative Procedures

The administrative procedures outlined in Section 11, *Ethical Considerations and Compliance with Good Clinical Practice,* of the ASC01 clinical protocol are incorporated by reference for this sub-study.