A DOUBLE-BLINDED, PLACEBO-CONTROLLED PILOT STUDY OF DMF IN PULMONARY ARTERIAL HYPERTENSION (PAH) ASSOCIATED WITH SYSTEMIC SCLEROSIS (SSC-PAH): THE EFFECT OF DMF ON CLINICAL DISEASE AND BIOMARKERS OF OXIDATIVE STRESS

Test drug:	Dimethyl fumarate (Tecfidera, DMF)
Study purpose:	Safety and efficacy
Clinical study phase:	1b
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Protocol Number:	US-BGT-16-11075

DATE: 14-May-2019

Version 7

FINAL

INVESTIGATORS SIGNED AGREEMENT OF STUDY PROTOCOL
Version 7.0
13-May-2019

I have read the foregoing protocol, "Dimethyl Fumarate In Systemic Sclerosis- Associated Pulmonary Arterial Hypertension," and agree to conduct the study according to the protocol and the applicable ICH guidelines and local regulations, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

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Principal Investigator's Signature Date

_____

Principal Investigator's Name (Print)

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Site Investigator (Print)

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Site-Investigator's Signature Date

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Communication Plan :
There will be an initial Teleconference before study start up and then monthly teleconferences there after.

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1. LIST OF ABBREVIATIONS

6MWD	6-minute walk distance
6MWT	6-minute walk test
AE	Adverse Event
AHI	Apnea–Hypopnea Index
ALT	Alanine aminotransferase
ARR	annualized relapse rate
AST	Serum aspartate aminotransferase
BCG	Bacille Calmette Guerin
BDI	Borg Dyspnea Index
BNP	B-type natriuretic peptide
C	Celsius
CC	Coordinating Center
CCR1	C-C chemokine receptor type 1
cm	centimeters
CRF	Case Report From
CXR	Chest x-ray
dcSSc	diffuse cutaneous systemic sclerosis
DMF	Dimethyl fumarate (Tecfidera, DMF)
DSMB	Data and Safety Monitoring Board
EDSS	Expanded Disability Status Scale
EKG	electrocardiogram
ES	Executive Secretary
Gd	gadolinium-enhancing
HAQ-DI	Health Assessment Questionnaire disability index
HBV	Hepatitis B
HCAR2	Hydroxycarboxylic Acid Receptor 2
HCV	Hepatitis C
HIF-1	hypoxia inducing factor
HIV	human immunodeficiency virus
HPH	Hypoxic pulmonary hypertension
HRCT	High resolution CT scan
HRQoL	HRQoL
ICF	Informed consent form
ICH	International conference on harmonization guidelines
ID	Infectious diseases
IL13RA1	interleukin 13 receptor subunit alpha 1
ILD	Interstitial lung disease

ITT	Intention to treat
IUD	Intrauterine device
IV	Intravenous therapy
JAK2	Janus kinase 2
kg	kilograms
lcSSc	limited cutaneous systemic sclerosis
LVEDP	Left ventricular end-diastolic pressure
MMF	metabolite, monomethyl fumarate
mPAP	Mean pulmonary artery pressure
MRC1	Mannose Receptor C-Type 1
MRI	Magnetic Resonance Imaging
mRNA	Messinger ribonucleic acid
MRss	Modified Rodnan Skin Score
NADPH	Nicotinamide adenine dinucleotide phosphate
NFkB	Nuclear factor
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases
NIH	National Institutes of Health
Nrf2	Nuclear factor (erythroid-derived 2)
NYHA	New York Heart Association
PAH	Pulmonary arterial hypertension
PASP	Pulmonary arterial systolic pressure
PBMC	peripheral blood mononuclear cell
PCWP	pulmonary capillary wedge pressure
PDE-5	Phosphodiesterase Type 5 (PDE5) Inhibitors
PH	Pulmonary Hypertension
PHI	Patient health information
PML	Progressive multifocal leukoencephalopathy
PO	Oral administration
PPD	Positive purified protein
PRO	Patient Reported outcomes
PVR	Pulmonary vascular resistance
RDMS	Registered Diagnostic Medical Sonographer
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RRMS	relapsing- remitting multiple sclerosis
SABR	Biogen Safety and Benefit Risk
SAE	Serious Adverse Event
SBP	Systolic blood pressure

SHAQ	Scleroderma Health Assessment Questionnaire
SOC	Standard of Care
PRO-SRSS	Systemic Sclerosis Skin Questionnaire
SSc	Systemic sclerosis
SSc-PAH	Systemic sclerosis Pulmonary arterial hypertension
SUSARs	Suspected unexpected serious adverse reaction
TB	tuberculosis
TGFb	Transforming growth factor beta
TLC	total lung capacity
TNF	Tumor necrosis factor
UPMC	University of Pittsburgh Medical Center
US	United States
VAS	Visual Analog Scale
WHO	World Health Organization

2. SYNOPSIS

Tracking Number:	PRO16070614
Protocol Title:	A double-blinded, placebo-controlled pilot study of DMF in pulmonary arterial hypertension (PAH) associated with systemic sclerosis (SSc-PAH): The effect of DMF on clinical disease and biomarkers of oxidative stress.
Version Number:	5
Name of Study Treatment:	Dimethyl fumarate (Tecfidera, DMF/ Placebo)
Study Indication:	Systemic Sclerosis with Pulmonary Hypertension (SSc-PAH)
Phase of Development:	1b
Rationale for the Study:	Given the poor treatment options for patients with SSc-PAH, new therapies are urgently needed. Ideally, a medication that could be added to and complement the current standard of care, i.e. vasodilators, would be optimal. DMF, a newly approved drug for multiple sclerosis, is known to reduce oxidative damage and down-modulate NFκB signaling, and has shown activity in preclinical studies of PAH, making it an attractive candidate for the treatment of SSc-PAH. Showing clinical efficacy of DMF and/or a strong effect on biomarkers of oxidative stress in SSc-PAH would enable a larger, controlled clinical trial. In theory, efficacy could extend to additional organ involvement in patients with SSc. Thus, this clinical trial will test the effect of DMF on biomarkers of oxidative stress, and also on novel PBMC mRNA and serum proteins biomarkers of SSc-PAH. Showing that DMF inhibits biomarkers of oxidative stress and affects biomarkers and clinical measure of disease severity could facilitate a registration enabling trial.
Study Objectives and Endpoints:	**Primary Objective:** • To assess the safety of the treatment with DMF in patients with SSc-PAH. • To assess the efficacy in this pilot trial will be improvement in 6-minute walk distance (6MWD).

	Primary Endpoint: • Safety will be assessed by the incidence of serious adverse events (SAEs) and all adverse events in DMF compared to placebo treated patients. • The efficacy will be assessed by the change in 6MWD at 24 weeks from baseline in DMF compared to placebo treated patients (the study is powered to detect an improvement of 30-40 M in the 6MWD). The additional objectives and endpoints of this study in this study population are as follows: **Secondary Objectives:** • Study the effect of DMF on time to clinical worsening: determined by: Change in SOC therapy for PAH; Hospitalization for PAH; Death; Need for transplantation; Need for atrial septostomy; Decline in 6MWD by 15% from the baseline 6MWD; Decline in WHO/NYHA functional class. • Study the effect of DMF on isoprostane and other serum markers of oxidative stress, including lipid, DNA, and prostaglandin targets. • Study the effect of DMF on PAH associated biomarker gene expression by peripheral blood mononuclear cells, including IL13RA1, CCR1, JAK2 and MRC1. • Study the effect of DMF on serum B-type natriuretic peptide (BNP), Endostatin, Endothelin-1, Endoglin, Vascular Endothelial Growth Factor, von Willebrand Factor, and Vascular Cellular Adhesion Molecule 1. • Study whether DMF affects skin mRNA biomarkers. • Study the effect of DMF on Digital Ulcer Net Burden **Secondary Endpoints:** • The change in time to clinical worsening in DMF compared to placebo treated patients. • The change from baseline of serum markers of oxidative stress at 24 weeks, comparing DMF to placebo treated patients. • The change from baseline of PAH associated biomarkers at 24 weeks, comparing DMF to placebo treated patients

	• The change from baseline in proteomic biomarkers, including BNP, at 24 weeks, comparing DMF to placebo treated patients. • The change in skin nMRA biomarkers at 24 weeks, comparing DMF to placebo treated patients compared to baseline. • The change in Digital Ulcer Net Burden at 24 weeks, comparing DMF to placebo treated patients compared to baseline.

Study Design:	Randomized, double-blind, placebo-controlled study
Study Location and Number of Sites:	Approximately five sites in the US.
Number of Planned Subjects:	34 subjects will be enrolled in the study.
Study Population:	This study will be conducted in subjects with SSc-PAH
Treatment Groups	Two cohorts 17 subjects in each cohort, randomized 1:1 to receive DMF 240mg bid or placebo
Duration of Treatment and Follow-up:	Study duration for each subject will be 40 weeks including a 4-week screening period, 24 weeks of drug and 12-week safety follow-up after the last dose.

3. SCHEDULE OF EVENTS

Schedule of Events

Study Period	Screening	Visit 1	Phone Call	Phone Call	Visit 2	Visit 3	Visit 4/ ET	Visit 5 Safety f/u
Study Week	< 4 weeks	Week 0	Week 1	Week 4	Week 8	Week 16	Week 24	Week 36
Window (in Days)		0	± 1 day	± 3 days	± 10 days	± 10 days	± 10 days	± 10 days
Informed Consent	X							
Eligibility (i/exclusion)	X	X						
Demographics	X							
Medical History	X							
SAFETY ASSESSMENTS								
Physical Examination	X	X			X	X	X	X
Height	X							
Vital Signs (blood pressure, heart rate, weight)	X	X			X	X	X	X
Laboratory Tests (CBC Diff w/ Platelets, CMP, BNP, Urinalysis)[a]	X	X			X	X	X	X
HIV serologies, HCV/HBV serologies	X							
Urine Dipstick Pregnancy Test[b]	X	X			X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
EFFICACY ASSESSMENTS								
mRSS	X	X			X	X	X	X
Blood Collection for Biomarkers		X			X	X	X	X
NYHA Assessment	X	X			X	X	X	X
Digital Ulcer Assessment	X	X			X	X	X	
Six minute Walk Test[c]	X	X			X	X	X	X
Study Period	**Screening**	**Visit 1**	**Phone Call**	**Phone Call**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
Study Week	< 4 weeks	Week 0	Week 1	Week 4	Week 8	Week 16	Week 24	Week 36
Skin Biopsy [d]		X					X	
Patient Reported Outcomes (SHAQ, Raynauds VAS, PRO-SRSS)		X			X	X	X	X

[a] All laboratory samples will be analyzed at local lab

[b] For women of child-bearing potential

[c] 6MWT: Subject should complete assessment with same amount of supplemental oxygen used to complete the Screening 6MWT unless safety will be impacted

[d] One 3mm skin biopsy will be obtained from the mid dorsal surface of the forearm., removing a small cylinder of skin.

4. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a particularly lethal complication of systemic sclerosis (SSc). Although vasodilators have emerged as efficacious therapies, many patients do not respond or respond only partially to currently available therapeutics, and so there remains a very high medical need for new treatments and new approaches to therapy for this disease. In particular, it is important to understand the role of inflammation and immunity in promoting SSc-PAH and the potential for drugs that do not rely on vasodilation to treat this disease. We will investigate the anti-oxidant, DMF, in a double-blind placebo controlled trial of 34 SSc-PAH patients. The study medication will be added to stable background PAH medication(s). Subjects will be dosed for 24 weeks, will undergo examination every 8 weeks, and will be finally evaluated 12 weeks after completion of treatment.

DMF/Tecfidera. DMF is marketed under the trade name Tecfidera, a registered trademark of Biogen. DMF is approved by the Food and Drug Administration and by the European Commission for the treatment of patients with relapsing and relapsing-remitting forms, respectively, of multiple sclerosis.

Mechanism of Action. The mechanism of action of DMF in multiple sclerosis is unknown, but DMF and the metabolite, monomethyl fumarate (MMF), activate the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway in vitro and in vivo in animals and humans. The Nrf2 pathway is involved in the cellular response to oxidative stress, and activation of this pathway may represent the major therapeutic mechanism. However, DMF it has several other activities that may be as important for its therapeutic effect. First, DMF can dramatically inhibit NFκB mediated triggering of the production of inflammatory mediators such as TNF and IL-6 [1]. While NFκB translocation to the nucleus is inhibited, the actual molecular mechanism of this effect is incompletely understood. Second, DMF can indirectly affect hypoxia inducing factor (HIF-1), a transcription factor that orchestrates the response to hypoxia and increases cell survival in response to a hypoxic environment, possibly by stabilizing HIF-1 via inhibition of prolyl-hydrolases that tag HIF-1 for removal [2]. Third, DMF increases the activity of a cystine-glutamate membrane exchange protein called System Xc [2]. System Xc governs the import of cystine into the cytoplasm, which is crucial for synthesis of glutathione, the primary redox regulator in the cell. Like Nrf2, increased System Xc function is considered to be an accommodation to increased oxidative stress [3]. Fourth, DMF is a potent agonist of the nicotinic acid receptor, HCAR2 (or Gpr109a) [4]. HCAR2 is essential for the protective effects of DMF in an experimental multiple sclerosis model [5]. Furthermore, HCAR2 is induced upon macrophage activation and has a major role in sensing microbiome-derived butyrate in the gut [6]. HCAR2 activation by DMF in macrophages leads to a "dampening or suppressive" program in macrophages [5]. Thus, in addition to ameliorating oxidative stress, DMF has important effects on macrophage activity. All of these effects could be beneficial in the setting of SSc-PAH.

Clinical Studies of DMF in Multiple Sclerosis. The efficacy and safety of DMF in patients with relapsing- remitting multiple sclerosis (RRMS) have been demonstrated in two studies. Both studies included patients who had experienced at least 1 relapse over the year preceding the trial or had a brain Magnetic Resonance Imaging (MRI) scan demonstrating at least one

gadolinium-enhancing (Gd+) lesion within 6 weeks of randomization. The Expanded Disability Status Scale (EDSS) was also assessed and patients could have scores ranging from 0 to 5. Neurological evaluations were performed at baseline, every 3 months, and at the time of suspected relapse. MRI evaluations were performed at baseline, month 6, and year 1 and 2 in a subset of patients (44% in Study 1 and 48% in Study 2).

Study 1 was a 2-year randomized, double-blind, placebo-controlled study in 1234 patients with RRMS. The primary endpoint was the proportion of patients relapsed at 2 years. Additional endpoints at 2 years included the number of new or newly enlarging T2 hyperintense lesions, number of new T1 hypointense lesions, number of Gd+ lesions, annualized relapse rate (ARR), and time to confirmed disability progression. Patients were randomized to receive DMF 240 mg twice a day, DMF 240 mg three times a day, or placebo for up to 2 years. DMF had a statistically significant effect on all of the endpoints. Study 2 was a 2-year multicenter, randomized, double-blind, placebo-controlled study that also included an open-label comparator arm in patients with RRMS. The primary endpoint was the annualized relapse rate at 2 years. Additional endpoints at 2 years included the number of new or newly enlarging T2 hyperintense lesions, number of T1 hypointense lesions, number of Gd+ lesions, proportion of patients relapsed, and time to confirmed disability progression. Patients were randomized to receive DMF 240 mg twice a day, DMF 240 mg three times a day, an open-label comparator, or placebo for up to 2 years. DMF had a statistically significant effect on the relapse and MRI endpoints. There was no statistically significant effect on disability progression. In both of these studies DMF 240 mg three times daily resulted in no additional benefit over the DMF 240 mg twice daily.

Risks of DMF: Flushing and GI reactions (abdominal pain, diarrhea, and nausea) are the most common reactions, especially at the initiation of therapy, and may decrease over time. DMF can decrease lymphocyte counts and can cause liver injury. DMF can cause anaphylaxis and angioedema after the first dose or at any time during treatment. Progressive multifocal leukoencephalopathy (PML) has occurred rarely in patients taking DMF.

Flushing. In clinical trials, 40% of DMF treated patients experienced flushing. Flushing symptoms generally began soon after initiating DMF and usually improved or resolved over time. In the majority of patients who experienced flushing, it was mild or moderate in severity. The incidence of flushing may be reduced by administration of DMF with food. Alternatively, administration of non-enteric coated aspirin (up to a dose of 325 mg) 30 minutes prior to DMF dosing may reduce the incidence or severity of flushing.

Lymphopenia. DMF may decrease lymphocyte counts. In the multiple sclerosis placebo controlled trials, mean lymphocyte counts decreased by approximately 30% during the first year of treatment with DMF and then remained stable. Four weeks after stopping DMF, mean lymphocyte counts increased but did not return to baseline. 6% of DMF versus <1% of placebo treated patients developed lymphocyte counts lymphocyte counts $<0.5 \times 10^9$/L. There was no increased incidence of serious infections observed in patients treated with DMF in the placebo controlled trials, although one patient in an extension study developed PML in the setting of prolonged lymphopenia with lymphocyte counts generally $<0.5 \times 10^9$/L for 3.5 years.

Liver Injury. Clinically significant cases of liver injury have been reported in patients treated with DMF in the postmarketing setting. The onset has ranged from a few days to several months after initiation of treatment with DMF. These abnormalities resolved upon treatment discontinuation.

Progressive Multifocal Leukoencephalopathy. Progressive multifocal leukoencephalopathy (PML) has occurred in patients with multiple sclerosis treated with DMF. PML is an opportunistic viral infection caused by the JC virus. A fatal case of PML occurred in a patient who received DMF for 4 years, associated with prolonged lymphopenia in a clinical trial. PML has also occurred in the postmarketing setting in the presence of lymphopenia. The majority of cases occurred in patients with lymphocyte counts $<0.5 \times 10^9/L$.

There are no adequate and well-controlled studies in of use of DMF in pregnant women, and it is not known whether DMF is excreted in human milk. In a placebo controlled thorough QT study performed in healthy subjects, there was no evidence that DMF causes QT interval prolongation of clinical significance.

DMF Pharmacokinetics. After oral administration in humans, DMF is extensively metabolized by esterases, which are ubiquitous in the gastrointestinal tract, blood, and tissues, before it reaches the systemic circulation. As DMF is not quantifiable in plasma following oral administration, all pharmacokinetic analyses have tested plasma MMF concentrations. Human plasma protein binding of MMF is 27-45% and independent of concentration. Metabolism of MMF occurs through the tricarboxylic acid cycle, with no involvement of the cytochrome P450 system. MMF, fumaric and citric acid, and glucose are the major metabolites in plasma. Renal and fecal elimination are minor routes of elimination, accounting for 16% and 1% of the dose respectively. The terminal half-life of MMF is approximately 1 hour and no circulating MMF is present at 24 hours in the majority of individuals. Accumulation of MMF does not occur with multiple doses of DMF.

Potential Benefits: The study subjects may receive a benefit from the study drug. Finding a new therapy for SSc-PAH could clearly benefit patients with SSc-PAH. Better understanding biomarkers could lead to better study designs and facilitate development of other treatments in the future. This benefit outweighs the small risk of this trial.

4.1. Background

Systemic Sclerosis. Scleroderma, also known as systemic sclerosis (SSc), is a multisystem disease affecting skin and, more variably, other tissues, commonly including joints, muscles, lungs, the gastrointestinal tract and kidneys. SSc is an autoimmune disease characterized by fibrosis and vasculopathy of various internal organs. It is a complex, multifactorial disease with very limited treatment.

Although SSc can affect almost any part of the body, skin disease is the most consistent clinical manifestation. Skin disease typically starts in the hands with an edematous phase of hand swelling lasting one to several months. The skin then progressively thickens and tethers to underlying tissues. In diffuse cutaneous SSc (dcSSc), skin thickening, induration and tethering typically extend proximally up the arm and can involve the torso, abdomen, face and legs. Patients with limited cutaneous SSc (lcSSc) have skin disease limited to below the elbow and face and neck as well as other characteristic clinical features. SSc skin pathology (diffuse and limited cutaneous SSc) shows fibrosis and variable perivascular lymphocyte infiltration in the deep reticular dermis.

SSc affects multiple other body systems. Most severe complications are seen more frequently in dcSSc with considerable morbidity and mortality [7]. Lung disease manifests as

interstitial fibrosis or pulmonary arterial hypertension (PAH, more common in lcSSc). Lung disease remains the leading cause of death among SSc patients. Gastrointestinal disease primarily results from dysmotility. In the esophagus and stomach this most commonly leads to esophagitis. In the small and large bowel this most commonly leads to constipation, bowel obstruction and/or malnutrition. Renal disease is primarily manifest as accelerated hypertension and renal insufficiency. Angiotension converting enzyme inhibitors are generally though not uniformly effective for treating this manifestation, which previously led to significant mortality.

Other important clinical manifestations include cold-induced vasospastic disease in extremities (Raynaud's phenomenon) and digital ulcers. SSc can also have cardiac manifestations. Pericarditis is the most frequent cardiac manifestation. Subclinical pericarditis is common with large effusions developing occasionally. Myocardial involvement with low-grade myocardial fibrosis is relatively common, but not frequently of clinical importance [8]. Fibrosis most commonly manifests as the appearance of a septal infarction pattern on EKG in patients with normal coronary arteries, or as ventricular conduction delays. Occasionally myocardial fibrosis leads to heart failure. Cardiac arrhythmias are seen in ~5% of patients with SSc. Most common are atrial or ventricular ectopy, generally not associated with more serious rhythm disturbances. However, thallium perfusion defects are associated with sudden cardiac death [9].

Current treatment for SSc is limited [10]. For most disease manifestations treatment is primarily symptomatic and generally inadequate. The exception is renal disease, scleroderma renal crisis, once a major cause of mortality in SSc patients, can often be treated successfully with angiotensin converting enzyme inhibitors. Pulmonary complications now represent the major cause of mortality. Cyclophosphamide provides some benefit in patients with interstitial lung disease (ILD), the most lethal complication of SSc. However, the effect of this agent on SSc-associated ILD is modest and transient [11,12]. More recently, autologous stem cell transplant has emerged as an effective therapy for this disease complication.

Bowel hypomotility also leads to considerable morbidity and sometimes mortality. Esophageal hypomotility is treated, frequently without success, with pro-motility and acid-blocking agents. Dysmotility of the lower bowel and its complications are even more difficult to treat with pro-motility agents providing modest relief in some patients and antibiotics helping in cases of small bowel overgrowth. Thus there are limited therapeutic alternatives for SSc patients faced with progressive lung or bowel disease.

Pulmonary arterial hypertension (PAH) also leads to considerable mortality in SSc patients. PAH may respond to vasodilators such as epoprostanol and bosentan, but frequently responses are incomplete and mortality still high [13]. SSc-PAH is particularly difficult to manage, with a 3-year mortality rate of ~60% following diagnosis [14]. When compared to other subgroups of PAH patients, SSc-PAH patients respond sub-optimally to approved PAH therapies [15].

While the etiology of SSc is unknown several observations suggest that oxidative stress plays a key role. Notably, increased levels of markers of DNA and lipid oxidation are present in the blood and urine of SSc patients [16-19]. Oxidative processes can enhance TGFβ and Wnt pathways promoting fibrosis [20-24]. Indeed, the bleomycin model of pulmonary fibrosis relies on oxidative DNA damage [25-27], and agents inducing oxidative stress can induce skin fibrosis and lung fibrosis [28]. Oxidative stress has also been implicated in several animal model of PAH [29]. In SSc-PAH, multiple lines of evidence implicate oxidative damage including increased production of reactive oxygen species (ROS) and nitrogen species (RNS), as well as decreased NO bioavailability and reduced downstream responsiveness to NO stimulation [30,31]. Dysregulation of ROS/NO redox homeostasis impairs vascular function leading to abnormal cell proliferation and

obliteration of the vasculature. Thus inhibition of oxidative stress might be efficacious as a therapy for SSc-PAH.

4.2. Rationale

All currently approved medications for PAH are vasodilators despite increasing evidence that the immune system is an important and possibly essential contributor to PAH and SSc. Not surprisingly, although vasodilators prolong survival, mortality is still very high. Adventitial macrophages appear particularly important in PAH pathogenesis [32]. Activated macrophages through myeloperoxidase and NADPH oxidase produce mediators of oxidative stress, such as superoxide, hydrogen peroxide and hypochlorous acid [33]. Oxidative stress can lead to endothelial cell dysfunction [34]. This will be the first study in PAH or SSc-PAH to examine whether blocking the observed changes in oxidative stress improves markers of oxidative stress and clinical disease. The combination of potential pathways targeted by DMF is highly unique and appropriate for SSc-PAH. We are not aware of any reports testing the effect of DMF or other inhibitors of oxidative stress in SSc or PAH. Although bardoxolone, an unapproved drug with similar activity as DMF on Nrf2, is under study for PAH, it has been associated with heart failure and may not have an adequate safety profile [35], particularly for SSc-PAH patients, already at high risk for cardiac complications. In addition, DMF has distinctive activities compared to bardoxolone. These drugs show numerous differences in both Nrf2-dependent and Nrf2-independent gene expression [36].

This study will explore how biomarkers might be effectively utilized in clinical trials of SSc-PAH as pharmacodynamic and predictive biomarkers. The effect of DMF on novel mRNA and protein biomarkers previously described in SSc-PAH patients, as well as a panel of conventional oxidative stress and PAH biomarkers, will be assessed. Peripheral blood mononuclear cells (PBMCs) from patients with SSc-PAH express a cluster of macrophage related genes that are tightly linked to the presence of PAH [37-39]. Novel protein biomarkers of SSc-PAH have been identified in previous studies [39]. Additionally, upregulated markers of oxidative stress are found in the serum in SSc patients [19] and in PBMCs from SSc-PAH patients [38]. Although DMF is known to block oxidative stress and affect NFκB activation, markers of these pathways were not examined in the trials leading to approval of DMF for multiple sclerosis. Thus, both testing biomarkers of disease as well as testing biomarker targets of DMF are both important aspects of the planned study.

Given the poor treatment options for these patients, new therapies are urgently needed. Ideally, a medication that could be added to and complement the current standard of care, i.e. vasodilators, would be optimal. Since DMF is known to reduce oxidative damage and down-modulate NFκB signaling, it is an attractive candidate for the treatment of SSc-PAH. In theory, efficacy could extend to additional organ involvement in SSc, such as the skin and interstitial lung disease. Showing clinical efficacy and/or a strong effect on biomarkers of oxidative stress on SSc-PAH in patients treated with DMF would enable a larger, controlled clinical trial.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1. Primary Objective and Endpoint

Primary Objective:

- To assess the safety of the treatment with DMF in patients with SSc-PAH.
- To assess the efficacy in this pilot trial will be improvement in 6-minute walk distance (6MWD).

Primary Endpoint:

- Safety will be assessed by the incidence of serious adverse events (SAEs) and all adverse events in DMF compared to placebo treated patients.
- The efficacy will be assessed by the change in 6MWD at 24 weeks from baseline in DMF compared to placebo treated patients (the study is powered to detect an improvement of 30-40 M in the 6MWD).

5.2. Additional Objectives and Endpoints

Secondary Objectives:

- Study the effect of DMF on time to clinical worsening: determined by: Change in SOC therapy for PAH; Hospitalization for PAH; Death; Need for transplantation; Need for atrial septostomy; Decline in 6MWD by 15% from the baseline 6MWD; Decline in WHO/NYHA functional class.
- Study the effect of DMF on isoprostane and other serum markers of oxidative stress, including lipid, DNA, and prostaglandin targets.
- Study the effect of DMF on PAH associated biomarker gene expression by peripheral blood mononuclear cells, including IL13RA1, CCR1, JAK2 and MRC1.
- Study the effect of DMF on serum B-type natriuretic peptide (BNP), Endostatin, Endothelin-1, Endoglin, Vascular Endothelial Growth Factor, von Willebrand Factor, and Vascular Cellular Adhesion Molecule 1.
- Study whether DMF affects skin mRNA biomarkers.
- Study the effect of DMF on Digital Ulcer Net Burden

Secondary Endpoints:

- The change in time to clinical worsening in DMF compared to placebo treated patients.
- The change from baseline of serum markers of oxidative stress at 24 weeks, comparing DMF to placebo treated patients.
- The change from baseline of PAH associated biomarkers at 24 weeks, comparing DMF to placebo treated patients

- The change from baseline in proteomic biomarkers, including BNP, at 24 weeks, comparing DMF to placebo treated patients.
- The change in skin nMRA biomarkers at 24 weeks, comparing DMF to placebo treated patients compared to baseline.
- The change in Digital Ulcer Net Burden at 24 weeks, comparing DMF to placebo treated patients compared to baseline.

6. STUDY DESIGN

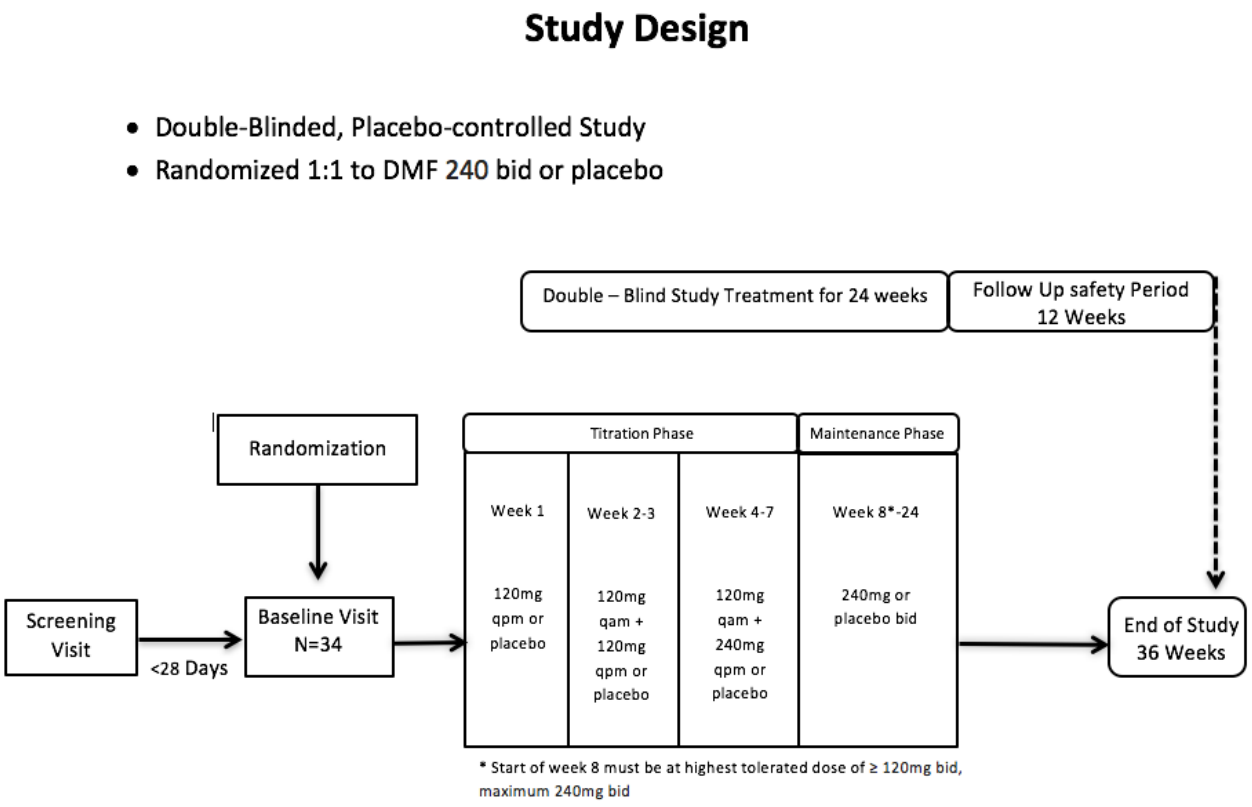
6.1. Study Overview

This is a double-blinded, placebo-controlled study of DMF in 34 SSc-PAH patients, anticipating 10% dropout to achieve 28 patients completing the study. The study medication will be added to stable background PAH medication(s). Subjects will be dosed for 24 weeks, will undergo examination every 8 weeks, and will be finally evaluated 12 weeks after completion of treatment. Subjects will be recruited from approximately 5 sites in the US and will have two cohorts, randomized 1:1 to receive a starting dose of 120 DMF qpm or placebo for 7 days then follow the up-titration schedule to a maintenance dose of 240mg bid or placebo (or the highest tolerated dose of ≥ 120mg bid by the start of Week 8) for the remainder of the study. Subjects who withdraw from the study will not be replaced.

6.2. Overall Study Duration and Follow-Up

Study duration for each subject will be 40 weeks including a 4- week screening period, 24 weeks of drug and 12-week safety follow-up after the last dose.

6.2.1. Study Flow Diagram



6.3. Early Termination of Study

The Principal Investigator, after consultation with NIAMS and Biogen (who supplies the study drug), may terminate this study at any time. Study termination would be considered in the event of unanticipated serious adverse events in consultation with the data safety monitoring board, or in the event of a major new therapeutic advance outside of the study.

All adverse events will be reviewed by the DSMB as part of their semiannual review. Any recurrent unanticipated serious adverse event will trigger ad hoc DSMB review within 14 days of submission. The PI will be responsible for informing the DSMB of such events within 48 hours, so that a timely meeting can be arranged by teleconference. The PI at his/her discretion may temporarily halt the study pending this review. The DSMB will then decide whether to terminate the study. In this case study medication dosing would be suspended and final study safety visits would be continued.

7. STUDY POPULATION

7.1. Inclusion Criteria

To be eligible to participate in this study, candidates must meet the following eligibility criteria at the time of randomization:

1. Signed inform consent prior to any study-mandated procedures
2. Adult patients 18-80 years of age
3. Clinical diagnosis of systemic sclerosis (either limited or diffuse cutaneous disease).
4. World Health Organization Group 1 PAH associated with scleroderma (SSc-PAH)
5. WHO functional Class II-III
6. Subject must be able to maintain O2 saturation ≥ 90% at rest (with or without oxygen). Oxygen use is permitted.
7. Screening 6MWD of at least 150 meters but not greater than 450 meters
8. Subjects must have been treated with background medical therapy for PAH (prostanoid, endothelin receptor antagonist, PDE-5 inhibitor, and/or guanylate cyclase stimulators) and have been on stable dose(s) of those medical therapy(ies) for a minimum of 8 weeks prior to randomization with no expectation of change for 24 weeks after randomization.
9. Lymphocyte count must be >500/microliter
10. Subjects must have either:

    A: Right heart catheterization showing mPAP≥ 25 mmHg and PCWP or left ventricular end diastolic pressure ≤15mm Hg a pulmonary vascular resistance of ≥400 dynes/cm-5 (5 Wood units) within 12 months of study entry.

    OR

    B: Right heart catheterization showing PAH (mPAP≥ 25 mmHg and PCWP or left ventricular end diastolic pressure ≤15mm Hg and pulmonary vascular resistance ≥240 dynes/cm-5 (3 Wood units)) within 3 months of study entry and no change in therapy since that catheterization.

7.2. Exclusion Criteria

Candidates will be excluded from study entry if any of the following exclusion criteria exist at the time of randomization:

1. Pulmonary hypertension associated with
    a. PAH of any etiology other than scleroderma
    b. PH of any etiology other than WHO Group I PAH
    c. Pulmonary venous hypertension defined as PCWP or LVEDP >15 mHg

   d. Untreated Sleep Apnea with AHI >20 or SaO2 Nadir <87%
   e. Chronic Thromboembolic Disease
   f. Sarcoidosis

2. Moderate or severe interstitial lung disease as characterized by a forced vital capacity (FVC) of < 70% predicted and/or moderate or severe ILD by HPH, except if FVC is between 60-70% predicted and the most recent standard of care HRCT shows only mild ILD, or the FVC is between 50%-60% predicted and the most recent standard of care HRCT shows no ILD. Patients with FVC < 70% predicted should be excluded if there is clinical evidence of weakness or other factor the site investigator feels might adversely affect the 6MWD.
3. Participation in a clinical investigational study within the previous 30 days
4. Moderate to severe hepatic impairment (e.g., Child-Pugh Class B or C)
5. Renal failure defined as:
   a. estimated creatinine clearance <30 m/min
   b. serum creatinine>2.5 mg/dl
6. Serum aspartate aminotransferase (AST) and or alanine aminotransferase (ALT) 2 times the upper limit of normal
7. Bilirubin > 2x ULN.
8. Absolute neutrophil count (ANC) < 1,500/mm^3 (or < 1.5 x 10^9/L).
9. Platelet count < 100,000/mm^3 (or < 100 x 10^9/L).
10. Hemoglobin < 9 g/dL.
11. Persistent hypotension with systolic blood pressure (SBP) < 90 mmHg.
12. Recently started (< 8 weeks prior to randomization) or planned cardio-pulmonary rehabilitation program based on exercise
13. Pregnant or lactating women
11. 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.
12. Positive serology for hepatitis B defined by positive HBV surface antigen and/or positive HBV core antibody, total.
13. Positive serology for HCV antibody, unless treated and cured of HCV.
14. Evidence of active infection requiring IV or PO antibiotics within 2 weeks of randomization.
15. Evidence of active or latent tuberculosis (TB) within 30 days of screening, including any of the following:

a. active TB: a history of active TB unless completion of treatment for active TB has been documented.

b. latent TB (a positive PPD and/or QuantiFERON® test, a negative chest x-ray, and no symptoms or risk factors), unless one month of prophylaxis has been completed prior to inclusion.

c. an indeterminate QuantiFERON® unless followed by a negative QuantiFERON®, or by a subsequent negative PPD and consultation with and clearance by local infectious disease (ID) department.

16. Recent administration of a live vaccine (< 8 weeks) or any other immunization within 4 weeks of treatment.

17. 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.

18. History of non-compliance with other medical therapies.

19. A history of alcohol or drug abuse within 1 year of randomization.

20. Planned treatment or treatment with another investigational drug within 1 month prior to start.

8. STUDY PROCEDURES

Once the investigational site has been activated for study participation, subjects may be enrolled if they have met the inclusion criteria in Section 7.1 and have not been excluded based on the exclusion criteria in Section 7.2. Biogen will be supplying sites with study drug directly.

8.1. Screening, Enrollment, Re-screen and Randomization of Subjects

Subjects must be consented before any screening tests or assessments are performed. At the time of consent, the subject will be enrolled into the study.

Subjects will be randomized after all screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in Sections 7.1 and 7.2. No subject may begin treatment prior to randomization and assignment of a unique subject identification number. Any subject identification numbers that are assigned will not be reused even if the subject does not receive treatment.

Subjects will be randomized to receive DMF 120mg qpm or placebo for 7 days then up-titrate to DMF 240mg bid (or highest tolerated dose of ≥ 120mg bid) or placebo in a 1:1 ratio for the remainder of the treatment period. Subjects who withdraw from the study will not be replaced.

The maximum allowable time between screening and the first administration of study treatment is 28 days. If visit 1 does not occur in this time frame, the subject would be required to be re-screened. All study procedures outlined in the study schema would need to be repeated with the exception of the HIV, HCV and HBV serologies.

The study blind will be maintained using the following procedures: Almac, a third party contracted by Biogen will be supplying clinical sites with pre-labeled study drug and placebo. The unblinded staff at the Coordinating Center will provide the site pharmacist with an unblinded manifest once shipment is received from Almac. Blinding of study results will be maintained until data lock. Patient treatment assignment will be unblinded only if required for safety after discussion with Dr. Lafyatis. Data lock will occur after all patients have completed the final study visit and all translational studies have been completed. Thus, study investigators will remain blinded to treatment assignment until all clinical and translational data have been collected.

8.2. Follow-up

Safety follow-up will be conducted for all subjects at 3 months after the last dose of study treatment has been administered. This period of follow-up is supported by pharmacokinetic modeling, which demonstrated that serum drug levels will be completely cleared within several days. If a subject is withdrawn from study drug treatment, a safety follow-up visit will be conducted at 3 months from the date of withdrawal from the study medication.

8.3. Stopping Rules

Subjects must be withdrawn from the study for any one of the following reasons:

- The subject becomes pregnant.

- The subject desires to discontinue study treatment under this protocol.
- The subject experiences a medical emergency that necessitates permanent discontinuation of study treatment and/or unblinding of the subject's treatment assignment.
- Stopping rules per patient are is lymphocyte counts are <500 mcL at any two visits.
- At the First Sign or Symptom Suggestive of Progressive Multifocal Leukoencephalopathy, Dmf Will Be Withheld, And Appropriate Diagnostic Evaluation Performed.
- Patients Showing Persistent Elevation in Transaminases > 3x The Upper Limit Of Normal Will Be Discontinued From Treatment.
- Subject is unable to tolerate study drug at 120 mg bid at the start of Week 8

Subjects may also be discontinued from treatment at the discretion of the Principal Investigator.

The reason for the subject's withdrawal from the study must be recorded in the subject's case report form (CRF).

9. STUDY TREATMENT

Biogen will be supplying sites directly with Tacfidera/Placebo unblinded and the site pharmacist will be responsible for labeling the study drug with a unique identifier that corresponds with a predetermined randomization assignment. The study treatment will be dispensed to the subject by the (pharmacist or study coordinator) or designee per the sites institution for study drug dispensing policies.

9.1. Dosing Regimen and Administration

DMF or placebo will be given as a pill to be taken twice a day. The first week of the study the subject will receive 120mg DMF tablets or similar appearing placebo tablets to be taken one tablet per day, and after the first week the subject will take one 120mg DMF tablets twice per day for two weeks. For the next month, weeks four through eight, the subject will take 120mg DMF tablet every morning and 240mg DMF tablet every evening. At weeks 8 – 24 the subject will enter the maintenance phase and will take 240 DMF tablet twice a day. By the start of week 8, the subject must be at a minimum of 120 mg DMF tablets twice a day. If a subject is unable to tolerate the maximum dose of 240mg twice a day, the subject may continue 120mg twice daily or highest tolerated dose for the remainder of the maintenance period. DMF or placebo can be taken with or without food. Missed doses should not be made up. The next dose should be taken as scheduled.

Administration of DMF with food may reduce the incidence of flushing. Alternatively, administration of non-enteric coated aspirin (up to a dose of 325 mg) 30 minutes prior to DMF dosing may reduce the incidence or severity of flushing.

Gastroenterology adverse events, although reported to be mostly mild to moderate in severity, are experienced by a significant proportion of patients receiving DMF. Using a Delphi technique, neurologists reached consensus on several strategies to manage nausea, vomiting, abdominal pain, and diarrhea when using DMF: namely co-administration with food (particularly a high-fat meal such as peanut butter, yogurt, and cheese), dose titration up to 4 weeks when initiating DMF therapy, temporary dose reduction to 120 mg twice daily for 2–4 weeks, and use of specific symptom-directed therapies.

Ondansetron (Zofran) is useful for reducing the impact of nausea and vomiting, as well as, bismuth subsalicylate, promethazine and antacids. Abdominal pain can be managed with bismuth subsalicylate, antacids, and anti-secretory drug treatment (dicyclomine hydrochloride or others), while diarrhea can be managed with loperamide and diphenoxylate/atropine.

For the SSc-PAH DMF study the following approach should be taken if patients develop gastrointestinal side effects. First, when the medication is started the patient should be educated about the benefit of taking DMF with food, particularly high fat meal, such as peanut butter, yogurt or cheese. This should be reinforced at a one-week follow-up telephone call just before they increase their dose. Patients should be instructed to call if gastrointestinal or other side effects develop at the initial or with the increased dose. If taking the medication with food does not ameliorate nausea/vomiting, or if patient is having continuing nausea at the 120 mg BID dose then patients should be instructed to stay at the 120 mg BID dose for up to 8 weeks before increasing the dose to 120 mg every morning and 240 mg every evening. During the time of dose

reduction, the study physician should try the above methods and medications to increase the dose to 240 mg BID. If the patient still cannot tolerate a minimum of 120mg BID then the patient should be discontinued from the study.

Patients showing gastrointestinal adverse events such as nausea, vomiting, diarrhea, abdominal pain, or dyspepsia will be treated symptomatically or discontinued from treatment depending on the severity and patient tolerability at the discretion of the investigator.

9.2. Drug Ordering and Accountability

Initial Orders

After site activation occurs, the initial drug supply will need to be ordered from Almac**.** Almac is a third party contracted by Biogen for the distribution of study drug and placebo. Detailed instructions and forms for the pharmacists will be in the pharmacy manual that will be provided by the Coordinating Center.

Re-Supply

Subsequent drug supply will need to be ordered from Almac**.** Detailed instructions and forms for the pharmacists will be in the pharmacy binder that will be provided by the Coordinating Center.

Method of Assigning Subjects to Treatment

Patients will be randomized after all screening assessments have been completed, entered into the EDC database and the investigator has verified that eligibility criteria have been met. The Coordinating Center will verify eligibility prior to any subject being randomized. A written letter of approval will be sent to the clinical site once review and approval has occurred.The site pharmacist will be responsible for randomizing the subject in the EDC database; no participant may begin treatment prior to randomization. Eligible participants will be randomized to DMF or placebo in a 1:1 manner. The Coordinating Center Data Manager in consultation with the Study Statistician will prepare the randomization schedule, using computer-generated block randomization with the block size(s) known only by the Coordinating Center Data Manager and Study Statistician. A secure web-based application will be used by the site pharmacist to enter participant information (e.g., participant ID, stratification factor(s)) and to obtain the treatment assignment.

9.3. Treatment Compliance

Compliance with treatment dosing will be monitored and recorded by site staff at each study visit by counting all returned study medication.

9.4. DMF/Placebo Stability and Storage

DMF/ placebo will be supplied in 120 mg capsules in a blister pack/wallet directly to each site pharmacy from Biogen. The drug will be shipped unlabeled and will be labeled by each site

pharmacy. Each site pharmacist will be responsible for labeling the drug with a unique identifier that corresponds with a predetermined randomization assignment.

120mg DMF/ placebo for initiation, dispensed in blister packs/wallets

The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity), as described below:

- The investigational agent should be stored between 15°C and 30°C (59°F and 85°F) and protected from light. Opened wallets should be discarded at the expiration date and patients instructed in the proper storage of the drug when it is taken home.

The study site personnel or their designee (pharmacist or coordinator) at each site will be responsible for correct storage and handling of the study drug.

Study drug will not be used beyond the initial expiration date on the drug packaging. The label will include conditions for storage, lot number, and other pertinent information such as Sponsor and caution statement.

The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study participants and only from official study sites by authorized personnel, as dictated by local regulations.

Study site staff should refer to the MOOP for specific instructions on the handling, preparation, administration, and disposal of the study treatment.

9.4.1. DMF/Placebo Handling and Disposal

Study treatment must only be dispensed by a Pharmacist or appropriate trained staff. Study treatment is to be dispensed only to subjects enrolled in this study. Once study treatment is prepared for a subject, it can only be administered to that subject.

The study site must maintain accurate records demonstrating dates and amount of study drug received, to whom they are dispensed (subject-by-subject accounting), and accounts of any study drug accidentally or deliberately destroyed. Unless otherwise notified, the study sites must save all unused DMF /Placebo supplies for drug accountability. Subjects will be expected to return all unused drug to the site at either study termination or withdrawal.

The study sites will destroy the study drug per their institutions destruction policy at the end of the trial. The sites must send the documentation of the study drug destruction at the end of the trial to the Coordinating Center. Sites will be required to send the Coordinating Center a copy of their institutions drug destruction policy.

9.5. DMF/Placebo Accountability

The clinical site will maintain an Investigational Drug Accountability Log. The following information will be recorded:

- Total amount of drug received, date of drug receipt, initials of person receiving

- Identification of study subject to whom study product was dispensed, date of dispensing, quantity dispensed, and initials of person responsible for dispensing
- Amount of study product returned to the site from the study subject, date of return and initials of person receiving/recording the return
- Record any study drug accidentally or deliberately destroyed
- Balance of study drug on site

10. DATA COLLECTION

10.1. Assessments

Study-related procedures and outcome measures that will be performed as part of this protocol are listed below. Specific times at which each test will be performed are summarized in the time and events table (section 3).

10.2. Safety Assessments

- **Complete medical history.** Medical history will be performed as per standard medical care.
- **Physical examination.** A standard complete physical examination will be performed, with the addition of the assessment of modified Rodnan Skin Score, digital ulcers.
- **Vital signs.** Vital signs will include: pulse, blood pressure, respiratory rate, temperature (C º), height (cm), and weight (kg).
- **Safety Labs.** Safety labs (Complete Blood Count (CBC), Complete Metabolic Panel (CMP), B-Type Natriuretic Peptide (BNP) and Urinalysis will be done at every visit and sent to the local lab for processing. A Urine Dipstick pregnancy test will be done on all WOCBP at every visit before any study treatment is dispensed.

10.3. Efficacy Assessments

10.3.1. 6-Minute Walk Distance

The 6MWT[40] measures the distance an individual is able to walk over a total of six minutes on a hard, flat surface. The goal is for the individual to walk as far as possible in six minutes. The individual is allowed to self-pace and rest as needed as they traverse back and forth along a marked walkway.

10.3.2. NYHA Classification

The New York Heart Association (NYHA) Functional Classification provides a simple way of classifying the extent of heart failure. It places patients in one of four categories based on how much they are limited during physical activity; the limitations/symptoms are in regard to normal breathing and varying degrees in shortness of breath and/or angina.

10.3.3. Modified Rodnan skin score

The Modified Rodnan skin score (MRss) is a validated physical examination method for estimating skin induration. It is correlated with biopsy measures of skin thickness and reflects prognosis and visceral involvement, especially in early disease [41],[42]. It is scored on a 0 (normal) to 3+ (severe induration) ordinal scales over 17 body areas, with a maximum score of 51 and is used to categorize severity of SSc. It has been extensively used as primary/ secondary outcome in RCTs [43], [44], [45]. This will be collected at every study visit.

10.3.4. Skin Biopsies

Skin biopsies will be collected from enrolled participants to evaluate transcriptional changes following treatment with DMF at Visit 1 and 4. One 3mm skin biopsy will be obtained from the mid dorsal surface of the forearm. If affected skin is located in that area, then the affected skin should be used. A biopsy will be performed whether affected skin is present or not. Skin biopsies from future visits should be conducted on the same arm.

10.3.5. Biomarkers of Oxidative stress and PAH.

- **Oxidative stress Biomarkers.** Circulating biomarkers of oxidative stress in plasma (8-isoprostane (total and free) and Total anti-oxidant capacity), and in serum (TBAR and 8-hydroxydeoxyguanosine) will be measured at baseline 8-, 16-, 24-, and 36-week study visits
- **Plasma and serum proteins.** Circulating protein biomarker will be measured in plasma (NT-ProBNP, IL-6, CCL2/MCP-1) or serum (Endoglin, Endostatin, Endothelin-1, vWF, VEGF, VCAM, FSTL3, Spondin-1, Jam-C, CCL28, Midkine) at baseline 8-, 16-, 24-, and 36-week study visits.
- **Peripheral Blood Mononuclear Cell (PBMC) RNA.** PBMC RNA will be assessed for IL13RA1, CCR1, JAK2, MRC1, BIP, ATF6 and DNAJB1 at baseline 8-, 16-, 24-, and 36-week study visits.

10.3.6. Biomarkers of skin mRNA.

Biomarkers for the effect of DMF on skin will be assessed by gene expression analyses

10.3.7. Digital ulcer assessment

Digital ulcers are defined as a full thickness skin lesion with loss of epithelium. Ulcers should be > 3mm in maximal diameter. Healing is defined by re-epithelialization with loss of pain and exudate. Pitting scars and hyperkeratotic lesions are always excluded. Also, eschar is not considered as DU. This will be collected at Screening through Week 24 visits.

Digital ulcer will be assessed by the following methods:

- Visual analog score for patient-reported severity of digital ulcers
- Ulcer count:
  - Total ulcer counts
  - Distal counts: distal (fingertip) – any ulcer including skin area distal to proximal interphalangeal (PIP) joint

1. Non-ischemic ulcers over the PIP and MCP will also be evaluated for healing as secondary outcomes

10.3.8. Patient-Reported Outcomes (PROs)

Three patient-reported outcomes (PROs)—the SHAQ, Raynauds VAS, and Systemic Sclerosis Skin Questionnaire—will be completed at every visit by all subjects in the study.

10.3.9. Scleroderma Health Assessment Questionnaire (SHAQ)

The SHAQ consists of 8 domains from the Health Assessment Questionnaire disability index (HAQ-DI), a HRQoL instrument that measures self-reported function in 8 domains of activity in 20 weighted responses and a VAS of pain experienced in the past week. It additionally measures 5 domains specific to scleroderma using a continuous VAS: Raynaud's phenomenon, digital tip ulcers, lung symptoms, gastrointestinal symptoms, and a global patient assessment[46]. The VAS subscales of the SHAQ were shown to be significantly correlated with objective parameters,[46] and was responsive to change in a cohort and in a Raynaud's phenomenon trial in SSc.[46,47]

10.3.10. Raynaud's attacks assessment

Raynaud's attacks will be assessed using the following individual outcome measures: in Raynaud's condition score and patient assessment of Raynaud's phenomenon using the VAS[48]

The Raynaud's condition score is a daily patient-assessment of Raynaud's phenomenon activity using a 0-10 ordinal scale. It incorporates the cumulative frequency, duration, severity and impact of Raynaud's phenomenon attacks, reflecting the overall degree that Raynaud's has affected use of the patient's hands[48]. The Raynaud's condition score, along with details of the frequency and duration of Raynaud's attacks, will be incorporated into the daily diary that subjects will be asked to complete for 1 week (7 days) at the time points shown in the schedule of assessments.

The patient and physician assessment assess the severity of Raynaud's phenomenon in the past week using a 0-100 VAS.

10.3.11. Systemic Sclerosis Skin Questionnaire (PRO-SRSS)

Patient assessment of skin disease activity will be done at specified visits for each enrolled subject. The assessment at each specified visit will be performed with a questionnaire of 22

questions about how scleroderma affects the skin and how those skin problems affect how the person feels and does things. Each question is followed by seven boxes with numbers 0-6, spaced equidistance in between. The boxes are anchored by two verbal descriptors, one of "Not at all" (box labeled 0) and one of "Very Much" (box labeled 6). The subject will select a box labeled by an integer in response to each question.

11. PROTOCOL DEVIATION DEFINITIONS

Protocol deviations occur when there is non-adherence to the protocol, including failure to follow informed consent, safety surveillance and enrollment procedures, or to adhere to Good Clinical Practices (GCP).

Deviations may occur when there is non-adherence to study procedures or schedules by either the subject or site personnel, as specified by the protocol.

11.1.1. MINOR DEVIATIONS

A minor protocol deviation is one that does not have the potential to impact subject safety or risk, compromise the integrity of the study data, or affect the subject's willingness to participate in the study.

11.1.2. MAJOR DEVIATIONS

Major protocol deviations are variations from protocol-directed conduct of a clinical trial that are considered to affect the safety of the subject, the ability of the trial to evaluate the efficacy of the study regimens, or failure of the investigator to provide subject protection as required by GCP.

11.1.3. INSTRUCTIONS FOR DOCUMENTING DEVIATIONS

All protocol deviations should be recorded, as they are discovered, for your record keeping and submission to the CC who will submit to the University of Pittsburgh IRB per their requirements.

All Protocol deviations should be reported to the coordinating center via FAX: (412) 648-9643 or scan & email: krk99@pitt.edu and entered into the EDC.

A sample of the protocol deviation log is presented as **Appendix F with Required Source Document**.

12. SAFETY DEFINITIONS, MONITORING, AND REPORTING

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 those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly. Reporting of Adverse Events and Serious Adverse Events/Events of Special Interest) to the sponsor (NIAMS through KAI) and Biogen. Appropriate notifications will also be made to the site investigators, Institutional Review Boards (IRBs), Institutional Ethics Committees (IECs), 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, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03 (June 14, 2010): https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Throughout the course of the study, every effort will be made to remain alert to possible adverse events (AEs). If an AE occurs, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention will be provided. At the signing of the ICF, each subject must be given the names and telephone numbers of study site staff for reporting AEs and medical emergencies.

A sample of the AE, SAE and MEDWATCH form is shown in **Appendix E**.

12.1. Definitions

12.1.1. Adverse Event

Any untoward or unfavorable medical occurrence 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.html#Q2)

12.1.2. Adverse Reaction and Suspected Adverse Reaction (SAR)

Suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the investigational 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) and ICH E2A).

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

12.1.3. Unexpected Adverse Event/Reaction

A SAR is considered "expected" when it is listed in the investigator brochure, the package insert, or the protocol. A SAR is considered "unexpected" when its nature (specificity), severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the investigator brochure, the package insert, or the protocol (21 CFR 312.32(a) and ICH E2A). A serious unexpected suspected adverse reaction is referred to as a SUSAR. For this study, expectedness will be determined by product information provided in the investigator brochure, package insert, and protocol for Dimethyl fumarate (Tecfidera, DMF).

12.1.4. Serious Adverse Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor (NIAMS) 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 Sponsor (NIAMS) 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.

Elective hospitalizations are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.1.5. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the study site must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study.

- The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the time of the procedure or treatment.
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission.

12.2. Grading and Attribution of Adverse Events

12.2.1. 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 (CTCAE) Version 4.03 (June 14, 2010). 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.

Expectedness of all AEs will be determined by the Principal Investigator according to the approved local label.

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.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events if related to a study-mandated procedure, treatment, or change in treatment (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 as a result of the event/result.

12.2.2. Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE eCRF. Final determination of attribution for safety events that may be eligible for expedited reporting to the FDA will be determined by the sponsor, NIAMS. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided below.

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.

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.

12.3. Monitoring and Recording Events

12.3.1. Adverse Event Guidelines

All adverse events will be collected and will be presented in aggregate in the monitoring body periodic reports.

At a minimum, the following criteria should be used as a guide for recording Adverse Events. These guidelines are not all inclusive and the recording of Adverse Events remains at the discretion of the investigator. A symptom or condition that is present but does not reach one of these levels may still be recorded as an adverse event.

1) A symptom or event that requires discontinuation of study medication.
2) A worsening of an existing condition.
3) A newly diagnosed symptom or event that requires a written prescription for treatment.
4) A newly diagnosed symptom or event that results in a referral to another provider.
5) Any grade 3 or 4 event according to the NCI Common Toxicity Criteria.

An adverse event is considered a **serious adverse event** if one or more of the following apply:

1. results in subject hospitalization or prolongs existing hospitalization
2. event is life-threatening
3. results in death
4. results in significant or permanent disability
5. requires medical intervention to prevent permanent damage
6. results in a congenital anomaly or birth defect

12.3.2. Adverse Event Reporting

If a subject experiences an adverse event (AE) or serious adverse event (SAE), complete the Adverse Event Form (AE).

All AEs are collected, analyzed, and monitored by using an Adverse Event Form, a sample of which is shown in **Appendix E**. AEs and/or laboratory abnormalities identified in the protocol as critical to participant safety must be reported to NIAMS, and the DSMB through KAI and will also be reported to Biogen at the time of each DSMB meeting, typically biannually. All AEs experienced by the participant during the time frame specified in the protocol from the time of signed consent through the end of the study are to be reported, as outlined in the protocol.

12.3.3. Unanticipated Problems

Unanticipated Problems are not included in the 45 CFR part 46, but are defined by the OHRP as any incident, experience or outcome that meets all of the following requirements:

(1) Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

(2) Related or possibly related to participation in the research. Possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

(3) Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

OHRP recognizes that it may be difficult to determine whether a particular incident, experience, or outcome is unexpected and whether it is related or possibly related to participation in the research. OHRP notes that an incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in the research protocol or informed consent process/document, or other corrective actions in order to protect the safety, welfare, or rights of participants or others.

Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include:

- Changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects; modification of inclusion

or exclusion criteria to mitigate the newly identified risks; implementation of additional procedures for monitoring subjects; suspension of enrollment of new subjects; suspension of research procedures in currently enrolled subjects; modification of informed consent documents to include a description of newly recognized risks; and provision of additional information about newly recognized risks to previously enrolled subjects.

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem. Furthermore, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subject's research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs. All unanticipated problems that involved participant safety should be reported to the NIAMS and the DSMB through KAI and Biogen within 48 hours of the Investigator becoming aware for the event. For further information see http://www.hhs.gov/ohrp/policy/advevntguid.html.

12.3.4. Serious Adverse Event Reporting

An AE is considered serious if one or more of the following criteria are met:

1) Results in death
2) Is life-threatening
3) Requires or prolongs in subject hospitalization (i.e. elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs)
4) Is disabling (i.e. resulted in a substantial disruption of the ability to carry out normal life functions)
5) Is a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a subject exposed to the trial drug prior to conception or during pregnancy)
6) Does not meet any of the above serious criteria but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Any SAE experienced by a subject after the subject signs the informed consent form (ICF) and before End of Study/Premature Withdrawal/Final Follow-up Visit, is to be recorded on an SAE Form, regardless of the event relationship to DMF.

Any SAE ongoing when the subject completes the study or discontinues from the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status.

12.3.5. Immediate Reporting of Serious Adverse Events

It is the Principal Investigator's responsibility to ensure that the SAE reporting information and procedures are used and followed appropriately.

Site Investigators must report SAEs to Dr. Lafyatis (PI) within 24 hours of becoming aware of the event.

If the subject experiences a Serious Adverse Event:

The Clinical Site will:

1. Complete the Serious Adverse Event form. (SAE Form can be found in **Appendix E**)

2. Fax the completed SAE Form to the Coordinating Center at the University of Pittsburgh within 24 hours of knowledge of the event

Fax: (412) 648-9643

3. Call the Coordinating Center (Kristi Kong) during office-hours (including weekends; between 9:00 am and 4:00 pm EST M-F; and holidays)

Cell: (267) 371-0521

The Coordinating Center at the University of Pittsburgh will:

1. The coordinating Center will submit to the Central IRB per the University of Pittsburgh IRB submitting guidelines. (These guidelines can be found in **Appendix G** with the SOP's)
2. The Coordinating Center will submit the SAE form to NIAMS and the DSMB through KAI within 48 hours of becoming aware of the event.
3. Dr. Robert Lafyatis will be responsible for reporting to the FDA.

Reporting Information for SAEs
All SAEs regardless of relatedness must be reported to the NIAMS and the DSMB through KAI within 48 hours of the investigator becoming aware of the event Any Serious Event that occurs between the time that the subject has signed informed consent and Final Follow-up Visit must be reported to Biogen within 24 hours of the study site staff becoming aware of the event A report ***must be submitted*** to Biogen SABR or designee regardless of the relationship to DMF/Placebo. To report initial or follow-up information on a Serious Event, email a completed SAE report to the following: **Email:** KAIforNIAMS@kai-resssearch.com **Biogen:** Jason Mendoza, Email: jason.mendoza@biogen.com, Fax: 888-299-8459 **Email:** BiogenPSPVgroup@ppdi.com

12.3.6. Deaths

Death is an outcome of an event. The event that resulted in death should be recorded and reported on the appropriate CRF. All causes of death must be reported as SAEs. The Investigator should make every effort to obtain and send death certificates and autopsy reports to Biogen.

12.3.7. Suspected Unexpected Serious Adverse Reactions

A Suspected Unexpected Serious Adverse Reaction is known as a SUSAR is when during a clinical trial for a certain drug there may be serious adverse reactions in subjects given the drug, that may or may not be dose related, but are unexpected, as they are not consistent with current information. Reporting a SUSAR is an important aspect of clinical trials involving drugs.

The Principal Investigator will notify Biogen and all participating investigators in an IND safety report of potentially serious risks from this clinical trial no later than 15 calendar days after we receive the safety information and determine the information qualifies for reporting. Events must be reported to Biogen if they meet the definitions for **all** of the following: (1) Suspected adverse reaction (2) Serious and (3) Unexpected. The blind should ordinarily be broken for IND safety reports submitted to FDA. Knowledge of the treatment received is necessary for interpreting the event, may be essential for the medical management of the subject, and may provide critical safety information about a drug that could have implications for the ongoing conduct of the trial (e.g., monitoring, informed consent). In general, if the blind is broken and a subject with an adverse event that would meet the criteria for reporting as a single event was receiving placebo, the event should not be reported in an IND safety report because there is not a reasonable possibility that the drug caused the adverse event. Follow-up IND safety report will be used for any follow-up information. A "7-day IND safety report" will be used for unexpected fatal or life threatening adverse reaction reports.

The Clinical Center is responsible for notifying the Coordinating Center and the Principal Investigator is responsible for notifying the University of Pittsburgh Institutional Review Board

12.4. Procedures for Handling Special Situations

12.4.1. Reporting Pregnancy

The Investigator should refer to the approved local label for guidance if female subjects become pregnant or are considering becoming pregnant during the study.

12.4.2. Overdose

An overdose is any dose of study treatment given to a subject or taken by a subject that exceeds the dose described in the protocol. Overdoses are not considered AEs; however, all overdoses should be recorded on an Overdose Form and faxed to <Biogen SABR or designee> within 24 hours. An overdose should be reported even if it does not result in an AE. Overdoses do not need to be recorded in the CRF; dosing information is recorded on a CRF.

12.4.3. Medical Emergency

In a medical emergency requiring immediate attention, study site staff will apply appropriate medical intervention, according to current standards of care.

12.4.4. Unblinding for Medical Emergencies

• This is a double-blind study. The study staff (except for select staff at the IDS CC) and the patient are blinded to the treatment assignment.

• Blinding is critical to the integrity of this clinical study. However, in the event of a medical emergency or pregnancy in a participant, in which knowledge of the investigational product is critical to the participant's management, the blind for that participant may be broken.

• Before breaking the blind of an individual participant's treatment, the investigator should have determined that the information is necessary, i.e., that it will alter the participant's immediate management. A discussion with the protocol chairs is encouraged prior to proceeding with unblinding. The investigator holds sole responsibility for the decision to unblind in case of emergency. In many cases, particularly when the emergency is not investigational product-related, the problem may be properly managed by assuming that the participant is receiving active product without the need for unblinding. NIAMS and the DSMB through KAI should be informed in the event of any emergency unblinding.

12.5. Principal Investigator Responsibilities

The Sponsor- Investigator's responsibilities include the following (refer to Section 12.3.1for details):

- Review all AEs to determine seriousness and fulfillment of collection criteria
- Monitor and record all SAEs, regardless of the relationship to DMF/Placebo.
- Determine the relationship of each SAE to DMF/Placebo.
- Determine the onset and resolution dates of each SAE
- Record all pregnancies.
- Complete the appropriate form for each SAE and fax or email it to Biogen SABR or designee within 24 hours of the study site staff becoming aware of the event.
- Pursue SAE follow-up information actively and persistently. Follow-up information must be reported to Biogen SABR or designee within 24 hours of the study site staff becoming aware of new information.
- Ensure all SAE reports are supported by documentation in the subjects' medical records.
- Report SAEs to local ethics committees, Health Authorities, other Investigators and entities as required by local law.

13. STATISTICAL CONSIDERATIONS

13.1. Sample Size Justification

While setting the sample size in a pilot trial via formal power calculations is not necessary, providing a sample size justification is useful as we assess aspects of an anticipated phase 3 trial of DMF among patients with systemic sclerosis associated pulmonary arterial hypertension, such as recruitment rates, adherence to DMF, and the estimates of variance from a pilot trial of DMF. We estimated the optimal sample size of 28 for a two armed parallel group randomized controlled clinical trial with a two-sided type I error rate of 5% and a power of 80% using approaches proposed by Whitehead and colleagues[49]. We recognize that sample size for a pilot trial can be justified based on expected effect size. Published rules of thumb and mathematical modeling also factor into sample size justification, but recommendations are variable. Alternative recommendations are a pilot randomized controlled trial should have 9, 11 and 14 patients in each arm for treatment with expected effect size of 0.5, 0.4, and 0.3 respectively, using the 80% non-central t-distribution approach[49]. Our proposal to include 34 patients (allowing for a 20% drop out rate) in this study to allow us to estimate the efficacy with a reasonable degree of certainty.

The pilot study sample size calculation is based on an anticipated future main superiority clinical trial, for an anticipated detectable effect size of 0.3. For a main trial powered at 80%, type I error rate of 5%, and an effect size of 0.3, 14 patients/arm is the theoretical optimal pilot size trial for a main trial of 188 patients/arm using a non-central t-distribution approach [1]. This sample size (188) is in line with the sample size for most current phase 3 trials for PAH. The main trial size is limited by the size of the available SSc-PAH patient population.

13.2. Interim Analysis

Interim analysis of the study will be conducted due to pre-specified stopping rules as outlined in the protocol, or as determined necessary by the DSMB to assess safety concerns.

13.3. Endpoint Analysis

Intention to treat (ITT) analysis will be undertaken to evaluate the effect of DMF.

First, we will compare the baseline characteristics using χ^2 test for categorical or nominal variables and t-test for continuous variables between treatment and placebo groups.

Second, we will assess whether treatment of DMF will significantly improve the 6MWD, the primary outcome variable in this proposal, among patients with SSc-PAH. Specifically, for each patient we will calculate the difference in 6MWD assessed at 24 weeks from that assessed at baseline. If the change in 6MWD is normally distributed we will calculate the mean of 6MWD change for each treatment group and compare the difference in means of 6MWD change between two groups using linear regression model. If 6MWD is not normally distributed, we will estimate the median value of 6MWD change for each group and test if median value is different using

quantile regression model. We will perform multivariable regression model to adjust for potential confounder If the baseline characteristics are not evenly distributed between two groups.

Third, since the 6MWD will be assessed repeatedly at baseline, 8-week, 16-week and 24-week visits we will examine the effect of DMF on the changes in 6MWD at each follow-up visit (i.e., 8-, 16-, and 24-week) from that at baseline using mixed-effects regression model.
We will take the same statistical analysis approach to examine the effect of DMF on each biomarker measured for this pilot trial.

Fourth, since almost all previous studies have examined the cross-sectional association between levels of specific biomarkers of SSc-PAH and clinical outcome (i.e., 6MWD) we do not know whether changes in levels of these biomarkers will also correspond to changes in clinical outcome, such as 6MWD. In this pilot trial, we will collect data on both biomarker and clinical outcomes (i.e., 6MWD) simultaneously at four time points (i.e., baseline, 8-, 16-, and 24-week). Thus, it will allow us to assess whether change in each biomarker is associated with change in 6MWD. Specifically, we will assess whether the levels of a specific biomarker assessed at baseline (i.e., cross-sectional association) and its change over time (i.e., longitudinal association) are associated with a change in 6MWD using mixed-effects linear regression models. In the mixed effect model the time will be considered as a categorical variable. We will model both treatment assignment and time as the fixed effects, and subjects as a random effect. Missing values for covariates and outcome variable will be imputed by a sequential regression method based on a set of covariates as predictors [50]

Finally, number of patients with side effects and number of patients of non-adherence will be tabulated and compared between the two groups using χ^2 test.

The safety outcomes will be reported in summary statistics in DMF and placebo treated patients, separately, including frequency or mean of each safety measurement. If necessary we will compare the proportions of safety outcome using Chi-square test or difference in means of outcome (e.g., lymphocyte counts) using t-test between two groups.

Secondary outcomes will be analyzed by reporting descriptive statistics in placebo and DMF treated groups, including proportions or means. We will compare the difference in proportions as well as its 95% confidence intervals using Chi-square test or difference in means as well as its confidence intervals between DMF and Placebo groups.

14. ETHICAL REQUIREMENTS

The Principal Investigator must comply with all instructions, regulations, and agreements in this protocol and applicable International Conference on Harmonisation (ICH) guidelines and conduct the study according to local regulations. The subject's privacy; physical, mental, and social integrity; and the confidentiality of his or her personal information will be strictly respected in accordance with the World Medical Association Declaration of Helsinki.

14.1. Ethics Committee

The Principal Investigator must obtain ethics committee approval of the protocol, ICF, and other required study documents prior to starting the study.

It is the responsibility of the Investigator(s) to ensure that all aspects of institutional review are conducted in accordance with current governmental regulations.

Biogen must receive a letter documenting ethics committee approval, which specifically identifies the protocol, tracking number, and ICF version, prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the ethics committee at required intervals and not less than annually. At the completion or termination of the study, the investigational site must submit a close-out letter to the ethics committee.

14.2. Subject Information and Consent

Prior to performing any study-related activities under this protocol, including screening tests and assessments, written informed consent with the approved ICF must be obtained from the subject or subject's legally authorized representative, as applicable, in accordance with local practice and regulations. Per regulation a physician (PI or CO-I) must obtain informed consent.

The background of the proposed study, the procedures, the benefits and risks of the study, and that study participation is voluntary for the subject must be explained to the subject (or the subject's legally authorized representative). The subject must be given sufficient time to consider whether to participate in the study.

A copy of the signed and dated ICF must be given to the subject or the subject's legally authorized representative. Confirmation of a subject's informed consent must also be documented in the subject's research record prior to any testing under this protocol, including screening tests and assessments.

The signed ICF will be retained with the study records. Local regulations must be complied with in respect to the final disposition of the original (wet signature) and copies of the signed and dated ICFs.

14.3. Subject Data Protection

Prior to any testing under this protocol, including screening tests and assessments, candidates must also provide all authorizations required by local law (e.g., PHI authorization in North America).

The subject will not be identified by name in the CRF or in any study reports, and these reports will be used for research purposes only. Ethics committees and various government health agencies may inspect the records of this study. Every effort will be made to keep the subject's personal medical data confidential.

14.4. Compensation for Injury

The Principal Investigator must maintain appropriate insurance coverage for clinical trials and follow applicable local compensation laws.

14.5. Conflict of Interest

Throughout the course of the study, any real or perceived COI will be discussed at all monitoring meetings. If applicable, potential COI's will be reported to the HRPO COI office as soon as they are discovered. Any potential conflicts of interest will be disclosed to the potential participant before the subject makes a decision to participate in the study. If any new COI concerns occur during the course of the study, they will be fully disclosed to all participants.

14.6. Registration of Study and Disclosure of Study Results

The Principal Investigator will register the study and post study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations (i.e. Clinicaltrials.gov, PubMed)

15. ADMINISTRATIVE PROCEDURES

15.1. Study Site Initiation

Investigators must not screen or enroll any subjects into the study prior to all prerequisite study document completion and agreement by the Principal Investigator and Biogen.

15.2. Study Funding

This study is funded by NIAMS. Biogen will be supplying the drug/placebo. All financial details are provided in the separate contract(s) between NIAMS, the institution, Principal Investigator, and Biogen.

15.3. Study Completion

Participant notification: The Principal Investigator and study staff will send a letter to all participants to thank them for their participation and to notify them that the study is over, inform them of the treatment group they were in during the first 3 months and we will ask whether they would like to be informed of the results. This will be by return postcard and upon publication of the findings the participants will be sent a lay summary and the abstract from the publication.

Close-out activities

- Verification that study procedures have been completed, data have been collected, and study intervention(s) and supplies are returned to the pharmacist and discarded as per guidance from Biogen.
- Completion of all data queries
- Correspondence and study files will be stored and accessible for external audits
- Study records will all be maintained at each participating site.
- All IRBs will be notified of the study's completion
- Preparation of a final report for the DSMB and NIAMS

All records will be maintained at each participating site for 3 years per NIH guidelines for a grant-funded study.

The ethics committee must be notified of completion or termination of the protocol. Within 3 months of protocol completion or termination, the investigator must provide a final clinical summary report to the ethics committee. The Principal Investigator will maintain an accurate and complete record of all submissions made to the ethics committee, including a list of all reports and documents submitted.

15.4. Publications

Details are included in the clinical trial agreement for this study.

16. FURTHER REQUIREMENTS AND GENERAL INFORMATION

16.1.1. Electronic Data Capture

Subject information will be captured and managed by study sites on electronic CRFs by RDMS developed and supported by University of Pittsburgh, Arthritis and Autoimmunity Center.

16.1.2. Local Laboratories for Laboratory Assessments

Local laboratories for each site will analyze all hematology, blood chemistry, urine samples collected for safety laboratory assessments for this study.

16.2. Data Safety Monitoring Board

The NIAMS will appoint a 4-5 member Data and Safety Monitoring Board (DSMB). The board acts in an advisory capacity to the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH) to review participant safety and progress. Routine meetings of the DSMB are generally held two times a year (or at other intervals determined by the DSMB), in-person or via conference call. To maintain the independent nature of the DSMB, the PI and study staff should only communicate DSMB-related requests, questions or concerns through the NIAMS or KAI Research the Executive Secretary (ES). The DSMB members should only communicate with the PI through the ES and the NIAMS

16.3. Changes to Final Study Protocol

All protocol amendments must be submitted to the ethics committee and Regulatory Authorities as required by local law. Protocol modifications that affect subject safety, the scope of the investigation, or the scientific quality of the study must be approved by the ethics committee before implementation of such modifications to the conduct of the study. If required by local law, such modifications must also be approved by the appropriate regulatory agency prior to implementation.

In the event of a protocol modification, the subject consent form may require similar modifications (see Sections 14.1 and 14.2).

It is the responsibility of the Principal Investigator to submit all revisions to the protocol to Biogen before submitting to their ethics committee.

16.4. Ethics Committee Notification of Study Completion or Termination

Where required, the Health Authorities and ethics committees must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

16.5. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations.

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