Phase II Investigation of Antimycobacterial Therapy on Progressive, Pulmonary Sarcoidosis

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Investigation of the Efficacy of Antimycobacterial Therapy on Pulmonary Sarcoidosis Phase II Randomized, Double-blind, Placebo-controlled Trial

CLEAR: **C**oncomitant **L**evaquin, **E**thambutol, **A**zithromycin, **R**ifamycin

Protocol Version 10.0

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[redacted]

Abbreviations

6MWT:	Six Minute Walk Test
AE:	Adverse Event
ALT:	Alanine transaminase
AMC:	Albany Medical Center
AST:	Aspartate Aminotransferase
ATS:	American Thoracic Society
AUC:	Area under the curve
BAL:	Bronchoalveolar lavage
BUN:	Blood urea nitrogen
CBC:	Complete Blood Count
CC:	Cleveland Clinic
CCC:	Clinical Coordinating Center
CMP:	Complete Metabolic Profile
CLEAR:	Concomitant Levaquin, Ethambutol, Azithromycin and Rifamycin
CT:	Computed tomography, CT scan
DCC:	Data Coordinating Center (unblinded biostatistician)
DLCO:	Carbon monoxide diffusing capacity
DNA:	Deoxyribonucleic acid
DSMB:	Data and Safety Monitoring Board
ERS:	European Respiratory Society
ESAT-6	Early secretory target mycobacterium tuberculosis, mycobacterium antigen
FAS:	Fatigue Assessment Score
FVC:	Forced Vital Capacity
IDSA:	Infectious Diseases Society of America
IgG:	Immunoglobulin
IV:	Intravenous
KSQ:	King Sarcoidosis Questionnaire
MTB:	M. Tuberculosis Complex
MUSC:	Medical University of South Carolina
Ng:	Nanogram
OSU	Ohio State University
Pd1:	Programmed cell death 1
PBMC:	Peripheral blood moncuclear cell
PFT:	Pulmonary function test
PO:	By mouth or orally
PPD:	TB skin test, purified protein derivative
QD:	Every day
RNA:	Ribonucleic acid
SAE:	Serious Adverse Event
SD:	Standard Deviation
SGRQ:	St. George's Respiratory Questionnaire
UC:	University Cincinnati
ULN:	Upper limit of normal
UP:	Unanticipated Problem
VU:	Vanderbilt University
WASOG:	World Association of Sarcoidosis and Other Granulomatous Disorders

Protocol Synopsis

Primary Objective: To assess the efficacy and safety of oral CLEAR therapy in patients with confirmed progressive pulmonary sarcoidosis.

Hypothesis: The CLEAR regimen will improve the absolute FVC percent predicted in chronic pulmonary sarcoidosis participants by augmenting T cell responses through the normalization of p56Lck expression and IL-2 production.

Study Design:

- Multi-center, prospective, double-blind, randomized, placebo-controlled clinical trial.
- A maximum of 128 patients will be randomized, 64 in each arm.
- Participants will be randomized at a ratio of 1:1 (stratifying on use of prednisone and by site) to receive either CLEAR therapy or identical-appearing placebo, in addition to the participant's standard care therapy.
- Treatment will continue for a maximum of 112 days.

Inclusion Criteria:

1. Patients with sarcoidosis as defined by the ATS/ERS/WASOG statement on sarcoidosis as defined by the clinical presentation consistent with sarcoidosis, as well as biopsy demonstrating granulomas, and no alternative for the cause of the granulomas, such as tuberculosis for at least one year prior to randomization.
2. Evidence of disease progression as defined by at least one of the following three criteria:
   a) Decline of absolute percent predicted of FVC (FVC ≥45% or higher of predicted value) or DLCO of at least 5% on serial measurements (DLCO range >35%, if measured);
   b) Radiographic progression in chest imaging on side by side comparison;
   c) Change in dyspnea score, as measured by Transition Dyspnea Index (TDI);
3. Positive peripheral immune responses to ESAT-6 as a biomarker of response to CLEAR regimen.
4. Possess evidence of parenchymal or nodal disease on chest radiograph.

Exclusion Criteria:

1. Inability to obtain consent
2. Age less than 18 years
3. Female participants of childbearing potential not willing to use one of the following methods of birth control for the duration of the study and 90 days after study completion: condoms, sponge, foams, jellies, diaphragm, non-hormonal intrauterine device, a vasectomized sole partner or abstinence. **Note: Oral contraceptive pills are not effective birth control when taking rifamycin.** A negative urine pregnancy test at screening visit if female of childbearing potential
4. FVC predicted value is < 45%.
5. End-stage fibrotic pulmonary disease.
6. Significant underlying liver disease.
7. Allergy or intolerance to any of the antibiotics within the CLEAR regimen.
8. Allergy or intolerance to albuterol
9. Poor venous access for obtaining blood samples
10. History of active tuberculosis, close contact with a person with active tuberculosis within the 6 months prior to the screening visit or has a positive PPD.
11. Significant disorder, other than sarcoidosis, that would complicate the treatment evaluation, (such as respiratory, cardiac, neurologic, musculoskeletal or seizure disorders)
12. Use of an investigational drug within 30 days prior to screening or within 5 half-lives of the agent, whichever is longer.

13. Currently receiving >40mg prednisone.
14. ALT or AST >5 times upper limit of normal (ULN)
15. Leukopenia, as defined by WBC <3.0 cells/mm^3 or absolute neutrophil count <1000
16. Breast feeding.
17. Color perception impairment as defined by the inability to differentiate colors per personal history or history of optic neuritis from any cause, including from sarcoidosis.
18. If patient is on immunomodulators, they must be on regimen for ≥ 3-month period and on a stable dose for > 4 weeks.
19. Family or personal history of long QT interval
20. Most recent nuclear medicine scan or echocardiogram (if done), demonstrating cardiac ejection fraction <35%
21. Participant has persistent or active infection(s) requiring hospitalization or treatment with antibiotics, antiretrovirals, or antifungals within 30 days prior to baseline. Minocycline and doxycycline are not considered antibiotics when used to treat sarcoidosis.
22. Any significant finding in the patient's medical history or physical or psychiatric exam prior to or after randomization that, in the opinion of the investigator, would affect patient safety or compliance or ability to deliver the study drug according to protocol.
23. On medications that, in the opinion of the investigator, would affect patient safety when taken with the antibiotics of the CLEAR regimen
24. History of or receiving treatment for pulmonary hypertension. Receiving biologic medication within the 6 months prior to screening visit

Primary Endpoint

- Determine the effect of CLEAR therapy versus placebo on the change in percent predicted absolute forced vital capacity (FVC) in participants with pulmonary sarcoidosis, comparing baseline with performance after completion of 16 weeks of therapy.

Sample Size:

A maximum of 128 participants will be randomized into one of two arms: CLEAR therapy or Placebo. We need a sample size of 51 completed participants per arm to have 90% power to detect a 5% difference (5% in CLEAR vs 0% in placebo) in change of FVC percent predicted from baseline. To factor in a 20% drop-out rate, we plan to enroll 64 participants per arm.

Safety Monitoring:

The DSMB will receive a safety analysis once 15 patients have been enrolled. We also plan to conduct 1 interim analysis, after 50 participants have completed the study. Interim analysis will be provided to the DSMB by the independent DSMB statistician or his/her designee. The safety monitoring plan is described fully in the Safety Monitoring section of the protocol.

Study Design

1. Background

1.1 Introduction

Sarcoidosis is a granulomatous disease of world-wide prevalence, most commonly involving the lung, skin, lymph node and eyes [1]. Diseases such as tuberculosis and chronic beryllium disease illustrate that granulomatous inflammation may or may not have an infectious etiology. Recent investigations of sarcoidosis specimens suggest that infectious agents, specifically mycobacteria, have a role in sarcoidosis pathogenesis. Independent laboratories from around the world have reported molecular and immunologic evidence supporting a significant association between mycobacteria and sarcoidosis.

Previously, we reported evidence of mycobacterial 16S rRNA or RNA polymerase B in 60% of the sarcoidosis granulomas and in none of the controls ($p<0.00002$, chi square) [2]. Sequence analysis of the 16S rRNA and *rpoB* amplicons revealed the presence of a novel *Mycobacterium*, genetically most similar to *M. tuberculosis* complex (MTB) (99% positional identity). Song et al noted IgG antibodies to recombinant MTB katG in sera from 48% of sarcoidosis patients compared to 0% in sera from PPD negative controls ($p=0.0059$). Using matrix-assisted laser desorption/ionization time of flight mass spectrometry, they found MTB katG peptides in 75% of sarcoidosis specimens compared to 14% of control specimens ($p=0.0006$); in situ hybridization localized MTB katG and 16S rRNA DNA to the inside the sarcoidosis granuloma [3]. Analysis of Polish sarcoidosis lymph nodes revealed MTB complex heat shock protein (hsp) 70, hsp65, and hsp16 [3]. We were the first to report Th-1 immune responses to mycobacterial virulence factors, ESAT-6, katG, Ag85A, and superoxide dismutase A (sodA) in sarcoidosis peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) [4-7]. Most recently an independent group detected Th-1 immune responses to mycobacterial katG whole protein in sarcoidosis BAL among Swedish and American sarcoidosis specimens [8].

Among Japanese researchers there continues to be a quantitative difference in *Propionibacterium acnes* DNA among sarcoidosis and controls [12], suggesting there may be other bacteria capable of inducing sarcoidosis pathophysiology**.** Many P. acnes have been detected in sarcoid lymph nodes using quantitative PCR and in sarcoid granulomas by in situ hybridization. P. acnes trigger factor protein causes a cellular immune response only in sarcoid patients and induces pulmonary granulomas in mice sensitized with the protein and adjuvant, but only those with latent P. acnes infection in their lungs. Eradication of P. acnes by antibiotics prevents the development of granulomas in this experimental model[9, 10] .

Although emerging data from several independent laboratories suggest a role for mycobacteria, it is unclear whether this is a response to poorly degraded antigen, as is seen in hypersensitivity pneumonitis, or persistent, viable *Mycobacterium* species. Three clinical observations support that sarcoidosis is due to an active *Mycobacterium*: 1) Transplantation of human sarcoidosis organs leads to disease transmission between donor and recipient [11, 12]; 2) cellular immune responses against mycobacterial virulence factors are detected in sarcoidosis diagnostic BAL at presentation and are quantitatively similar to those seen in patients with active mycobacterial infection [13]; 3) the immune responses detected thus far are directed against microbial antigens that are secreted during active mycobacterial replication [14-16].

1.2 Study Rationale

The purpose of this study is to assess the efficacy of CLEAR therapy for sarcoidosis patients with progressively worsening symptoms. Given that the morbidity and mortality has proven to be significant in patients with sarcoidosis, we believe there is real opportunity for improved clinical outcomes if the right interventional agent can be identified. In choosing change in absolve FVC as the primary outcome;

we will be able to detect changes in clinical outcomes that are important to patients and to the scientific community. We anticipate that these improvements will reduce or resolve the necessity of immunosuppressant therapy in these participants.

1.3 Proposed Therapeutic Intervention and Mechanism of Action

In studies conducted at Vanderbilt, mycobacterial DNA, as well as immune responses to mycobacterial virulence factors were detected in participants with pulmonary sarcoidosis. Most recently mycobacterial RNA in sarcoidosis biopsies was detected, and viable mycobacteria were visualized within a sarcoidosis clinical specimen using reporter mycobacteriophages. In known mycobacterial infections, antibiotic regimens such as macrolides (clarithromycin or azithromycin), rifamycins, ethambutol are used[17]. Macrolides and rifampin inhibit protein synthesis by RNA dependent and DNA dependent mechanisms respectively; ethambutol inhibits mycobacterial cell wall formation by its action on mycolic acid synthesis, and quinolones inhibit DNA gyrase (bacterial topolsomerase II), an enzyme required for DNA replication, transcription, repair and recombination. New regimens using quinolones have been shown to decrease the length of therapy[18]. Both macrolides and quinolones concentrate in macrophages containing mycobacteria. The Phase I Pulmonary Trial also demonstrates improvement in T cell biologic function, as evidenced by increase in Th1 cytokine expression and proliferation, as well as reversal of T cell anergy demonstrated by decreased PD-1 expression.

1.4 Peer Reviewed Clinical Trials

Previous reports of efficacy of antimicrobials in sarcoidosis subjects are limited to case reports. To date there have been only two peer-reviewed clinical trials of antibiotic therapy in sarcoidosis patients. One demonstrated clinical benefit of antimycobacterial therapy in sarcoidosis subjects [19] ; the other reported improvement in FVC among sarcoidosis subjects completing an 8 week regimen[20].

1.5. Rationale for selecting this antibiotic regimen.

There have been numerous trials regarding the optimal medical regimen for treatment of cutaneous and pulmonary mycobacterial disease. These regimens typically involve the use of macrolides such as clarithromycin, in addition to ethambutol and rifampin[17, 18]. In addition, it has been noted that quinolones also aid in treatment of mycobacterial diseases. In January 2008, at Vanderbilt, a case study was performed in a sarcoidosis patient in hospice who had with painful cutaneous lesions. Biopsy of the lesions revealed noncaseating granulomas, which was read by the pathologist as consistent with sarcoidosis. Molecular analysis of 16 sarcoidosis specimens for mutations in mycobacterial rpoB (active site for rifampin), and DNA gyrase (levaquin) had been previously performed. No mutations that confer resistance were detected. Azithromycin was included because it concentrates in host macrophages, where we suspect viable mycobacteria reside. Ethambutol was included because of its inhibition of cell wall synthesis. The patient was started on a regimen of levaquin, ethambutol, azithromycin and rifampin. He experienced complete resolution of his lesions by six weeks, and they did not recur during the remaining six months of his life. Recently a clinical trial of this regimen in cutaneous sarcoidosis patients was conducted at Vanderbilt. The results were compelling in that macroscopic and pathologic improvement in these lesions was observed, after completion of the regimen (Drake, Vanderbilt). Vanderbilt has completed a Phase I Pulmonary Trial of the CLEAR regimen on 15 pulmonary sarcoidosis participants. We have seen clinically significant improvement in absolute forced vital capacity, six minute walk test, and perception of dyspnea (Saint George's Respiratory Questionnaire).

1.6 Regimen Dose Selection

The antibiotic dosing regimens of levaquin, ethambutol, azithromycin and rifamycin were chosen based on what have been shown to be efficacious in other mycobacterial diseases, such as tuberculosis and *M. avium* infection[21].

2. Objectives

2.1 Primary Objective

To assess the efficacy and safety of oral CLEAR therapy in patients with confirmed progressive pulmonary sarcoidosis.

2.2 Hypothesis

The CLEAR regimen will improve absolute FVC percent predicted in chronic pulmonary sarcoidosis participants by augmenting T cell responses through the normalization of p56Lck expression and IL-2 production.

3. Endpoints

3.1 Primary Endpoint

- Determine the effect of CLEAR therapy versus placebo on the change in predicted absolute forced vital capacity (FVC) in participants with pulmonary sarcoidosis, comparing baseline with performance after completion of 16 weeks of therapy.

3.2 Secondary Endpoints:

- Change in FEV1
- Radiographic improvement in sarcoidosis lung disease by frontal chest x-ray . Local investigators will score chest x-rays. This analysis correlates roentgenographic findings with physiologic parameters [22].
- Change in 6 minute walk distance, oxygen saturation and level of dyspnea.
- Change in the Saint George's Respiratory Questionnaire (SGRQ; King's Sarcoidosis Questionnaire (KSQ) for the assessment of health status; The Fatigue Assessment Scale (FAS).
- Necessity of escalating immunosuppressive therapy secondary to continued clinical deterioration, after study enrollment.
- Safety profile of regimen as evidenced by adverse events and abnormal lab values, tolerability and toxicity of the treatment regimen including comparison of reported adverse events and abnormal laboratory values compared to placebo.
- Increase in T cell biologic function through normalization of p56Lck and IL-2 expression.
- Decrease in peripheral and pulmonary T cell responses against ESAT-6 at 16 weeks compared to baseline.

4. Study Population

4.1 General Considerations

Study entry is open to males and females, age 18 years or older, of any ethnic background, who meet study eligibility criteria. The gender, ethnicity, and socioeconomic background of study participants are expected to mirror that of the population served by the study sites, and that of the population most affected by pulmonary sarcoidosis.

4.2 Special Populations

The demographic profiles of Vanderbilt show that the aggregate patient population contains representative proportions of minorities and women. Recruitment of minorities and women will be monitored by the research coordinator. If necessary, additional recruitment efforts will be made with collaborators to ensure that the aggregate patient sample contains appropriate gender and minority

subsets. Pregnant women will be excluded because of the lack of safety data for antimycobacterial therapy use during pregnancy.

This study will not enroll prisoners. However, it is possible that a participant will be incarcerated after enrollment. If an enrolled individual is incarcerated, then study medications will be stopped and the participant will be treated for pulmonary sarcoidosis according to the standards of the institution in which s/he is incarcerated. While incarcerated, individuals will not be followed in the study. When the individual is no longer incarcerated, study treatment and/or study follow-up may continue, at the discretion of the investigator.

4.3 Recruitment

Patients with pulmonary sarcoidosis will be recruited from pulmonary and dermatology clinics and may be patients who have participated in previous sarcoidosis studies. The research coordinator will screen research registry patient lists to identify potential candidates for enrollment. Permission to approach patients and/or their families will be requested by the attending physicians. Community physicians will be made aware using an IRB-approved recruitment flyer. Other recruitment methods may be used specific to each site such as ResearchMatch. Each of the participating sites have large numbers of sarcoidosis patients and have experienced no difficulty in enrolling participants in the past. All patients contacted for screening will be entered into a screening log. If the patient is not enrolled, the screening log will include information explaining why enrollment did not occur (inclusion/exclusion criteria, attending physician denial, patient refusal, lack of immune responses to ESAT-6, etc.).

4.4 Sample Size

A maximum of 128 participants will be randomized into one of two arms: CLEAR therapy or Placebo. We need a sample size of 51 completed participants per arm to have 90% power to detect a 5% difference (5% in CLEAR vs 0% in placebo) in change of FVC percent predicted from baseline. To factor in a 20% drop-out rate, we plan to enroll 64 participants per arm.

4.5 Inclusion/Exclusion Criteria

4.5.1 Inclusion Criteria

1. Patients with sarcoidosis as defined by the ATS/ERS/WASOG statement on sarcoidosis as defined by the clinical presentation consistent with sarcoidosis, as well as biopsy demonstrating granulomas, and no alternative for the cause of the granulomas, such as tuberculosis for at least one year prior to randomization. Tuberculosis must be ruled out by negative histology and culture.
2. Evidence of disease progression as defined by at least one of the following three criteria:
   a) Decline of absolute percent predicted of FVC (FVC ≥ 45% or higher of predicted value) <u>or</u> DLCO of at least 5% on serial measurements (DLCO range >35%, if measured);
   b) Radiographic progression in chest imaging on side by side comparison;
   c) Change in dyspnea score, as measured by Transition Dyspnea Index (TDI);
3. Positive peripheral immune responses to ESAT-6 as a biomarker of response to CLEAR regimen.
4. Possess evidence of parenchymal or nodal disease on chest radiograph.

4.5.2 Exclusion Criteria:

1. Inability to obtain consent
2. Age less than 18 years of age
3. Female participants of childbearing potential not willing use one of the following methods of birth control for the duration of the study and 90 days after study completion: condoms, sponge, foams, jellies, diaphragm, or non-hormonal intrauterine device, a vasectomized sole partner or abstinence. **Note: Oral contraceptive pills are <u>not</u> effective birth control when taking rifamycin.** Females of childbearing potential must have a negative urine pregnancy test at screening visit

4. FVC predicted value is < 45%.
5. End-stage fibrotic pulmonary disease
6. Significant underlying liver disease
7. Allergy or intolerance to any of the antibiotics within the CLEAR regimen.
8. Allergy or intolerance to albuterol
9. Poor venous access for obtaining blood samples
10. History of active tuberculosis, close contact with a person with active tuberculosis within the 6 months prior to the screening visit or has a positive PPD.
11. Significant disorder, other than sarcoidosis, that would complicate the treatment evaluation, such as respiratory, cardiac, neurologic, musculoskeletal or seizure disorders.
12. Use of an investigational drug within 30 days prior to screening or within 5 half-lives of the agent, whichever is longer.
13. Currently receiving >40mg prednisone.
14. ALT or AST ≥5 times upper limit of normal (ULN)
15. Leukopenia, as defined by WBC <3.0 cells/mm^3 or absolute neutrophil count <1000 mm^3
16. Breast feeding.
17. Color perception impairment as defined by the inability to differentiate colors per personal history or history of optic neuritis from any cause, including from sarcoidosis.
18. If patient is on immunomodulators, they must be on regimen for ≥3-month period and on a stable dose for ≥ 4 weeks.
19. Family or personal history of long QT interval
20. Most recent nuclear medicine scan or echocardiogram (if done), demonstrating cardiac ejection fraction <35%
21. Participant has persistent or active infections requiring hospitalization or treatment with antibiotics, antiretrovirals, or antifungals within 30 days of baseline. Minocycline and doxycycline are not considered antibiotics when used to treat sarcoidosis.
22. Any significant finding in the patient's medical history or physical or psychiatric exam prior to or after randomization that, in the opinion of the investigator, would affect patient safety or compliance or ability to deliver the study drug according to protocol.
23. On medications that, in the opinion of the investigator, would affect patient safety when taken with the antibiotics of the CLEAR regimen
24. History of or receiving treatment for pulmonary hypertension. Receiving biologic medication within the 6 months prior to screening visit

4.5.3 Rationale for exclusions

Patients less than 18 years old are excluded because we are not sure that sarcoidosis is the same syndrome in children. No cited studies to date have been performed in children. Exclusion criteria are related to exacerbation of drug allergy or possibly drug toxicity. Concomitant obstructive lung disease could hinder detection of clinical improvement. Patients who are negative for ESAT-6 immune responses will not be included because Phase I trial demonstrated minimal or no improvement in FVC with the CLEAR regimen. Ethambutol is associated with optic neuropathy including optic neuritis or retrobulbar neuritis occurring in association with Ethambutol therapy may be characterized by one or more of the following events: decreased visual acuity, scotoma, color blindness, and/or visual defect. Optic neuropathy has not been reported in participants on therapy <90 days. Levaquin can prolong the QT interval or induce Torsades de Pointe. Other exclusions are related either to safety or complexity and potential for interfering with assessing a response to CLEAR therapy.

5. Study Design

This is a multi-center, randomized, double-blind, placebo controlled, prospective investigation of the efficacy of antimycobacterial therapy in pulmonary sarcoidosis, using **C**oncomitant **L**evaquin, **E**thambutol, **A**zithromycin and **Rifamycin* (CLEAR)**. The objective is to assess the efficacy and safety of oral CLEAR therapy in participants with clinically active pulmonary sarcoidosis. We anticipate that

CLEAR therapy will increase pulmonary function as evidence in by an increase in the absolute forced vital capacity. We anticipate that these improvements will reduce or resolve the necessity of immunosuppressant therapy in these participants.

* Either Rifampin or Rifabutin, but not both, will be administered based on whether the patient is on other medications that are metabolized by the cytochrome P450 pathway. Rifampin is a potent inducer of cytochrome P450; rifabutin is a potent rifamycin antibiotic, but has much less induction of cytochrome P450. Rifabutin should be used if rifampin drug interactions are possible. If the patient is on cytochrome P450 metabolized drugs the patient will receive Rifabutin. If not, the patient may receive Rifampin or Rifabutin. See **Appendix A** for known drug interactions with Rifampin or **Appendix B** for known drug interactions with Rifabutin. If the drug in question is not listed, please use Lexi-Comp software to look up compatibility or contact the CCC.

5.1 Blinding and Randomization

Participants will be randomized to either CLEAR therapy or placebo at a ratio of 1 to 1 stratifying by site and use of prednisone ≥ 10 mg or not and using a permuted-block randomization algorithm with random block sizes. Randomization schedules will be generated by a statistician who is not involved with the conduct of the clinical trial. The Vanderbilt Investigational Pharmacy will provide each site with the study drugs and site-specific treatment assignments. The placebos and colored capsules will be obtained by each site. Pills of identical appearance containing antibiotics (each a different color) or placebo will be prepared and dispensed by each site's study pharmacy. A participant's treatment assignment will only be revealed outside the pharmacy if a clinical emergency necessitates unblinding their treatment or if requested by the DSMB. Neither participants, clinical providers, nor those influencing recruitment decisions or interpretation of data will be aware of the treatment that is assigned or the order that treatments will be allocated.

Patients will be considered enrolled upon randomization. Patients receiving any amount of study drug will be considered treated. Patients discontinued from receipt of study drug for any reason, will continue to follow all other protocol requirements for the entire 180 day study period to the extent that the informed consent is still active.

Only the investigational pharmacists and the DSMB will be unblinded to randomization assignments. There are safety issues associated the CLEAR regimen; for example, levaquin and prolongation of the QT interval. If there is a significant safety issue thought related to study drug, all four study drugs within the regimen should be stopped and the patient followed until stabilization or resolution of this issue. Efforts will be made to unblind only individuals not associated with the research and who need to know, such as an attending physician. Should un-blinding of an individual become necessary during the study, the investigator at the clinical site should contact the Vanderbilt Clinical Coordinating Center (CCC) for approval and specific unblinding instructions.

Matching placebo will be provided for each of the CLEAR therapies. At the site, only the unblinded pharmacist should know the randomization assignment for an individual patient. Riboflavin will be added to the placebos for Rifampin and Rifabutin to assure participants are not unblinded due to lack of discoloration of urine, tears.

5.2 Study Drug Information

5.2.1 Levaquin

The mechanism of action of levofloxacin and other fluoroquinolone antimicrobials involves inhibition of DNA gyrase (bacterial topolsomerase II), an enzyme required for DNA replication, transcription, repair and recombination. Levofloxacin is rapidly and essentially completely absorbed after oral administration. Peak plasma concentrations are usually attained one to two hours after oral dosing. The absolute

bioavailability of a 500 mg tablet and a 750 mg tablet of levofloxacin are both approximately 99%, demonstrating complete oral absorption of levofloxacin. Following a single intravenous dose of levofloxacin to healthy volunteers, the mean ±SD peak plasma concentration attained was 6.2 ±1.0 µg/mL after a 500 mg dose infused over 60 minutes and 11.5 ±4.0 µg/mL after a 750 mg dose infused over 90 minutes. Levofloxacin oral solution and tablet formulations are bioequivalent[23].
Levofloxacin pharmacokinetics are linear and predictable after single and multiple oral or i.v. dosing regimens. Steady-state conditions are reached within 48 hours following a 500 mg or 750 mg once-daily dosage regimen. The mean ±SD peak and trough plasma concentrations attained following multiple once-daily oral dosage regimens were approximately 5.7 ±1.4 and 0.5 ±0.2 µg/mL after the 500 mg doses, and 8.6 ±1.9 and 1.1 ±0.4 µg/mL after the 750 mg doses, respectively. The mean ±SD peak and trough plasma concentrations attained following multiple once-daily i.v. regimens were approximately 6.4 ±0.8 and 0.6 ±0.2 µg/mL after the 500 mg doses, and 12.1 ±4.1 and 1.3 ±0.71 µg/mL after the 750 mg doses, respectively.
Oral administration of 500 mg levaquin with food prolongs the time to peak concentration by approximately 1 hour and decreases the peak concentration by approximately 14% following tablet and approximately 25% following oral solution administration. Therefore, levaquin tablets can be administered without regard to food.

Adverse Drug reactions for Levaquin

Common (≥3%): in US clinical trials were nausea, headache, diarrhea, insomnia, constipation, and dizziness. An increased chance of problems with the joints and tissues around the joints has been observed in pediatric patients receiving levaquin and the safety in pediatric patients treated for more than 14 days has not been studied.

Uncommon (<3%): tear of a tendon, increased risk of tendinitis, tendon rupture in all ages, allergic reaction, liver function problems, nervous system effects (tremors, anxiety, lightheadedness, depression, insomnia (trouble sleeping), diarrhea, nerve problems such as numbness or tingling in your hands/feet, abnormal heart rhythms, changes in your blood sugar levels, sensitivity to light, nausea, headache, constipation and dizziness.

Rare: (<1%): prolonged the QT interval or induced Torsades de Pointes (it should not be used in patients with known prolonged QT intervals or a family history of such), increased risk of tendinitis and tendon rupture in all ages. This risk is further increased in older patients usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart, or lung transplants [21], shortness of breath, abdominal pain, vomiting, upset stomach, rash, itching, suicidal thoughts, and swelling in your arms or legs, chest pain.

5.2.2 Ethambutol

Ethambutol works by obstructing the formation of cell wall. Mycolic acids attach to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall. Ethambutol diffuses into actively growing mycobacterium cells such as tubercle bacilli. Ethambutol appears to inhibit the synthesis of one or more metabolites, thus causing impairment of cell metabolism, arrest of multiplication, and cell death. In regards to its pharmacokinetics, Ethambutol is about 75 to 80% absorbed after an oral dose. Absorption is rapid and does not seem to be affected by food.

Following a single oral dose of 25 mg/kg of body weight, ethambutol attains a peak serum level of 2 to 5 µg/mL 2 to 4 hours after administration. No drug accumulation has been observed with consecutive single daily doses of 25 mg/kg in patients with normal kidney function, although marked accumulation has been demonstrated in patients with renal insufficiency. Serum concentrations are undetectable 24 hours after

the last dose except in some patients with abnormal renal function[24].

Ethambutol distributes widely into body fluids and tissues. Concentrations in erythrocytes may reach 2 to 3 times the plasma concentrations. It also appears in the lungs, kidneys, urine, and saliva, and to lesser extents in pleural and ascitic fluids. Ethambutol crosses the placenta. CSF concentrations reaching 10 to 50% of serum concentrations may occur with inflamed meninges. Ethambutol is not highly bound to plasma proteins. Its volume of distribution is about 1.6 L/kg.

The half-life of ethambutol is about 3 to 4 hours in patients with normal renal function; it may be as long as 7 to 8 hours in patients with renal insufficiency and 18 to 20 hours in the anephric patient.

Within 24 hours after oral administration, approximately 50% of the initial dose is excreted unchanged in the urine, while an additional 8 to 15% appears as inactive metabolites. The main metabolic path appears to be an initial oxidation of the alcohol to an aldehydic intermediate, followed by conversion to a dicarboxylic acid. From 20 to 22% of the initial dose is excreted in the feces as unchanged drug.

Adverse Drug Reactions for Ethambutol

Common (>10%): Appetite loss; disorientation; dizziness; general body discomfort; headache; nausea, stomach upset, vomiting

Uncommon (<3%): Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); chest pain or tightness; confusion; dark urine; easy bruising or bleeding; fever, chills, or sore throat; hallucinations; joint pain, swelling, or severe tenderness; lower back pain; numbness or tingling of the hands, legs, or feet; severe stomach pain; swollen glands in the neck or armpit; vision loss or other vision changes; yellowing of the skin or eyes.

Rare (<1%): decreases in visual acuity, including irreversible blindness, which appear to be due to optic neuritis. Optic neuropathy including optic neuritis or retrobulbar neuritis occurring in association with ethambutol therapy may be characterized by one or more of the following events: decreased visual acuity, scotoma, color blindness, and/or visual defect. These events have also been reported in the absence of a diagnosis of optic or retrobulbar neuritis. Optic neuropathy has not been reported in participants on therapy <90 days.

5.2.3Azithromycin

Macrolides inhibit RNA-dependent protein synthesis by reversibly binding to the 50 S ribosomal subunits of susceptible microorganisms. Its pharmacokinetics are as follows: The bioavailability of azithromycin after oral administration is approximately 40%. Administration with food, particularly a high fat meal, increases the maximum concentration, but does not affect the overall absorption of the drug. In a study of adults receiving a standard regimen of 500 mg on day 1 followed by 250 mg on days 2-5, the maximum concentration was 0.24 mcg/ml, with a time to achieve maximum concentration of 3.2 hours and an average area under the concentration curve (AUC) of 2.1 mcg·hr/ml. Azithromycin is widely distributed throughout the body, with a volume of distribution in adults of 31.1 L/kg. Protein binding is concentration dependent, ranging from 7 to 51%. Azithromycin penetrates well into the lungs, tonsils, and middle ear fluid, with concentrations exceeding those in the blood. Although azithromycin has extensive tissue distribution, only minimal concentrations have been measured in cerebrospinal fluid. The primary route of elimination of azithromycin is through biliary excretion, as unchanged drug. Only 6-14% of a dose is excreted unchanged in the urine. Azithromycin has an apparent clearance of 630 ml/min in adults, with a terminal elimination half-life of 68 hours[25].

Adverse Drug Reactions for Azithromycin

Common (>10%): Diarrhea; headache; loose stools; nausea; stomach pain; upset stomach; vomiting.

Uncommon (<3%): Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue; unusual hoarseness); bloody stools; changes in hearing or hearing loss; chest pain; eye or vision problems; irregular heartbeat; muscle weakness; pounding in the chest; red, swollen, blistered, or peeling skin; ringing in the ears; seizure; severe diarrhea; stomach cramps/pain; trouble speaking or swallowing; yellowing of the skin or eyes, decreased hemoglobin, hematocrit, lymphocytes, neutrophils and blood glucose; elevated serum creatinine phosphokinase, potassium, ALT, GGT, AST, BUN, creatinine, blood glucose, platelet count, lymphocytes, neutrophils and eosinophils;

Rare (<1%): leukopenia, neutropenia, decreased sodium, potassium, platelet count, elevated monocytes, basophils, bicarbonate, serum alkaline phosphatase, bilirubin, LDH and phosphate. The majority of participants with elevated serum creatinine also had abnormal values at baseline.

5.2.4 Rifamycins (Rifampin or Rifabutin)

5.2.4a Rifampin

Rifampin inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme. Rifampin is readily absorbed from the gastrointestinal tract. Peak serum concentrations in healthy adults and pediatric populations vary widely from individual to individual. Following a single 600 mg oral dose of rifampin in healthy adults, the peak serum concentration averages 7 mcg/mL but may vary from 4 to 32 mcg/mL. Absorption of rifampin is reduced by about 30% when the drug is ingested with food.
Rifampin is widely distributed throughout the body. It is present in effective concentrations in many organs and body fluids, including cerebrospinal fluid. Rifampin is rapidly eliminated in the bile, and an enterohepatic circulation ensues. During this process, rifampin undergoes progressive deacetylation so that nearly all the drug in the bile is in this form in about 6 hours. This metabolite is microbiologically active. Intestinal reabsorption is reduced by deacetylation, and elimination is facilitated. Up to 30% of a dose is excreted in the urine, with about half of this being unchanged drug. Rifampin is about 80% protein bound. Most of the unbound fraction is not ionized and, therefore, diffuses freely into tissues.
In healthy adults, the mean biological half-life of rifampin in serum averages 3.35 ± 0.66 hours after a 600 mg oral dose, with increases up to 5.08 ± 2.45 hours reported after a 900 mg dose. With repeated administration, the half-life decreases and reaches average values of approximately 2 to 3 hours. The half-life does not differ in patients with renal failure at doses not exceeding 600 mg daily, and consequently, no dosage adjustment is required. Following a single 900 mg oral dose of rifampin in patients with varying degrees of renal insufficiency, the mean half-life increased from 3.6 hours in healthy adults to 5.0, 7.3, and 11.0 hours in patients with glomerular filtration rates of 30 to 50 mL/min, less than 30 mL/min, and in anuric patients, respectively.

5.2.4b Rifabutin

Rifabutin is an antimycobacterial agent. It is a semisynthetic ansamycin antibiotic derived from rifamycin S. Mycobutin capsules for oral administration contain 150 mg of rifabutin per capsule, along with the inactive ingredients microcrystalline cellulose, magnesium stearate, red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Following a single oral dose of 300 mg to nine healthy adult volunteers, rifabutin was readily absorbed from the gastrointestinal tract with mean (±SD) peak plasma levels (Cmax) of 375 (±267) ng/ml (range: 141-1033 ng/ml) attained in 3.3 (±0.9) hours (Tmax range: 2-4 hours). Plasma concentrations post-Cmax declined in an apparent biphasic manner. Kinetic dose-proportionality has been established over the 300-600 mg dose range in nine healthy adult volunteers (crossover design) and in 16 early symptomatic human immunodeficiency virus (HIV)-positive patients over a 300-900 mg dose range. Rifabutin was slowly eliminated from plasma in seven healthy adult volunteers, presumably because of distribution-limited elimination, with a mean terminal half-life of 45 (±17) hours (range: 16-69 hours). Although the systemic levels of rifabutin following multiple dosing decreased by 38%, its terminal half-life remained unchanged. Rifabutin, due to its high

lipophilicity, demonstrates a high propensity for distribution and intracellular tissue uptake. Estimates of apparent steady-state distribution volume (9.3±1.5 L/kg) in five HIV-positive patients, following IV dosing, exceed total body water by approximately fifteenfold. Substantially higher intracellular tissue levels than those seen in plasma have been observed in both rat and man. The lung to plasma concentration ratio, obtained at 12 hours, was found to be approximately 6.5 in four surgical patients administered an oral dose. Mean rifabutin steady-state trough levels (Cp,minss; 24-hour post-dose) ranged from 50 to 65 ng/ml in HIV-positive patients and in healthy adult volunteers. About 85% of the drug is bound in a concentration-independent manner to plasma proteins over a concentration range of 0.05-1 μg/ml. Binding does not appear to be influenced by renal or hepatic dysfunction[26].

Because treatment with rifabutin may be associated with neutropenia, and more rarely thrombocytopenia, physicians should consider obtaining hematologic studies periodically in patients receiving rifabutin prophylaxis. Urine, feces, saliva, sputum, perspiration, tears, and skin may be colored brown-orange with rifabutin and some of its metabolites. Soft contact lenses may be permanently stained. Patients to be treated with rifabutin should be made aware of these possibilities.

Because of the structural similarity of rifabutin and rifampin, rifabutin may be expected to have some effect on these drugs as well. However, unlike rifampin, rifabutin appears not to affect the acetylation of isoniazid. When rifabutin was compared with rifampin in a study with 8 healthy normal volunteers, rifabutin appeared to be a less potent enzyme inducer than rifampin.

Adverse Drug Reaction for Rifamycins (Rifampin or Rifabutin)
Common (>10%): tired feeling; or red or orange colored urine, stools, tears, sweat, or saliva

Uncommon (<3%): Allergic reaction (hives; difficulty breathing; swelling of your face, lips, tongue, or throat), fever, chills, body aches, flu symptoms, joint pain or swelling, easy bruising or bleeding, weakness, urinating less than usual or not at all, nausea, stomach pain, loss of appetite, itching, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes), neutropenia (a low white blood cell count that may make it more likely for you to get infections).

Rare (<1%): Rifampin has been shown to produce liver dysfunction. Fatalities associated with jaundice have occurred in patients with liver disease and in patients taking rifampin with other hepatotoxic agents. Patients with impaired liver function should be given rifampin only in cases of necessity and then with caution and under strict medical supervision. In these patients, careful monitoring of liver function, especially SGPT/ALT and SGOT/AST should be carried out prior to therapy and then every 2 to 4 weeks during therapy. If signs of hepatocellular damage occur, rifampin should be withdrawn. Hematological side effects have included thrombocytopenia, leukopenia, hemolytic anemia, and decreased hemoglobin, in less than 1% of patients. Hyperbilirubinemia and hepatitis have been reported in up to 3% of patients. Approximately 50% of hepatotoxicity has been observed during the first month of therapy.

5.3 Study Drug Dosing and Administration:

The following oral CLEAR medications or matching placebo will be introduced as follows:

Day 0 Start: Azithromycin: 250mg po QD (or 500 3 days/week if toxicity) x 8 weeks *and*
Ethambutol: 1200 mg po QD for ≥50kg; <50kg 800 mg po QD (or 3 days/week if toxicity) x 8 weeks
Rifampin 600 mg QD or Rifabutin 300mg po QD (or 3 days/week if toxicity) x 16 weeks
Levaquin: 500 mg po QD (or 3 days/week if toxicity) x 16 weeks

Participants will be allowed to take their regimen three times/week instead of daily (which has been shown to be equally efficacious) if toxicity occurs that is thought to be drug related. In addition, if toxicity warrants, one or more drugs can be discontinued and the participant can continue to participate.

The site PI may also elect to stagger the start of study drugs to help identify which drugs may be eliciting which side effects. The study drugs would be introduced starting with Azithromycin and Ethambutol at the baseline visit (day 0) and introducing Rifampin (or Rifabutin) the following week (day 7), and Levaquin one week later (day 14) using the same dosages as above.

5.4 Interruptions in Study Drug Administration for Toxicity

Due to the large pill burden of seven capsules a day, participants may have toxicities such as nausea. If nausea persists, it is acceptable for a participant to take up to a one-time five day drug holiday while the nausea improves. If a drug holiday is taken, the drug schedule and subsequent visit schedule should be adjusted such that the full sixteen week course is completed.

An expected side effect of Levaquin is extreme joint pain and the investigator should work closely with their primary care physician to manage participant's pain if possible, so the drug does not have to be discontinued.

5.4.1 Drug Toxicity During 2-drug Phase

To avoid single drug therapy during the 8 weeks of 2-drug therapy, the following order of replacement drug should be followed:

If rifamycin is stopped due to toxicity:
1st - azithromycin + levaquin
2nd – ethambutol + levaquin
3rd – only levaquin

If Levaquin is stopped due to toxicity:
1st - azithromycin + rifamycin
2nd – ethambutol + rifamycin
3rd – only rifamycin

Single drug therapy should be avoided if at all possible.

5.5 Assessment of Drug Adherence:

Participants should be asked to return the pill bottles and medication diary at each visit. Pill counts should be conducted at each visit and reconciled with quantities dispensed, and a review of medication diary to assess adherence to prescribed regimen and the onset of new symptoms should be conducted. If a participant misses a dose, they will receive guidance on making up missed dose

5.6 Discontinuation of Study Drug Administration

Treatment will continue for a maximum of 112 doses after study enrollment or 16 weeks post-randomization, whichever comes first. Treatment will be discontinued in the following circumstances:

1. Death
2. Primary care provider or participant request
3. ALT is noted to exceed ten times the upper limit of normal
4. A serious adverse event has occurred and is thought to be related to study drug and in the opinion of the investigator or attending physician warrants discontinuation of the study drug, or any intolerable or unacceptable adverse event (including clinically significant abnormal labs thought to be due to study drug) that in the opinion of the investigator or attending physician warrants discontinuation
5. Positive pregnancy test
6. Non-compliance with the regimen and timing that might result in dropping out from the study

7. Development of an intolerable AE due to study participation as determined by the Investigator, participant, or both.
8. Development of an intercurrent illness, condition, or procedural complication, which would interfere with the participant's continued participation.

Participants who have stopped taking study drugs may continue to participant in the study and will following the appropriate procedures in the protocol.

Any participant who discontinues treatment for medical reasons, e.g. because of adverse events (AEs) or clinical laboratory abnormalities, should be followed up at medically appropriate intervals in order to evaluate the course and to ensure reversibility or stabilization of the abnormality or event. If a participant fails to return for a scheduled visit, a documented effort must be made to determine the reason. This information should be recorded in the study records.

5.7 Premature Withdrawal from Study Participation

At any time, participants may withdraw from the study (i.e., withdraw consent to participate) at their own request. Participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. No disadvantage will arise for any participant who withdraws consent for participation at any time or who is withdrawn from the study by the Investigator.

Reasons for discontinuation from the study will be recorded on the appropriate page of the CRF in any case and may include the following:

- Participant's request for withdrawal
- Investigator's decision that discontinuation is in the best interest of the participant
- Participant is lost to follow-up

Participants who elect to stop treatment will be asked to complete at minimum, follow-up spirometry. Any participant who discontinues treatment for medical reasons, e.g. because of adverse events (AEs) or clinical laboratory abnormalities, should be followed up at medically appropriate intervals in order to evaluate the course and to ensure reversibility or stabilization of the abnormality or event. If a participant fails to return for a scheduled visit, a documented effort must be made to determine the reason. This information should be recorded in the study records.

Participants whose clinicians feel that an escalation in their baseline therapy is warranted will be allowed to receive up to 14 days of increased dose of prednisone.

If at the end of that time, they still require an increased dose of prednisone, they will remain in the study and data collected as outlined in the events table, however, only their last Pulmonary Function Tests collected prior to increasing the dose of prednisone will be used for analysis.

Participants will not be allowed to begin on a new immunosuppressive drug for their sarcoidosis during the course of the study. Since these drugs take six months to become effective, this does not increase the risk to the patient. If the patient wishes to start a new therapy, they will be considered a treatment failure and they will be asked to come in to complete the end of study visit, which is the same as the month 4 visit.

6. Visit Specific Procedures

The following assessments will provide the basis for assessing protocol compliance and safety as well as differences between study arms for multiple efficacy variables. Data for each of the variables will be

recorded as shown below and in the Time-Events schedule (**Appendix B**), or until death or study withdrawal.

6.1 Screening Visit 1Assessments

- Consent Participant
- Demographic and Enrollment Data
- Pertinent Medical History (including comorbidities and current medication list)
- Physical Examination (including height, weight and gender)
- Urine Pregnancy Test (if female of childbearing potential)
- Screening Labs; Complete Metabolic Panel (CMP) including: sodium, potassium, HCO3, creatinine, glucose, calcium, BUN, total bilirubin, total protein, ALT, AST*
- Complete Blood Cell Count including: WBC, RBC, hemoglobin, hematocrit and platelet count*
- Immune responses against mycobacterial ESAT-6 (T cell recognition assay)
  *If this testing was done within the 14 days prior to this visit, these results may be used for this visit.

A screening log will be maintained for those potential participants who are contacted for screening in this study. This log will include the reason if the patient is not enrolled. Data entry into REDCap of screening log data will not include patient identifiers. Clinical sites will maintain the screening log in their research files.

6.2 Screening Visit 2 Assessments

- Spirometry (FVC, FEV1) and DLCO if possible
- Chest X ray*
- Six Minute Walk Test (6MWT)**, including Borg Dyspnea Score and oxygen saturation (there should be two 6MWTs performed at least one hour apart to confirm reproducibility of baseline assessments) (Exception: if participant is experienced with the 6MWT only 1 is necessary at baseline.)
- St. George's Respiratory Questionnaire
- King's Sarcoidosis Questionnaire
- Fatigue Assessment Score (FAS)

*If CXR was done within the 30 days prior to this visit, this result may be used for this visit.
**There should be two tests performed at least one hour apart to confirm reproducibility of baseline assessments.

6.3 Baseline Visit Assessments

After confirming eligibility (ESAT-6 eligible and inclusion and exclusion criteria are met) **and** prior to dosing, the following procedures and assessments will be performed:

- Bronchoscopy with bronchoalveolar lavage (BAL) for T cell study*
- Randomization (Site Pharmacy)

*The bronchoscopy should be conducted after the 6MWT to avoid any confounders of outcome. Site-specific routine bronchoscopy procedures, such as sedation and lavage volume, should be followed; shipping of $\geq$ 60cc of complete BAL fluid should be shipped overnight at room temperature to the Drake lab in the sample collection tubes provided. If 60cc are not available, each site should send what is available. If the baseline bronchoscopy is associated with adverse events, inability to obtain sufficient specimen, patient refuses, a second bronchoscopy should be omitted.

The assessment weeks will be as follows:

6.4 Therapy Week 4 Visit Assessment (+/- 7 days)

Week 4 assessments should be completed as near week 4 as possible +/- 7 days.

- Physical Assessment (record any new signs, symptoms, adverse events since last visit)
- Urine pregnancy test, if female of childbearing potential
- Complete metabolic profile and complete blood count
- Blood for T cell recognition of mycobacterial virulence factors and T cell function
- Spirometry (record FVC and FEV1 data pre and post bronchodilator, if available) and DLCO (if available)
- One 6MWT, Borg Dyspnea Score and oxygen saturation
- Review of medication diary and pill count/reconciliation
- Assess for AEs and record

6.5 Week 8 Visit Assessment (+/- 7 days)

Week 8 assessments should be completed as near week 8 as possible +/- 7 days.

- Physical Assessment (record any new signs, symptoms, adverse events since last visit)
- Urine pregnancy test, if female of childbearing potential
- Complete metabolic profile and complete blood count
- Blood for T cell recognition of mycobacterial virulence factors and T cell function
- Spirometry (record FVC and FEV1 data pre and post bronchodilator, if available) and DLCO (if available)
- One 6MWT, Borg Dyspnea Score and oxygen saturation
- Review of medication diary and pill count/reconciliation
- Assess for and record AE's

6.6 Week 16 Visit Assessment (+/- 7 days)

Week 16 assessments should be completed as near week 16 as possible +/- 7 days.

- Physical Assessment (record any new signs or symptoms since last visit)
- Urine Pregnancy test, if female of childbearing potential
- Complete metabolic profile and CBC
- Blood for T cell recognition of mycobacterial virulence factors (ESAT-6) and T cell function
- Spirometry (record FVC and FEV1 data pre and post bronchodilator, if available) and DLCO (if available)
- Bronchoscopy with Bronchoalveolar Lavage (BAL) for T cell study
- One 6MWT and oxygen saturation and Borg Dyspnea Score
- St. George's Respiratory Questionnaire
- Fatigue Assessment Score (FAS)
- Kings Sarcoidosis Questionnaire
- Review of medication diary and pill count/reconciliation
- Assess for and record AE's;

6.7 Week 24 Visit Assessment (+/- 7 days)

Week 24 assessments should be completed as near week 24 as possible +/- 7 days.

- Physical Assessment (record any new signs or symptoms since last visit)
- Blood for T cell recognition of mycobacterial virulence factors (ESAT-6) and T cell function
- Spirometry (record FVC and FEV1 data pre and post bronchodilator, if available) and DLCO (if available)
- One 6MWT and oxygen saturation and Borg Dyspnea Score
- Assess for and record AE's

This visit should be at least 168 days following the date of the first dose of study drug, but can be later, if contact with the participant is intermittently lost to follow-up. Document all attempts to contact the participant in the case report form. Document the date of last contact.

6.8 Assessments for Data Collection

Data will be collected to assess the following endpoints:

- FVC percent predicted, FVC, FEV1 at baseline, week 4, week 8, and week 16
- Radiographic assessment of sarcoidosis lung disease using the Muer scoring system at baseline, week 4, week 8, and week 16.
- Change in 6 minute walk distance and oxygen saturation from baseline to 16 weeks.
- Change in questionnaires from baseline to 16 weeks.
- Decrease in peripheral and pulmonary T cell response to mycobacterial antigens between baseline and 16 weeks, as well as T cell function assays.
- Adverse events, assessments and laboratory results for safety analysis.
- Standard of care therapies.

7. Statistical Considerations

7.1 Statistical Methods

7.1.1 Analysis of Primary Endpoint

The comparison between CLEAR and placebo arms will be made using the Students's t-test. Normality of the primary endpoint will be examined using Kolmogorov-Smirnov test. If normality is violated, the comparison between CLEAR and placebo arms will be made using the Wilcoxon rank sum test.

The primary endpoint of this study is the arithmetic difference between %FVC predicted value measured at baseline and week 16 for each patient randomized. For analysis purposes, we define the week 16 post-randomization %FVC predicted value measurement at the value closest to 16 weeks from randomization within a window of 12 weeks to 20 weeks from randomization. This more accurately represents the data collected at 16 weeks on study due to the variability of visit schedules resulting from staggering the start of study drugs and patient scheduling. To understand the trajectory of improvement with CLEAR therapy, we will also be measuring the %FVC predicted value at weeks 4 and 8. If the improvement is linear in time, the rate of improvement (slope of change) may be reported as a secondary endpoint in the study.

This analysis is an exploratory analysis. Mixed effect models will be used to analyze these repeated measures with a random subject effect and with the treatment (CLEAR versus placebo) and the time trend (baseline, week 4, week 8, and week 16) as fixed effects. For the error covariance, we will use autoregressive model of order 1 [AR(1)] or other plausible covariance structures. The focus of this study will be the treatment effect and the time trend of the endpoint; however, mixed effect models also provide the flexibility of controlling for and evaluating covariates such age, race, and gender.

7.1.2 Approaches to the Analysis

The following 2 approaches will be used for the analyses:

- **Intention-to-Treat** All patients with a post-treatment endpoint measurement will be included. This will be considered the primary analysis and performed for all endpoints.

- **Per Protocol** This will be performed as a secondary analysis for the primary endpoint only. A patient who is identified as a major protocol violator will not be included. Protocol violators will be considered as follows: 1) Realize that patient did not meet Inclusion Criteria after enrolled into study; 2) Find an Exclusion criteria after enrolled into the study; 3) Regimen was

permanently stopped before 4 weeks; 4) Exacerbation requiring greater than 2 weeks of prednisone escalation. Major protocol violators will also be defined based on criteria that will be identified prior to unblinding the database.

7.1.3 Multiplicity: Since there is only 1 primary comparison for 1 primary endpoint for each study aim, and other comparisons are secondary and supportive, no multiplicity adjustment will be applied.

7.1.4 Analysis of Secondary Endpoints

For continuous secondary endpoints, statistical analyses will be conducted using the same methods as described for the primary endpoint. For binary endpoints such as >10% improvement on FVC, the Pearson chi-square test or Fisher's exact test will be used to assess them between the two arms. If imbalance on certain important covariates which might impact these binary endpoints is observed between the treatment arms, logistic regression to control for these covariates may be conducted.
The safety endpoint such as death, AE, SAE, drug-related AE, drug-related SAE, abnormal lab values, tolerability and toxicity of the treatment regimen, will be summarized (count and percent) by treatment arms and for the entire study sample. Between CLEAR and placebo comparison on these endpoints will be made using the Pearson chi-square test or Fisher's exact test.

7.1.4 Missing Data Analyses

Every effort will be made to avoid missing data. Still, we plan to enroll 64 subjects per arm to achieve 51 completed subjects, as described in the Sample Size Justification. For missing data, we will explore the missing data mechanism whether it is missing completely at random, missing at random, or missing not at random. We will test whether missingness on the primary endpoint depends on covariates and whether the missing data mechanism is ignorable. After a good understanding about the missing data mechanism, we will adopt two strategies to deal with missing data. The multiple imputation method that incorporates predictive mean matching and flexible additive imputation models as implemented in the *aregImpute* function in the Hmisc package (Harrell, 2013, page 12) in R will be used. We will also conservatively impute missing data to perform sensitivity analyses. Imputing strategies could be last-value-carry-forward (we believe this imputation is conservative since subjects on placebo may get worse and subjects on CLEAR may get better if they stayed in the study, and we assume a flat carry-forward) or we could assume a spontaneous improvement rate for the placebo arm estimated from the non-missing data if that is observed. The primary analysis is the intention-to-treat (ITT) analysis of the complete data set. These additional analyses that deal with missing data are secondary and they are to corroborate study findings from the primary ITT analysis.

7.2 Randomization

Participants will be randomized to either CLEAR therapy or placebo at a ratio of 1 to 1 stratifying by site and use of prednisone $\geq$ 10 mg or not and using a permuted-block randomization algorithm with random block sizes. Treatment assignments of the study will be made using separate randomization schedules for each of the three participating sites. The randomization schedule will be prepared by the DCC prior to initiation of patient recruitment. Randomization schedules will be generated by a statistician who is not involved with the conduct of the clinical trial. All randomization schedules will be generated using R, will remain confidential and known only by the site pharmacists until the database is locked and randomization assignments no longer must be blinded. Randomization marks the patient's official entry into the Study. Once a participant has been started on the assigned study treatment, efforts will be made to conduct all evaluations irrespective of whether the participants complete the study treatment regimen or not, or how well the participants complies with the study treatment regimen. These efforts should continue until termination of the study. Data collections will continue even if medication must be stopped.
For the intention to treat analysis, participants will be considered treated when the first study drug is taken, regardless of the amount of study drug they receive. Participants discontinued from receipt of

study drug for any reason, will continue to follow all other protocol requirements for the entire study period to the extent that the informed consent is still active.

7.3 Sample Size Justification

Sample size is calculated for primary endpoint; change from baseline of FVC percent predicted. Using data from 34 participants treated with Bosentan[1,2] to treat pulmonary hypertension, we obtained the SD of 7.7 for the primary endpoint. We need a sample size of 51 completed participants per arm to have 90% power to detect a 5% difference (5% in CLEAR vs. 0% in placebo) in change of FVC percent predicted from baseline. To factor in a 20% dropout rate, we plan to enroll 64 subjects per arm. This brings the sample size to 128 participants. In the Phase I trial, we experienced a higher drop rate 30%, but review of the clinical data demonstrates that patients with advanced disease accounted for the majority of the study withdrawals. The Phase II trial will allow patients to drop one study drug, and will include a research nurse coordinator to encourage study participation. We anticipate that we will have not have as high a study withdrawal rate.

7.4 Interim Monitoring

The DSMB will receive a report of adverse events (blinded by arm) after 15 participants have completed study treatment to review. This review will be conducted by the chair of the DSMB, who will recommend either that the study continue or that there be a review of the first 15 participant's data by the DSMB committee. An interim safety analysis will also be performed after 50 participants complete study drug treatment. This interim analysis will be presented to the DSMB by the PI and the study statistician or his/her designee. The results will be blinded and presented on a coded study arm basis (i.e. A and B arm) unless the DSMB votes to receive unblinded data. The interim analysis will focus on the safety profile of the study treatments. These analyses will be conducted based on the methods specified in the Data Analysis Plan for the safety endpoints. Since the interim analysis is not designed to draw formal inference on these safety endpoints, the magnitude of the between-treatment differences and the p-values will be provided solely to help the DSMB to evaluate the safety profile. Although efficacy data will also be presented, no early stopping of the trial based on the efficacy endpoints is planned for this Phase II study, because it is unlikely that an efficacy study of less than 128 subjects will have credibility with the scientific community. The DSMB is further described in Section Data Safety Monitoring Board Charter and Plan.

7.5 Futility

There will be no formal futility analysis for efficacy.

7.6 Data Safety Monitoring Board Overview

7.6.1 Data and Safety Monitoring Board (DSMB)

The committee will consist of three members, in addition to the study statistician. DSMB will be comprised of adult specialists with extensive clinical experience in sarcoidosis/ interstitial lung disease and clinical trial research experience. Members will be independent and have no conflict of interests related to the study or drug of study. The study statistician will be an ex-officio (non-voting) member of the DSMB.

7.6.2 DSMB role and responsibilities

The DSMB role and responsibilities will involve conducting interim monitoring of accumulating data from research activities to assure the continuing safety of human participants, relevance of the study question, appropriateness of the study, and integrity of the accumulating data. The committee will also identify protocol violations that suggest clarification of changes to protocol are needed; identify unexpectedly high dropout rates that threaten the trial's ability to produce credible results; ensure the

credibility of the study; ensure the validity of study results as well as to protect the safety of trial participants.

7.6.3 Confidential Reports

All reports of efficacy and futility monitoring will be blinded with regard to treatment assignment and will be reviewed in a closed session of the DSMB meeting. The DSMB will discuss unblinded results in the closed session. Safety data will not be blinded to the DSMB. Unexpected adverse events and unanticipated events will be reported and reviewed on an ongoing basis by the DSMB chair who may request input from other DSMB members if thought necessary.

7.6.4 Scheduled meetings

The first meeting of the DSMB will occur at study initiation, with the purpose of approving the statistical monitoring plan. The second DSMB meeting will occur when the statistical report on data for 50 enrolled participants is available for review. The statistical center will be monitoring the data continuously and may call a DSMB meeting at any time after 25 participants have been accrued and when warranted by the data, as described above. The calendar timing of these meetings will be participant to the overall rate of recruitment. The open portion of DSMB reports will be made available to participating sites. Discussions and actions items from the closed portion of the meeting will remain confidential. In the event of slow patient recruitment, the DSMB will meet by teleconferencing every 6 months for year one, then annually to review the safety analyses conducted by the Data Coordinating Center (DCC) and determine whether the study should be (a) allowed to continue, (b) modified, or (c) discontinued.

All severe adverse events (SAEs) will be reported to the DSMB as well as to all the IRBs involved in the study. The DSMB may choose at any time to have an additional DSMB meeting to determine if a change in protocol or informed consent is needed based on SEAs. Also, the DSMB will comment on the SAEs in their yearly report; the committee will also supervise the preparation of the interim confidential reports.

8. Data Collection and Site Monitoring

8.1 Data Collection

Investigators or research coordinators will collect data and enter it directly into the web-based data entry system managed by the Clinical Coordinating Center or record on paper data forms for later entry into REDCap, a web-based data entry system.

A screening log will be maintained for those potential participants who are contacted for screening in this study. The log will include the reason if the potential participant was not enrolled. Data entry by coordinating center personnel into REDCap of screening log data will not include private information. Clinical sites will maintain the screening log in their research files.

8.1.1 Data Collection Training

Quality data collection and appropriate study conduct will require careful attention to the training of research personnel at the Clinical Core Coordinating Center and participating sites. Training sessions for the Study Coordinators and key personnel will be held prior to the initiation of patient recruitment. The protocol, forms, and other materials will be distributed to the appropriate personnel. Each center's personnel will be trained centrally in the study requirements, standardization measurement of height, weight, phlebotomy, and requirements for laboratory specimen collection including counseling on adherence, and in eliciting of information from study participants in a reproducible manner.

Training will be performed by Clinical Coordinating Center personnel. Inclusion and exclusion criteria, data to be collected and the procedures to be conducted at each visit will be reviewed in detail. Data collection forms and the nature of the required information will also be discussed in detail. Entering data

remotely, responding to data queries and general information about obtaining research quality data will also be covered during the training session.

8.2 Site Monitoring

Sites will be monitored on a regular basis by the Clinical Coordinating Center (CCC), to ensure that all regulatory requirements are met and to monitor the quality of the data collected. Records of Institutional Review Board approvals will be faxed or sent electronically to the CCC. REDCap database automated range checking may be utilized to alert data entry personnel when data out of range is entered. Data quality assurance checks will be performed to assure the data are as accurate as possible prior to locking the database. If erroneous or inappropriately missing data are noted from any one site, the CCC will develop a corrective action plan for the site(s) involved.

9. Risk Assessment

9.1 Risks of Antimycobacterial Therapy (Levaquin, Ethambutol, Azithromycin, and Rifamycin)

Potential risks of study drugs are described below. All of the prescribed medications are well tolerated and adverse events are reports in less than 0.7% of patients. We are using the current FDA recommended doses.

9.1.1 Levaquin:

Common (≥3%): in US clinical trials were nausea, headache, diarrhea, insomnia, constipation, and dizziness. An increased chance of problems with the joints and tissues around the joints has been observed in pediatric patients receiving levaquin and the safety in pediatric patients treated for more than 14 days has not been studied.

Uncommon (<3%): tear of a tendon, increased risk of tendinitis, tendon rupture in all ages allergic reaction, liver function problems, nervous system effects (tremors, anxiety, lightheadedness, depression, insomnia (trouble sleeping),diarrhea, nerve problems such as numbness or tingling in your hands/feet, abnormal heart rhythms, changes in your blood sugar levels, sensitivity to light, nausea, headache, constipation and dizziness.

Rare (<1%): prolonged the QT interval or induced Torsades de Pointes (it should not be used in patients with known prolonged QT intervals or a family history of such), increased risk of tendinitis and tendon rupture in all ages. This risk is further increased in older patients usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart, or lung transplants [21], shortness of breath, abdominal pain, vomiting, upset stomach, rash, itching, swelling in your arms or legs, chest pain.

9.1.2 Ethambutol:

Common (>10%): Appetite loss; disorientation; dizziness; general body discomfort; headache; nausea, stomach upset, vomiting

Uncommon (<3%): Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); chest pain or tightness; confusion; dark urine; easy bruising or bleeding; fever, chills, or sore throat; hallucinations; joint pain, swelling, or severe tenderness; lower back pain; numbness or tingling of the hands, legs, or feet; severe stomach pain; swollen glands in the neck or armpit; vision loss or other vision changes; yellowing of the skin or eyes.

Rare (<1%): decreases in visual acuity, including irreversible blindness, which appear to be due to optic neuritis. Optic neuropathy including optic neuritis or retrobulbar neuritis occurring in association with ethambutol therapy may be characterized by one or more of the following events: decreased visual acuity, scotoma, color blindness, and/or visual defect. These events have also been reported in the absence of a diagnosis of optic or retrobulbar neuritis. Optic neuropathy has not been reported in participants on therapy <90 days

9.1.3 Azithromycin:

Common (>10%): Diarrhea; headache; loose stools; nausea; stomach pain; upset stomach; vomiting.

Uncommon (<3%): Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue; unusual hoarseness); bloody stools; changes in hearing or hearing loss; chest pain; eye or vision problems; irregular heartbeat; muscle weakness; pounding in the chest; red, swollen, blistered, or peeling skin; ringing in the ears; seizure; severe diarrhea; stomach cramps/pain; trouble speaking or swallowing; yellowing of the skin or eyes; decreased hemoglobin, hematocrit, lymphocytes, neutrophils and blood glucose; elevated serum creatinine phosphokinase, potassium, ALT, GGT, AST, BUN, creatinine, blood glucose, platelet count, lymphocytes, neutrophils and eosinophils;

Rare (<1%): leukopenia, neutropenia, decreased sodium, potassium, platelet count, elevated monocytes, basophils, bicarbonate, serum alkaline phosphatase, bilirubin, LDH and phosphate. The majority of participants with elevated serum creatinine also had abnormal values at baseline.

9.1.4 Rifamycins (rifampin or rifabutin):

Common (>10%): tired feeling; or red or orange colored urine, stools, tears, sweat, or saliva

Uncommon (<3%): Allergic reaction (hives; difficulty breathing; swelling of your face, lips, tongue, or throat), fever, chills, body aches, flu symptoms, joint pain or swelling, easy bruising or bleeding, weakness, urinating less than usual or not at all, nausea, stomach pain, loss of appetite, itching, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes), neutropenia (a low white blood cell count that may make it more likely for you to get infections).

Rare (<1%): Rifampin has been shown to produce liver dysfunction. Fatalities associated with jaundice have occurred in patients with liver disease and in patients taking rifampin with other hepatotoxic agents. Patients with impaired liver function should be given rifampin only in cases of necessity and then with caution and under strict medical supervision. In these patients, careful monitoring of liver function, especially SGPT/ALT and SGOT/AST should be carried out prior to therapy and then every 2 to 4 weeks during therapy. If signs of hepatocellular damage occur, rifampin should be withdrawn. Hematological side effects have included thrombocytopenia, leukopenia, hemolytic anemia, and decreased hemoglobin, in less than 1% of patients. Hyperbilirubinemia and hepatitis have been reported in up to 3% of patients. Approximately 50% of hepatotoxicity has been observed during the first month of therapy.

9.2 Risks of Phlebotomy

All patients will have blood drawn for research purposes. Risks of drawing blood are uncommon and include bleeding and bruising, and a rare risk of fainting or infection.

9.3 Risks of Bronchoscopy

Bronchoscopy is very safe, but no procedure is entirely free of potential risk. The chance of serious complications is reported to be less than 0.05% (5 in 10,000). Local discomfort (coughing, gagging, and soreness of your nose and throat) are frequently unavoidable but can be greatly diminished with medication. Nosebleed, wheezing (about 1% chance), a decrease in blood oxygen requiring that requires wearing an oxygen mask, low grade fever (less than a 5% chance), lung infection (pneumonia) in less than 0.1%, and coughing up small flecks of blood for 24 hours after the procedure (as a result of minor trauma to the bronchial lining) may occur (less than a 5% chance). Vocal Cord spasm can result in hoarseness and bronchial spasm can cause wheezing or shortness of breath. Mild bronchitis is common after bronchoscopy. Occasionally, antibiotics and/or a short course of corticosteroids are required if patients are significantly symptomatic. More serious complications such as major bleeding, lung collapse,

vocalcord and bronchial spasm, and heart problems have been reported but are very rare. It is not expected that patients will experience all of these side effects.

9.4 Minimization of Risks

Federal regulations at 45 CFR 46.111(a) (1) requires that risks to subjects are minimized by using procedures which are consistent with sound research design. There are several elements of study design in the present protocol that meets this human subject protection requirement.

Several of the exclusion criteria prohibit participation of patients who might be at increased risk from the effects of antimycobacterial therapy. For example, patients who have intolerances or allergies to any drug in this regimen, pregnancy, and who are taking medications that may unfavorably interact with a drug in the regimen are excluded. Also, we will monitor for adverse effects to liver and muscle by monitoring ALT. We will stop study medications if the ALT rises to more than 10 times ULN.

9.5 Potential Benefits

There have been no peer-reviewed studies assessing the benefits of antibiotic therapy in sarcoidosis pathogenesis. The potential benefit of this proposed study is that it may alter the natural history of this disease for which there is no cure. It may result in less immunosuppressant therapy being used for sarcoidosis patients, and potentially complete resolution of their disease.

9.6 Risks versus Benefits

The risks of the proposed regimen are minimal, especially with the rigorous exclusion criteria. The benefits include short term therapy that is much less expensive, compared to immunosuppressives which are more costly and carry greater longer term toxicities. Phase I analysis supports that this regimen may alter the natural history of the disease in patients sarcoidosis, and obviate the necessity of immunosuppressant therapy.

10. Human Subjects Protection

10.1 Institutional Review Board Involvement

The protocol and the informed consent documents to be used in this study will be submitted to the investigator's local IRB for approval. Written documentation of approval of the protocol and the informed consent documents must be provided to CCC before starting the study.
The investigator will promptly report to their local IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without prior IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

10.2 Equitable Selection of Participants

The exclusion criteria for this study neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals from participation in the research.

10.3 Informed Consent

The investigator is responsible for ensuring that the patient or patient's legally authorized representative understands the risks and benefits of participating in the study, and answering any questions the patient may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's willingness to continue his or her participation in the trial. Prior to a participant's enrollment in the study, the investigator will ensure that the purpose of the study is explained to the patient and that written consent is obtained.

All study participants will be informed of the objectives of the study and the potential risks. The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study agent.

The patient will receive a copy of his/her signed consent form. The investigator will retain a copy of the signed consent forms. The investigator will follow local guidelines for seeking informed consent from persons who may need additional protections, such as those who do not read.

10.4 Sequence of Consent Procedures

It is recognized that the Site-specific Center and participating site IRB have official responsibility for determining informed consent procedures. Prototype informed consent forms have been developed for this study. A FDA-approved IND number is not indicated for this trial.

10.3 Confidentiality

At the beginning of the study, each patient will be assigned a unique identification number and a study code. In any individual tabulation, participants will be identified only by number. The medical records of participants in CLEAR Trial will be confidential. All procedures will be in compliance with HIPAA regulations.

To maintain confidentiality, evaluation forms and reports entered into the study database will be identified only by the participant's coded number. The coded number will be generated at random by a computer, and only the study investigators will have access identity of the participant corresponding to the codes. All electronic records will be kept in a locked, password protected computer. All computer entry and networking programs will be done with coded numbers only. All source documents containing the participant's identifiable information will be maintained in a locked cabinet inside a locked office. Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the Institutional Review Board, National Heart, Lung, and Blood Institute, the Federal Drug Administration or other authorized Federal Agencies, and the Clinical Coordinating Center.

Data will be entered into REDCap, an electronic web-based database, for storage and only research staff with appropriate permissions will be allowed to access the database. Data will be automatically sequestered by site so that individuals at any institution may see their own site's data, but not data collected at other sites. Primary study coordinators will have no access to case report form data views, but will instead have rights to use a single export module designed to enable export of all site data. No identifiable information, with the exception of dates will be collected. To allow for remote monitoring, source documents containing patient identifiers may be uploaded into REDCap, a HIPAA secure, password protected web-based database.

11. Adverse Event Reporting

Investigators will determine if any clinical adverse experiences occur during the period from enrollment through study month 6 or hospital discharge or death, whichever occurs first. The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of treatment of patients with sarcoidosis. If clinically important and unexpected adverse experiences occur, they will be recorded on the adverse event case report form.

11.1 Reportable Adverse Event

For this trial, a **reportable adverse event** is defined as:

- Any clinically important untoward medical occurrence in a patient receiving study drug or undergoing study procedures which is different from what is expected in the clinical course of a sarcoidosis patient or,
- Any clinically important, untoward medical occurrence that is thought to be associated with the study drug or procedures, regardless of the "expectedness" of the event for the course of a sarcoidosis patient.

11.2 Expected events for sarcoidosis

Expected events for sarcoidosis are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of sarcoidosis patients. Examples of adverse events that are expected in the course of this study include dyspnea upon exertion, arthralgias, myalgias, etc. Such events, which are often the focus of prevention efforts as part of sarcoidosis care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study drug or procedures, or unexpectedly severe or frequent for an individual sarcoidosis patient. All SAEs or clinically significant laboratory abnormalities which, in the opinion of the investigator, may be due to study drug or study procedures will be monitored until complete resolution, until the condition stabilizes, or until the participant is lost to follow-up.

Investigators will report all events that are **serious** AND **unexpected** AND **study-related**, **as defined in Manual of Operations and Procedures**, to the Clinical Coordinating Center by phone, fax or email within 24 hours of becoming aware of event. The local Institutional Review Board must also be notified in a timely manner. The investigator will then submit a detailed written report to the Clinical Coordinating Center and the Institutional Review Board no later than 5 calendar days after the investigator discovers the event.

The Clinical Coordinating Center will report all serious, unexpected, and study-related adverse events to the DSMB, by email, or telephone, within 7 calendar days of the CCC being notified of the event. A written report will be sent to the DSMB within 15 calendar days, and these reports will be sent to investigators for submission to their respective Institutional Review Boards. The DSMB will also review all adverse events during scheduled interim analyses. The Clinical Coordinating Center will distribute the written summary of the DSMB's periodic review of adverse events to investigators for submission to their respective Institutional Review Boards in accordance with reporting guidelines.

The Clinical Coordinating Center will also determine if the serious adverse event is unexpected for CLEAR therapy. Unexpected is defined as any event not listed in the package insert for the individual antibiotic. If the Clinical Coordinating Center determines that any serious and study-related adverse event is unexpected, the FDA will be notified within 7 calendar days. Such events may also meet the definition of Unanticipated Problems as described below.

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug within 24 hours. An unanticipated problem is defined as follows:

11.3 Unanticipated Problem (UP):

Unanticipated problem is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the participant population being studied;

- Related or possibly related to participation in the research, in this guidance document, 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;
- 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.

12. Study Mandated Deviations from Assigned Treatment Medication Stop Points.

12.1. Definition

- A stop point can only occur after the participant is randomized and denotes the occurrence of an event, which necessitates altering the interventions of the study (i.e., cessation of CLEAR therapy or placebo). Visits and data collection continue after stop points. Before a stop point is declared all possible measures will be taken to reverse the problem necessitating the stop point. If there is a necessary deviation from the randomized intervention, we will minimize the degree of the deviation if at all possible. If possible the participant will resume the intervention at a later time.
- General Stop Points:
    1. Pregnancy
    2. ALT >10x upper limit of normal
    3. Intolerable medication-related toxicity

12.2. Measurements at the Time of a Stop Point

When a Stop Point has been confirmed by Site PI, a measurement of complete metabolic profile, CBC, spirometry for FVC and chest CT radiography (non-contrasted CT), if the participant is not pregnant.

12.3. Follow-Up After Stop Point

- Following treatment failures, which determine the primary and main secondary outcomes, participant follow-up will include the following information:
    1. Labs tests including CMP, CBC
    2. Spirometry for FVC
    3. Non Contrasted Chest CT, if the participant is not pregnant.

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Appendix A: Time and Events Schedule

Data Collection:
Table. CLEAR II Trial Assessments

Event	Screening Visit 1	Screening Visit 2	Baseline Visit	Week 4 Visit ± 7 days	Week 8 Visit ± 7 days	Week 16 Visit ± 7 days	Week 24 Visit ± 7 days
Consent	X						
Medical History	X						
Physical Exam	X			X	X	X	X
Pregnancy test (if female)	X			X	X	X	
Blood Chemistry (CMP[1]; CBC[2])	X			X	X	X	
T cell recognition assay	X			X	X	X	X
Spirometry Test/DLCO		X		X	X	X	X
Chest X-ray		X				X	
6MWT[3]/Borg Dyspnea Score		X*		X	X	X	X
Questionnaires		X				X	
Bronchoscopy			X			X	
Compliance evaluation				X	X	X	
Adverse Events				X	X	X	X
CLEAR II Therapy Initiation			X				

* 6MWT should be done 2 times (1 hour apart) at this visit, remaining visits, only once (Exception: if participant is experienced with the 6MWT only 1 is necessary at baseline.)

[1]CMP=Complete metabolic profile; [2]CBC=Complete blood count; [3]6MWT=Six minute walk test