CHEST

# Phase II Investigation of the Efficacy of Antimycobacterial Therapy in Chronic Pulmonary Sarcoidosis

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**BACKGROUND:** A Phase I, single-center investigation found that 8 weeks of antimycobacterial therapy improved sarcoidosis FVC. Safety and efficacy assessments have not been performed in a multicenter cohort.

**RESEARCH QUESTION:** The objective of this study was to determine the safety and efficacy of antimycobacterial therapy on the physiological and immunologic end points of sarcoidosis.

**STUDY DESIGN AND METHODS:** In a double-blind, placebo-controlled, multicenter investigation, patients with pulmonary sarcoidosis were randomly assigned to receive 16 weeks of concomitant levofloxacin, ethambutol, azithromycin, and rifabutin (CLEAR) or matching placebo to investigate the effect on FVC. The primary outcome was a comparison of change in percentage of predicted FVC among patients randomized to receive CLEAR or placebo in addition to their baseline immunosuppressive regimen. Secondary outcomes included 6-min walk distance (6MWD), St. George's Respiratory Questionnaire (SGRQ) score, adverse events, and decrease in mycobacterial early secreted antigenic target of 6 kDa (ESAT-6) immune responses.

**RESULTS:** The intention-to-treat analysis revealed no significant differences in change in FVC among the 49 patients randomized to receive CLEAR (1.1% decrease) compared with the 48 randomized to receive placebo (0.02% increase) ($P = .64$). Physiological parameters such as the change in 6MWD were likewise similar ($P = .91$); change in SGRQ favored placebo (–8.0 for placebo vs –1.5 for CLEAR; $P = .028$). The per-protocol analysis revealed no significant change in FVC at 16 weeks between CLEAR and placebo. There was no significant change in 6MWD (36.4 m vs 6.3 m; $P = .24$) or SGRQ (–2.3 vs –7.0; $P = .14$). A decline in ESAT-6 immune responses at 16 weeks was noted among CLEAR-treated patients ($P = .0003$) but not patients receiving placebo ($P = .24$).

**INTERPRETATION:** Despite a significant decline in ESAT-6 immune responses, a 16-week CLEAR regimen provided no physiological benefit in FVC or 6MWD among patients with sarcoidosis.

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**KEY WORDS:** antimycobacterial therapy; ESAT-6; FVC; sarcoidosis

**ABBREVIATIONS:** 6MWD = 6-min walk distance; CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin; ESAT-6 = early secreted antigenic target of 6 kDa; SAE = severe adverse event; SGRQ = St. George's Respiratory Questionnaire

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Sarcoidosis is an idiopathic, granulomatous disease with limited therapeutic options.[1,2] Current guidelines recommend various forms of immunosuppression as a mainstay of treatment, although these agents carry significant toxicities and have suboptimal efficacy. Corticosteroids have been proposed as the drug of choice for the treatment of pulmonary sarcoidosis, but toxicities are common. In addition, although antimalarial, cytotoxic, and biologic agents exhibit efficacy, relapse following tapering or discontinuation of these agents, as well as their side effect profiles, underscore the necessity for safer, more effective options.[3,4]

Although no definitive agent has been identified in sarcoidosis granulomas, independent laboratories have reported the presence of mycobacterial proteins and DNA in sarcoidosis lesions.[5-7] In addition, several investigators have described immune responses against secreted mycobacterial virulence factors in patients with sarcoidosis.[7-9] Immune responses against these mycobacterial antigens disappear with spontaneous clinical resolution of pulmonary sarcoidosis,[8] as well as following administration of antimycobacterial therapy to patients with this disease.[10] The clinical utility of antimycobacterial therapy was suggested by an 8-week, single-blind randomized trial of concomitant Levaquin, azithromycin, ethambutol, and rifabutin (CLEAR) in cutaneous sarcoidosis; an open-label trial similarly reported improved FVC, 6-min walk distance (6MWD), and early secreted antigenic target of 6 kDa (ESAT-6) responses in pulmonary sarcoidosis.[10,11] Histologic evidence of granulomatous resolution following administration of the CLEAR regimen among patients with cutaneous sarcoidosis was also noted.[11] A case report of resolution of ocular sarcoidosis with the same regimen has also been reported.[12] We designed a Phase IIB study to further define the safety and efficacy of the CLEAR regimen in sarcoidosis patients with progressive pulmonary disease.

## Patients and Methods

### Trial Design and Objectives

This randomized, double-blind, placebo-controlled investigation compared a regimen of antimycobacterial therapy consisting of CLEAR vs a four-drug placebo regimen for 16 weeks. Each patient received 8 weeks of four drugs (induction phase), followed by 8 weeks of two drugs (consolidation phase). The primary end point was the absolute change in percentage of predicted FVC comparing baseline FVC vs FVC following completion of 16 weeks of therapy. The secondary end points included change in 6MWD, St. George's Respiratory Questionnaire (SGRQ) score, adverse events of grades 1 to 5, and in ESAT-6-specific immune responses.

### Protocol Development and Oversight

The study protocol was approved by the Vanderbilt University Medical Center Institutional Review Board by Health Sciences Committee 1 (#121532) and was registered at ClinicalTrials.gov.[13] This study was conducted in accordance with the amended Declaration of Helsinki, and written informed consent was obtained from all patients. The Data and Safety Monitoring Board for this study reviewed data throughout the study and performed the single planned interim analysis for safety and efficacy after 50 randomized patients had completed their 16-week regimen.

### Interventions

Patient were randomized to receive either an oral antibiotic regimen consisting of 8 weeks of daily levofloxacin 500 mg, ethambutol (1,200 mg for ≥ 50 kg; 800 mg for < 50 kg) once daily, azithromycin 250 mg, and rifabutin 300 mg vs a daily identical-appearing four-drug placebo regimen. For the last 8 weeks of the study, participants were given two of the four drugs based on their individual tolerance and toxicity during the first 8 weeks. The placebo regimen was administered in the same format. The levofloxacin, ethambutol, and azithromycin were paid for at full cost through the Vanderbilt Investigational Drug Pharmacy. Rifabutin was donated by the Pfizer Global Medical Grant Program (#53232269).

### Population Eligibility and Randomization

Adults aged ≥ 18 years with the diagnosis of sarcoidosis as defined by the 1999 American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Other Granulomatous Disorders statement on sarcoidosis were eligible for enrollment.[1] Participants were also selected based on demonstration of pulmonary disease progression according to at least one of the following three criteria: (1) decline of absolute percentage of predicted FVC or diffusing capacity for carbon monoxide of at least 5% on serial

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measurements over 24 months; (2) radiographic progression in chest imaging on side-by-side comparison; or (3) decline in dyspnea score, as measured by using the Transition Dyspnea Index. Prior to randomization, participants were assessed for peripheral immune responses to ESAT-6 or evidence of peripheral anergy as defined by absence of responses to phytohemagglutinin. If either of those conditions were present, the subject was enrolled. Finally, participants were required to have evidence of parenchymal or nodal disease on chest radiograph. Site-specific pulmonologists who were unaffiliated with the CLEAR trial read all site-specific lung function tests.

Sample size was calculated for the primary end point: change from baseline of FVC percent predicted. Using data from participants with chronic pulmonary sarcoidosis treated with infliximab,[14] we obtained the SD of 7.7 for the primary end point. A sample size of 51 completed participants per arm was needed to have 90% power to detect a 5% difference in change of FVC percent predicted from baseline.

The major exclusion criteria were as follows:

(1) Inability to obtain consent.
(2) Age $<$ 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 following study completion: condoms, sponge, foams, jellies, diaphragm, nonhormonal 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 result at screening visit was required if the female subject was of childbearing potential.
(4) FVC predicted value $<$ 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 TB, close contact with a person with active TB within the 6 months prior to the screening visit, or a positive purified protein derivative skin test result.
(11) Significant disorder, other than sarcoidosis, that would complicate the treatment evaluation (eg, respiratory, cardiac, neurologic, musculoskeletal, or seizure disorders).
(12) Use of an investigational drug within 30 days prior to screening or within five half-lives of the agent, whichever is longer.
(13) Currently receiving $>$ 40 mg of prednisone.
(14) Alanine aminotransferase or aspartate aminotransferase levels more than five times the upper limit of normal.
(15) Leukopenia, as defined by a WBC count $<$ 3.0 cells/mm$^3$ or absolute neutrophil count $<$ 1,000/μL.
(16) Breastfeeding.
(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 the patient is on immunomodulators, they must be on a regimen for 3 months or longer 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 performed) showing cardiac ejection fraction $<$ 35%.
(21) Participant has persistent or active infection(s) requiring hospitalization or treatment with antibiotic, antiretroviral, or antifungal agents 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 examination prior to or following 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) Taking 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.

Patients were assigned to receive the CLEAR or placebo regimen by using a block randomization stratified according to site and use of prednisone $\geq$ 10 mg or not. Randomization lists were generated and distributed to site pharmacies by a statistician at Vanderbilt University Medical Center not associated with the study.

### Measurement of Treatment Completion

Patients were requested to bring all remaining doses of every trial drug to each visit for pill counts. Treatment completion for the per-protocol analysis was defined as administration of at least 90% of the doses within 16 weeks.

### Measurement of Safety During Treatment

At each follow-up visit, participants were questioned and examined for adverse events. Suspected adverse events were investigated, managed, and reported according to a standardized protocol. Information about suspected adverse events was reviewed and graded by the site-specific principal investigator and clinical coordinator. The severity of adverse events was judged according to the Common Toxicity Criteria for Adverse Events version 4.0. The events were categorized as follows: an adverse event that was not related to a trial drug; an adverse event of grade 1 or 2 that was related to a trial drug (not serious); an adverse event of grade 3 or 4 that was related to a trial drug (generally considered to lead to trial drug discontinuation if related to a trial drug); or a grade 5 event (death) that was related to a trial drug. Each adverse event resulting in organ impairment, hospitalization, or death was defined as a serious adverse event (SAE). An SAE was reported and reviewed by the institutional review board, as well as the four-member Data Safety Monitoring Board. The board provided oversight regarding safety for continuation of the study.

### Oversight

This trial was approved by the Vanderbilt University Human Research Protection Program and by the institutional review board at each participating site. All the authors vouch for the accuracy and completeness of the data and analyses presented and for the compliance of the trial to the protocol.

### Statistical Analysis

This randomized Phase II clinical trial was conducted to determine if CLEAR elicits a statistically significant (two-sided value $P < .05$) improvement in respiratory performance, the 6MWD, SGRQ, and immune responses against ESAT-6. Ninety-seven patients were randomized to treatment from May 5, 2014, to December 13, 2018, of whom 49 patients were randomized to receive CLEAR and 48 to receive placebo. Data were cleaned and locked for final analysis on April 18, 2019. The primary end point was baseline to 16-week change in preinhaler FVC as percent predicted. We defined the 16-week postrandomization FVC percent predicted value measurement as the value closest to 16 weeks from randomization within a window of 12 to 20 weeks from randomization.

The primary comparison between the CLEAR and placebo arms was conducted on an intention-to-treat (as randomized) basis among patients with both baseline and 16-week outcomes (N = 97). Unless otherwise stated, mean, SE, and comparative *P* values were estimates with multiple imputation using predictive mean matching with aregImpute, as previously described.[15,16] The multiple imputation data sets were summarized according to Rubin's rules.[17] Unless otherwise noted, figures represent the mean change score ± the SE computed by using Rubin's rules for imputed data. Change scores for secondary end points were compared by using the linear model formulation of the two-sample test between groups over 100 imputed data sets. The paired Student *t* test for multiply imputed data was used to compare baseline and 16-week change scores within each treatment group at 8 and 16 weeks. All analyses were repeated for a per-protocol population of patients (n = 72). Analysis of ESAT-6 and adverse events was not based on imputed data. The ESAT-6 analysis was the only within-group comparison and was conducted by using a Student paired *t* test, comparing baseline with 16-week values within their randomization cohort. The number of subjects experiencing an SAE and separately an adverse event (adverse events not including SAEs) was compared between treatment arms by using the paired or unpaired Student *t* test.

## Results

### Trial Participants

We screened 446 potential patients from May 2014 through December 2018, of whom 97 were enrolled and randomized to treatment (Fig 1). The most common reasons for exclusion from study participation were as follows: (1) significant comorbid conditions; (2) a history of a drug interaction between one of the CLEAR antibiotics with a medication that the patient was currently receiving; (3) patient declined to participate; (4) pulmonary hypertension treatment; (5) concerns for noncompliance with study visits; and (6) Scadding stage IV fibrosis detected on a chest radiograph.

The demographic and clinical characteristics of all study participants according to their randomization group are shown in Table 1. Of the 97 enrolled subjects, baseline characteristics were well balanced across treatment arms. Approximately one-half of the population (n = 50 [52%]) was female, and the majority were White (n = 68 [70%]), with approximately one-third African American (n = 28 [29%]). A possible balance exception was sex (59% women in the placebo group and 44% women in the CLEAR group). Although not a planned analysis, analysis of covariance of sex ($P = .996$) with treatment group ($P = .903$) on nonimputed data, as with their interaction ($P = .678$), suggests no confounding by sex.

The clinical data were analyzed via intention-to-treat and per protocol. No subjects were excluded from the intention-to-treat analysis. In the per-protocol analysis, 25 (12 in the active arm and 13 in the placebo arm) of the 97 patients were excluded because of failure to take > 4 weeks of the prescribed regimen (CLEAR, n = 8; placebo, n = 4), alteration in clinical immunosuppressive regimen while on study drugs (CLEAR, n = 1; placebo, n = 3), initiation of non-study antibiotics during study participation (CLEAR, n = 1; placebo, n = 3), and found to be receiving antibiotics

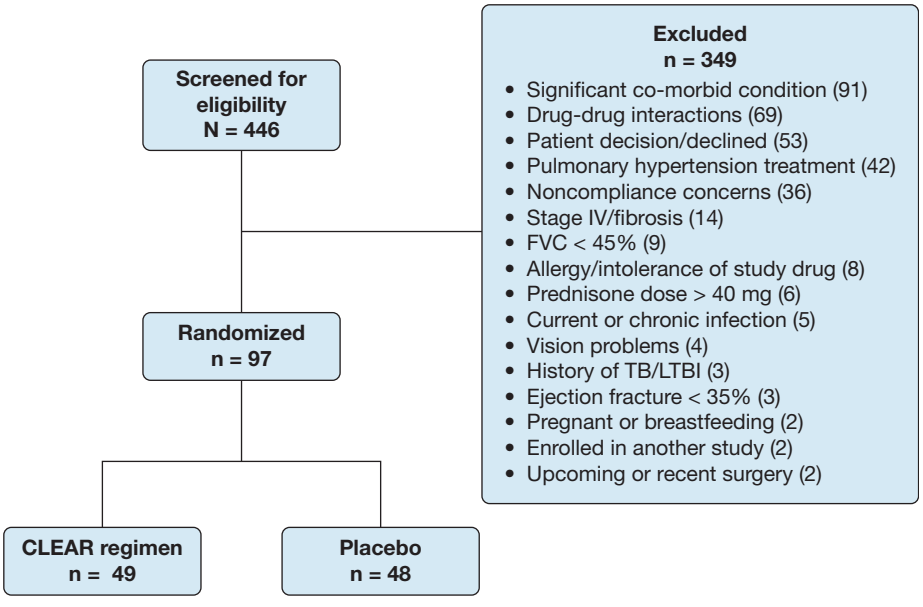


Figure 1 – *Consort Diagram of Phase IIB investigation of the efficacy of antimycobacterial therapy against progressive pulmonary sarcoidosis. CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin; LTBI = latent TB infection.*

**TABLE 1** ] Patient Demographic Characteristics According to Therapeutic Regimen

| Characteristic | CLEAR (n = 49) | Placebo (n = 48) | Combined (N = 97) |
|---|---|---|---|
| Age, mean ± SD, y | 54.5 ± 9.8 | 54.5 ± 9.8 | 54.5 ± 10 |
| Sex | | | |
| Male | 20 (41%) | 27 (56%) | 47 (48%) |
| Female | 29 (59%) | 21 (44%) | 50 (52%) |
| Race | | | |
| African American | 15 (31%) | 13 (27%) | 28 (29%) |
| White | 34 (69%) | 34 (71%) | 68 (70%) |
| Hawaiian or Pacific Islander | 0 (0) | 1 (2%) | 1 (1%) |
| Ethnicity | | | |
| Non-Hispanic/nor Latino | 48 (98%) | 46 (96%) | 94 (97%) |
| Hispanic/Latino | 1 (2%) | 2 (4%) | 3 (3%) |
| Baseline end points | | | |
| Preinhaler FVC % | 77.3 ± 13.7 | 75.5 ± 14.5 | 75.4 ± 14.1 |
| 6-min walk test, m[a] | 416.2 ± 140.7 | 416.4 ± 105.2 | 416.3 ± 123.5 |
| SGRQ activity[b] | 57.7 ± 23.2 | 55.5 ± 24.5 | 56.6 ± 23.8 |
| SGRQ impact[b] | 34.8 ± 22.2 | 32.9 ± 19.8 | 33.8 ± 20.9 |
| SGRQ symptoms[b] | 53.9 ± 23.8 | 50.5 ± 19.3 | 52.2 ± 21.7 |
| SGRQ total[b] | 44.9 ± 21.1 | 42.7 ± 18.7 | 43.8 ± 19.9 |
| Immunosuppression | | | |
| Patients on prednisone (mean dosage) | 23 (47%) (10 mg) | 19 (40%) (10 mg) | 42 (43%) (10 mg) |
| Patients on a DMA | 19 (39%) | 17 (35%) | 36 (37%) |
| Patients on a biologic agent | 1 (2%) | 1 (2%) | 2 (4%) |
| Patients on prednisone plus a DMA or biologic agent | 10 (20%) | 6 (13%) | 16 (16%) |
| Patients on any combination of prednisone, DMA, or biologic agent | 11 (22%) | 8 (17%) | 19 (20%) |
| ESAT-6 positivity | | | |
| Yes | 39 (80%) | 36 (75%) | 75 (77%) |
| Equivocal | 10 (20%) | 12 (25%) | 25 (23%) |

CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin; DMA = disease-modifying agent; ESAT-6 = early secreted antigenic target of 6 kDa.
[a]N = 48 for CLEAR and placebo groups.
[b]N = 48 and N = 47, for CLEAR and placebo groups, respectively.

with antimycobacterial therapy, such as trimethoprim-sulfamethoxazole, at the time of enrollment (CLEAR, n = 2; placebo, n = 3). The remaining 72 subjects were included in the per-protocol analysis.

### *Efficacy*

In the intention-to-treat analysis, there were no statistically significant differences in the primary end point (change from baseline to week 16 in pre-bronchodilator percent predicted FVC) between the CLEAR and placebo groups (CLEAR –1.06% vs placebo 0.02%; imputed two-sample Student *t* test, $P = .64$) (Fig 2A, Table 2). Eight-eight patients experienced follow-up during the 12- to 20-week postrandomization window. Median (interquartile range) and mean ± SD of time to the date of FVC percent measurement was 17 (16.1-18) weeks and 17.5 ± 1.9 weeks, respectively. Time to primary end point measurement was equivalent between treatment groups, with a median (interquartile range) of 17 (16.2-18) weeks for placebo and 17.2 (16.2-18.0) weeks for CLEAR II patients. Mean ± SD values were 17.5 ± 1.9 and 17.4 ± 1.8. Evaluation of other physiological parameters, such as 6MWD (placebo, 12.4 m; CLEAR, 9.8 m; imputed two-sample

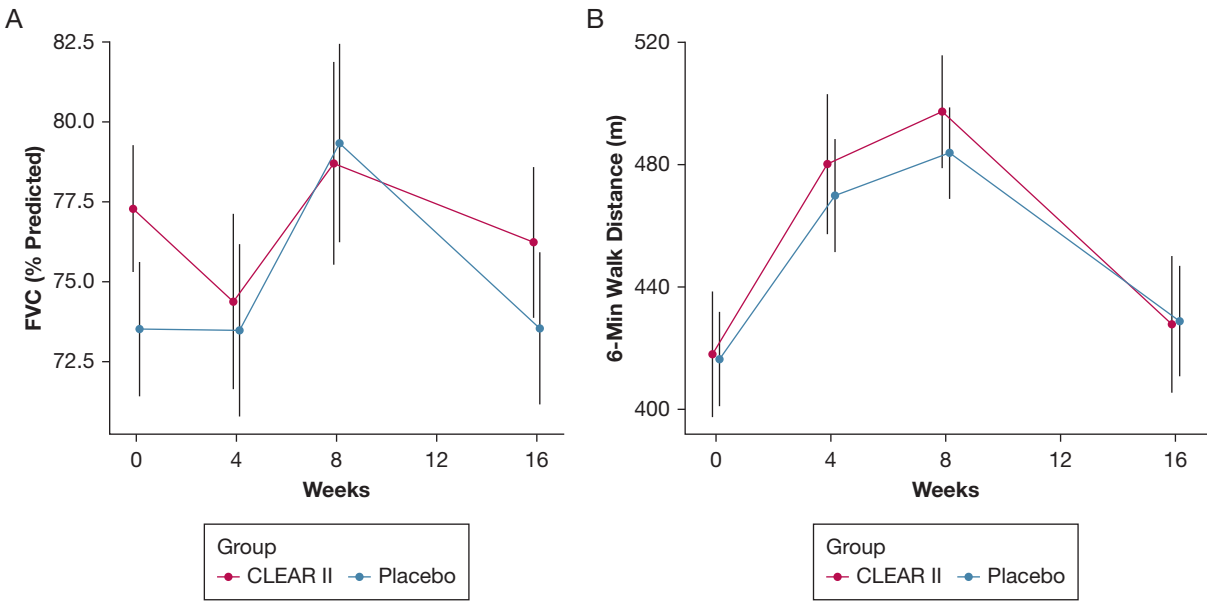


Figure 2 – *A-B, Graphic depiction of intention-to-treat assessment of physiological parameters such as FVC and 6-min walk distance in 97 subjects with sarcoidosis randomized to either the CLEAR regimen (active) or placebo. A, There were no statistically significant differences in the primary end point (change from baseline to week 16 in pre-bronchodilator percent predicted FVC) between 49 CLEAR and 48 placebo subjects (CLEAR –1.06% vs placebo 0.02%; imputed two-sample Student t test,* P = *.64). B, Evaluation of 6-min walk distance (placebo, 12.4 m; CLEAR, 9.8 m; imputed two-sample Student t test,* P = *0.91) revealed no statistically significant difference. CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin.*

Student *t* test, *P* = .91) revealed no statistically significant differences (Fig 2B). A negative change in the SGRQ score reflects an improvement in quality of life. The SRGQ did reveal statistically and clinically significant differences in favor of the placebo group (placebo, –7.97; CLEAR II, –1.52; *P* = .028, minimal clinically important difference = 4.0).

In the per-protocol analysis, 72 subjects were analyzed following removal of 25 patients prior to data analysis due to factors outlined in the protocol. Comparison of baseline vs week 16 end points of the remaining 72 patients revealed that there were no statistically differences in the primary end point (the change from baseline in percent predicted FVC) between 37 CLEAR-

**TABLE 2** ] Physiological and Qualitative End Point Analyses

| Variable | Placebo | CLEAR | *P* Value |
|---|---|---|---|
| Intent-to-treat population | | | |
| Preinhaler FVC % | 0.02 ± 1.47 | –1.06 ± 1.85 | .640 |
| 6-min walk distance | 12.40 ± 12.35 | 9.78 ± 19.31 | .908 |
| SGRQ activity | –7.15 ± 3.28 | –0.96 ± 2.87 | .162 |
| SGRQ impact | –7.45 ± 2.64 | –0.61 ± 2.78 | .057 |
| SGRQ symptoms | –10.15 ± 3.45 | –5.62 ± 3.22 | .331 |
| SGRQ total | –7.97 ± 2.01 | –1.52 ± 2.17 | .028 |
| Per-protocol group | | | |
| Preinhaler FVC % | 0.42 ± 1.48 | –0.74 ± 1.75 | .616 |
| 6-min walk distance | 6.27 ± 11.99 | 36.35 ± 22.32 | .242 |
| SGRQ activity | –4.25 ± 2.39 | –1.45 ± 2.91 | .458 |
| SGRQ impact | –7.97 ± 2.51 | –1,10 ± 2.62 | .061 |
| SGRQ symptoms | –9.78 ± 3.60 | –8.07 ± 3.39 | .730 |
| SGRQ total | –6.97 ± 2.13 | –2.32 ± 2.32 | .141 |

Imputed mean ± SE of baseline to 16-week differences in measurements. *P* values are from imputation-based two-sample Student *t* test. CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin; SGRQ = St. George's Respiratory Questionnaire.

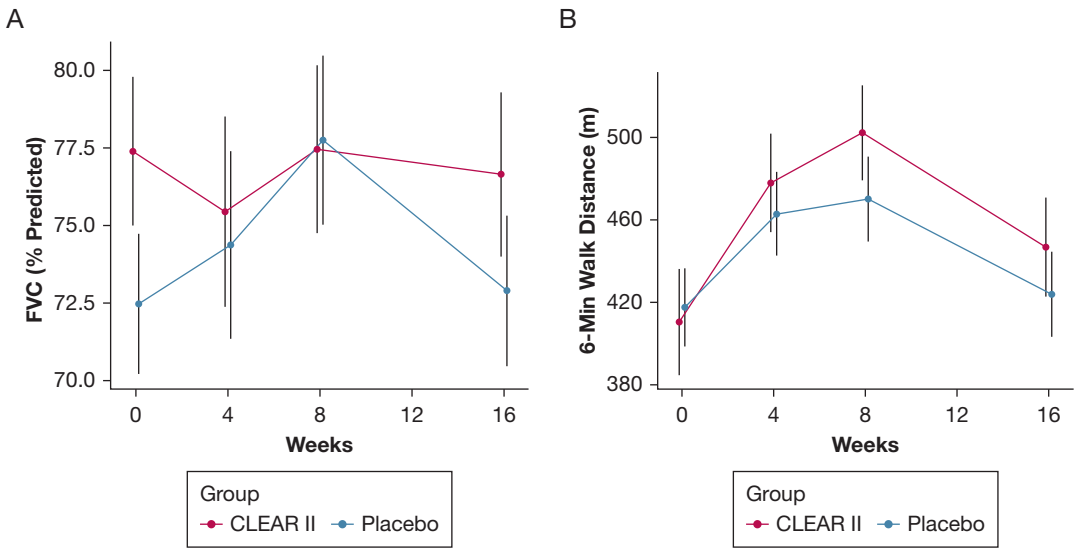


Figure 3 – *A-B, Graphic depiction of per-protocol assessment of physiologic parameters, such as FVC and placebo in 97 patients with sarcoidosis randomized to either the CLEAR regimen (active) or placebo. A, Comparison of baseline vs week 16 end points of 72 subjects revealed that there were no statistically differences in the primary end point (change from baseline in percent predicted FVC) between 37 CLEAR subjects compared with 35 placebo subjects (CLEAR –0.74% vs placebo 0.42%; imputed two-sample Student t test,* P = .62*). B, Evaluation of 6-min walk distance revealed no significant difference among patients randomized to the CLEAR or the placebo regimen (36.4 m vs 6.3 m; imputed two-sample Student t test,* P = .242*). CLEAR = concomitant Levaquin, ethambutol, azithromycin, and rifamycin.*

treated patients compared with 35 patients receiving placebo (CLEAR –0.74% vs placebo 0.42%; $P = .62$) (Fig 3A, Table 2). Evaluation of other physiological and qualitative end points revealed no significant differences; for example, there were no significant differences in the 6MWD of patients randomized to the CLEAR or placebo regimen (36.4 m vs 6.3 m; $P = .242$) (Fig 3B). The SGRQ also revealed no significant differences in the total score between the groups (placebo, –6.9; CLEAR, –2.3; imputed two-sample Student $t$ test, $P = .14$). CLEAR-treated patients had less improvement in activity, impact, and symptoms compared with patients receiving placebo.

Although there was no significant baseline difference in ESAT-6-specific spot-forming units between the two groups (unpaired Student $t$ test, $P = .48$) (Fig 4A), there was a significant difference in the spot-forming units following 16 weeks of therapy. There was no significant change in 38 subjects randomized to receive placebo (paired Student $t$ test, $P = .26$) (Fig 4B); however, a significant decline in the ESAT-6 spot-forming units among the 31 patients randomized to the CLEAR regimen (paired Student $t$ test, $P = .0003$) (Fig 4C). Only subjects for whom baseline and 16-week values were available were included in this analysis.

### *Safety*

At each follow-up visit, participants were evaluated for adverse events. A total of 75 adverse events were noted in 39 subjects (e-Table 1). Any adverse event resulting in organ impairment, hospitalization, or death was defined as an SAE. The number of SAEs was similar for CLEAR (n = 4) and placebo (n = 3) ($P = .72$) (Table 3). Three of the four SAEs in the CLEAR cohort were believed to be related to study drugs; none was believed to be related in the placebo cohort. There were no deaths in this trial.

## Discussion

In this randomized, double-blind, placebo-controlled trial of CLEAR therapy, we observed no benefit from the study intervention on pulmonary function, for both the intention-to-treat and per-protocol analyses. Most secondary end points, such as 6MWD and SGRQ, showed no significant improvement from CLEAR, and SGRQ was worse at the end of the CLEAR regimen. There was a significant decline in immune responses against ESAT-6 among the CLEAR-treated subjects, but no change was observed among those randomized to receive placebo (Figs 3, 4).

Although viable mycobacteria have been proposed to be causative agents for sarcoidosis, the current study provides no evidence to support that hypothesis. The current results are discordant from a randomized trial in which CLEAR therapy was beneficial for cutaneous sarcoidosis, and they also diverge from an uncontrolled report of CLEAR therapy.[10,11] One explanation for the failure of CLEAR therapy is that the underlying

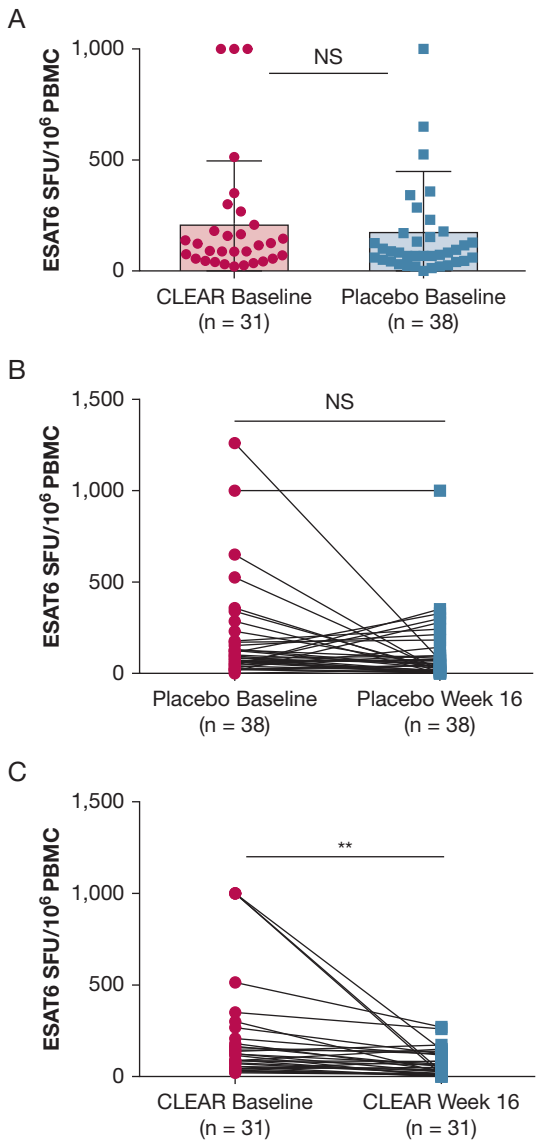


Figure 4 – *Comparison of ESAT-6 immune responses at baseline (n = 69 subjects) (A), as well as baseline to 16 weeks among patients with sarcoidosis randomized to either the placebo (n = 38 subjects) (B) or CLEAR (n = 31 subjects) (C) regimen. ESAT-6 = early secreted antigenic target of 6 kDa; NS = not significant; PBMC = peripheral blood mononuclear cells; SFU = spot-forming units.*

hypothesis regarding the cause of sarcoidosis is incorrect. This explanation accords with the failure of prior studies to culture mycobacteria from sarcoidosis tissues.[18,19]

Other explanations for the failure of CLEAR therapy should also be considered. The inclusion criteria were designed to enroll a population with actively progressing sarcoidosis, but the precision of the longitudinal data supporting progression, and the long time window for demonstrating progression, are both problematic and may have biased the study population to include patients with relatively stable, adequately controlled disease.[20] Progression in the placebo group on stable therapy would not be expected in a 16-week time frame. Active withdrawal of anti-sarcoidosis medications may be necessary to uncover relative treatment benefits if the effect size is modest, but this study required stable dosing throughout the treatment period. It is also possible that the treatment duration was too short to discern efficacy of therapy; medications such as methotrexate require up to 6 months to effect benefit in pulmonary sarcoidosis.[21] Antibiotic therapy may not result in improved FVC; one study noted that these patients may continue to experience an FVC decline, but it occurs at a significantly lower rate than patients who were not successfully treated.[22] Finally, FVC may be an insensitive marker of treatment effect; it has previously been shown to correlate poorly with symptoms and with chest imaging.[23,24] However, the absence of any observable clinical benefits argues that the end point chosen here is likely not the cause of the negative result.

Immune responses against mycobacterial antigens, such as ESAT-6 and katG, are present in patients with active sarcoidosis disease.[8,25,26] Independent investigators found that, using the ELISpot assay, antimycobacterial responses disappear with clinical resolution of sarcoidosis.[8] Immune responses against ESAT-6 have also been detected with active or latent TB, as well as other nontuberculous mycobacteria infections[27-29]; these responses decline with effective antimycobacterial therapy.[30-32] A significant decline in the ESAT-6 immune responses among subjects randomized to receive CLEAR (but not placebo) is provocative and of uncertain significance. It is unlikely that the decrease in ESAT-6 response in the CLEAR group is due to an immunosuppressant effect of one or more antibiotics in the treatment regimen because we previously reported improved immune function, including enhanced T-cell proliferative capacity and increased IL-2 and interferon-γ secretion, as well as augmented JAK-STAT signaling from sarcoidosis $CD4^+$ T cells, following completion of the CLEAR regimen.[11] The improvement in cellular immunity with CLEAR treatment of sarcoidosis could result in the augmented capacity to remove pathogenic microbial antigens such as ESAT-6. In addition, because the removal of microbial antigens such as ESAT-6 during treatment of mycobacterial infection reduces expression of profibrotic cytokines such as IL-17A, this mechanism may also mitigate the development of lung fibrosis during the treatment of sarcoidosis. The discrepancy between measurable declines in a virulence

**TABLE 3** ] SAEs Throughout the Study

| SAE Number | Event | Group | Institution | Date | Anticipated | Caused by Therapy |
|---|---|---|---|---|---|---|
| 1 | Leukopenia | Active | Cincinnati | 08/14/2016 | Yes | Probable |
| 2 | Pneumonia | Active | Cincinnati | 11/06/2014 | Yes | Probable |
| 3 | Neurosarcoidosis | Active | Vanderbilt | 12/28/2016 | No | Not related |
| 4 | Hypotension | Active | AMC | 01/29/2018 | Yes | Probable |
| 5 | Sepsis from abscess | Placebo | Cleveland | 07/18/2017 | No | Not related |
| 6 | Postoperative infection | Placebo | Cleveland | 11/14/2014 | No | Unlikely |
| 7 | Pneumonia | Placebo | Cleveland | 06/04/2018 | No | Not related |

AMC = Albany Medical College; SAE = severe adverse event.

factor associated with active mycobacterial replication (ESAT-6) and the negative results of the current study remain unexplained.

It may be that mycobacterial antigens are important initial triggers of some cases of sarcoidosis, as suggested by persistence of mycobacterial antigens in sarcoidosis tissues,[7] but viable infection is not integral to the perpetuation or progression of the disease. Also, because the study design did not obtain any samples to exclude the presence of infection in the subjects, it remains possible that the effects of CLEAR resulted in changes in the microbiome, which could then affect immune responses by changing the presence of microbial antigens. Future investigation regarding the impact of the CLEAR regimen on preventing further lung deterioration is warranted.

The current study did have some limitations. The number of subjects was relatively small. Twenty-five of the 97 patients were excluded from the per-protocol analysis, representing approximately 26% of the enrolled subjects. Twelve of those subjects (CLEAR, n = 8; placebo, n = 4) were excluded due to taking < 4 weeks of study medications, making it more difficult to assess the impact of 16 weeks of CLEAR therapy on the primary and secondary end points. Due to the pill burden and toxicities associated with a four-drug regimen, difficulty adhering to antimycobacterial therapy is well documented.[33-35] Toxicities, such as myalgias and arthralgias from azithromycin and levofloxacin, as well as fatigue from rifabutin, may have affected the SGRQ score, masking our ability to clearly delineate an anti-sarcoidosis effect. Higher SGRQ scores were noted among CLEAR-treated patients experiencing these toxicities. Another limitation is the failure to include patients with disease for < 1 year. We did not include patients within 1 year of diagnosis because it was believed that differentiation of drug efficacy from spontaneous resolution would be too difficult. However, improvement in lung function among patients with TB is more likely if patients are < 40 years of age and do not have chronic sequelae.[36] Future sarcoidosis clinical investigations should include patients within 1 year of their diagnosis, longer follow-up period assessment for potential steroid-sparing effect, and CT or PET scans. Several studies have shown that increased activity according to a PET scan is a useful predictor of treatment-responsive pulmonary sarcoidosis.[37,38] PET scanning was not included in the current study because the information at time of study implementation was incomplete, and the cost of this as an exploratory analysis was prohibitive.

## Conclusions

In a cohort of patients with progressive pulmonary sarcoidosis, the CLEAR therapy did not result in significant improvement in percent predicted FVC but, instead, was associated with significant declines in ESAT-6-specific immune responses.

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