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.

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. 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.

Although no definitive agent has been identified in sarcoidosis granulomas, independent laboratories have reported the presence of mycobacterial proteins and DNA in sarcoidosis lesions. In addition, several investigators have described immune responses against secreted mycobacterial virulence factors in patients with sarcoidosis. Immune responses against these mycobacterial antigens disappear with spontaneous clinical resolution of pulmonary sarcoidosis, as well as following administration of antimycobacterial therapy to patients with this disease. The clinical utility of antimycobacterial therapy was suggested by an 8-week, single-blind randomized trial of concomitant Levaoquin, 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. Histologic evidence of granulomatous resolution following administration of the CLEAR regimen among patients with cutaneous sarcoidosis was also noted. A case report of resolution of ocular sarcoidosis with the same regimen has also been reported. 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. 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 $ \geq 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 $\geq 18$ 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. 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
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 ximab,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.

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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. The multiple imputation data sets were summarized according to Rubin’s rules. 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.](chart.png)
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

### 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, m³              | 416.2 ± 140.7  | 416.4 ± 105.2   | 416.3 ± 123.5    |
| SGRQ activity²                   | 57.7 ± 23.2    | 55.5 ± 24.5     | 56.6 ± 23.8      |
| SGRQ impact³                     | 34.8 ± 22.2    | 32.9 ± 19.8     | 33.8 ± 20.9      |
| SGRQ symptoms⁴                   | 53.9 ± 23.8    | 50.5 ± 19.3     | 52.2 ± 21.7      |
| SGRQ total⁴                      | 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.

aN = 48 for CLEAR and placebo groups.

bN = 48 and N = 47, for CLEAR and placebo groups, respectively.
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 significant differences in the primary end point (the change from baseline in percent predicted FVC) between 37 CLEAR-

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**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.
treated patients compared with 35 patients receiving placebo (CLEAR –0.74% vs placebo 0.42%; imputed two-sample Student t test, \(P = .62\)) (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; imputed two-sample Student t test, \(P = .242\)) (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, \(P = .003\)) (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.\(^\text{10,11}\) One explanation for the failure of CLEAR therapy is that the underlying
A hypothesis regarding the cause of sarcoidosis is incorrect. This explanation accords with the failure of prior studies to culture mycobacteria from sarcoidosis tissues.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{8,25,26} Independent investigators found that, using the ELISpot assay, antimycobacterial responses disappear with clinical resolution of sarcoidosis.\textsuperscript{8} Immune responses against ESAT-6 have also been detected with active or latent TB, as well as other nontuberculous mycobacteria infections\textsuperscript{27-29}; these responses decline with effective antimycobacterial therapy.\textsuperscript{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-\(\gamma\) secretion, as well as augmented JAK-STAT signaling from sarcoidosis CD4\(^+\) T cells, following completion of the CLEAR regimen.\textsuperscript{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

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**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.
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 Toxocities, 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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Author contributions: W. P. D. had full access to all the data following study completion and assumes full responsibility for the integrity of the data and the accuracy of the data analysis. W. P. D., D. A. C., R. P. B., and G.R.B. were responsible for conception and design; K. A., D. A. C., R. P. B., M. A. J., E. D. C., E. J., and A. G. performed experiments; T. D., G. D. A., G. R. B., and W. P. D. contributed to analysis and interpretation; and W. P. D., D. A. C., R. P. B., M. A. J., E. D. C., E. J., T. D., K. A., A. G., A. K., A.S., and G. R. B. drafted the manuscript for important intellectual content.

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Additional information: The e-Table can be found in the Supplemental Materials section of the online article.

References

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee. February 1999. Am J Respir Crit Care Med. 1999;160(2):736-755.

2. Grunewald J, Grutters JC, Arkema EV, Saketko LA, Moller DR, Muller-Quernheim J. Sarcoidosis. Nat Rev Dis Primers. 2019;5(1):45.

3. Gottlieb JE, Israel HL, Steiner RM, Tirolo J, Patrick H. Outcome in sarcoidosis. The relationship of relapse to corticosteroid therapy. Chest. 1997;111(3):523-631.

4. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijser RS, Rijkers G, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. Eur Respir J. 2014;43(2):602-609.

5. Mitchell DN. Mycobacteria and sarcoidosis. Lancet. 1996;348(9030):768-769.

6. Oswald-Richter KA, Beachboard DC, Seeley EH, et al. Dual analysis for mycobacteria and proportion bacteria in sarcoidosis BAL. J Clin Immunol. 2012;32(5):1129-1140.

7. Song Z, Marzilli L, Greenlee BM, et al. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. J Exp Med. 2005;201(5):755-767.

8. Chen ES, Wahlstrom J, Song Z, et al. T cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. J Immunol. 2008;181(12):8784-8796.

9. Ahmadzai H, Cameron B, Chui JJ, Lloyd A, Wakefield D, Thomas PS. Peripheral blood responses to specific antigens and CD28 in sarcoidosis. Respir Med. 2012;106(5):707-714.

10. Drake WP, Richmond BW, Oswald-Richter K, et al. Effects of broad-spectrum antimycobacterial therapy on chronic pulmonary sarcoidosis. Sarcoidosis Vascul Dis Lung Dis. 2013;30(3):201-211.

11. Drake WP, Oswald-Richter K, Richmond BW, et al. Oral antimycobacterial therapy in chronic cutaneous sarcoidosis: a randomized, single-masked, placebo-controlled study. JAMA Dermatol. 2013;149(9):1040-1049.

12. Richmond BW, Richter K, King LE, Drake WP. Resolution of chronic ocular sarcoidosis with antimycobacterial therapy. Case Rep Intern Med. 2014;2014(12):5042.

13. National Institutes of Health Clinical Center. Phase II investigation of antimycobacterial therapy on progressive, pulmonary sarcoidosis. NCIT0204555. ClinicalTrials.gov. National Institutes of Health, 2013. Updated July 9, 2020. https://clinicaltrials.gov/ct2/show/NCT0204555.

14. Baughman RP, Drent M, Kavuru M, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med. 2006;174(7):795-802.

15. Jain NB, Ayers GD, Koudelkova H, et al. Operative vs nonoperative treatment for atrumatic rotator cuff tears: a trial protocol for the arthroscopic rotator cuff pragmatic randomized clinical trial. JAMA Netw Open. 2019;2(8):e199050.

16. Dickinson RN, Ayers GD, Archer KR, et al. Physical therapy versus natural history in outcomes of rotator cuff tears: The Rotator Cuff Outcomes Workgroup (ROW) cohort study. J Shoulder Elbow Surg. 2019;28(5):833-838.

17. Rubin DB. Multiple Imputation for Nonresponse in Surveys. Hoboken, NJ: Wiley-Interscience; 2004.

18. Brown ST, Brett I, Almenoff PL, et al. Recovery of cell wall-defective organisms from blood does not distinguish between patients with sarcoidosis and control subjects. Chest. 2003;123(2):413-417.

19. Milman N, Lisby G, Friis S, Kemp L. Prolonged culture for mycobacteria in mediastinal lymph nodes from patients with pulmonary sarcoidosis. A negative study. Sarcoidosis Vascul Dis Lung Dis. 2004;21(1):25-28.

20. Moller DR. Negative clinical trials in sarcoidosis: failed therapies or flawed study design? Eur Respir J. 2014;44(5):1123-1126.

21. Lower EE, Baughman RP. The use of low dose methotrexate in refractory sarcoidosis. Am J Med Sci. 1990;299(3):153-157.

22. Park HY, Jeong BH, Chon HR, Jeon K, Doley CL, Koh WJ. Lung function decline according to clinical course in nontuberculous mycobacterial lung disease. Chest. 2016;150(6):1222-1232.

23. Baughman RP, Drent M, Culver DA, et al. Endpoints for clinical trials of sarcoidosis. Sarcoidosis Vascul Dis Lung Dis. 2012;29(2):90-98.

24. Baughman RP, Nunes H, Swiss NJ, Lower EE. Established and experimental medical therapy of pulmonary sarcoidosis. Eur Respir J. 2013;41(6):1424-1438.

25. Oswald-Richter K, Sato H, Hajizadeh R, et al. Mycobacterial ESAT-6 and katG are recognized by sarcoidosis CD4+ T cells when presented by the American sarcoidosis susceptibility allele, DRB1*1101. J Clin Immunol. 2010;30(1):157-166.

26. Oswald-Richter KA, Beachboard DC, Zhan X, et al. Multiple mycobacterial antigens are targets of the adaptive immune response in pulmonary sarcoidosis. Respir Res. 2010;11:161.

27. Nyendak MR, Park B, Null MD, et al. Mycobacterium tuberculosis specific CD8 (+) T cells rapidly decline with antituberculosis treatment. PLoS One. 2013;8(12):e81564.

28. Scherrer S, Landolt P, Friedel U, Stephan R. Distribution and expression of ESAT-6 and CFP-10 in non-tuberculous mycobacteria isolated from lymph nodes of slaughtered cattle in Switzerland. J Vet Diagn Invest. 2019;31(2):217-221.

29. Johnston JC, Chiang L, Elwood K. Mycobacterium kansasii. Microbiol Spectr. 2017;5(1).

30. Liu C, Zhao Z, Fan J, et al. Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring. Proc Natl Acad Sci U S A. 2017;114(15):3969-3974.

31. Mattos AM, de Almeida CS, Franken KL, et al. Increased IgG1, IFN-gamma, TNF-alpha and IL-6 responses to Mycobacterium tuberculosis antigens in patients with tuberculosis are lower after chemotherapy. Int Immunol. 2010;22(9):775-782.

32. Clifford V, Tebruegge M, Zufferey C, et al. Mycobacteria-specific cytokine responses as correlates of treatment response in...
active and latent tuberculosis. *J Infect*. 2017;75(2):132-145.

33. De Schacht C, Mutaquiha C, Faria F, et al. Barriers to access and adherence to tuberculosis services, as perceived by patients: a qualitative study in Mozambique. *PLoS One*. 2019;14(7):e0219470.

34. Law S, Daftary A, O’Donnell M, Padayatchi N, Calzavara L, Menzies D. Interventions to improve retention-in-care and treatment adherence among patients with drug-resistant tuberculosis: a systematic review. *Eur Respir J*. 2019;53(1):1801030.

35. Wurie FB, Cooper V, Horne R, Hayward AC. Determinants of non-adherence to treatment for tuberculosis in high-income and middle-income settings: a systematic review protocol. *BMJ Open*. 2018;8(1):e019287.

36. Apostu M, Mihaescu T. Respiratory functional changes in pulmonary tuberculosis [in Romanian]. *Pneumologia*. 2013;62(3):148-157.

37. Vorselaars AD, Crommelin HA, Deneer VH, et al. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J*. 2015;46(1):175-185.

38. Schimmelpennink MC, Vorselaars ADM, van Beek FT, et al. Efficacy and safety of infliximab biosimilar Inflectra® in severe sarcoidosis. *Respir Med*. 2018;138S:S7-S13.