Original Investigation

Oral Antimycobacterial Therapy in Chronic Cutaneous Sarcoidosis
A Randomized, Single-Masked, Placebo-Controlled Study

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IMPORTANCE Sarcoidosis is a chronic granulomatous disease for which there are limited therapeutic options. This is the first randomized, placebo-controlled study to demonstrate that antimycobacterial therapy reduces lesion diameter and disease severity among patients with chronic cutaneous sarcoidosis.

OBJECTIVE To evaluate the safety and efficacy of once-daily antimycobacterial therapy on the resolution of chronic cutaneous sarcoidosis lesions.

DESIGN AND PARTICIPANTS A randomized, placebo-controlled, single-masked trial on 30 patients with symptomatic chronic cutaneous sarcoidosis lesions deemed to require therapeutic intervention.

SETTING A tertiary referral dermatology center in Nashville, Tennessee.

INTERVENTIONS Participants were randomized to receive either the oral concomitant levofloxacin, ethambutol, azithromycin, and rifampin (CLEAR) regimen or a comparative placebo regimen for 8 weeks with a 180-day follow-up.

MAIN OUTCOMES AND MEASURES Participants were monitored for absolute change in lesion diameter and decrease in granuloma burden, if present, on completion of therapy.

OBSERVATIONS In the intention-to-treat analysis, the CLEAR-treated group had a mean (SD) decrease in lesion diameter of −8.4 (14.0) mm compared with an increase of 0.07 (3.2) mm in the placebo-treated group (P = .05). The CLEAR group had a significant reduction in granuloma burden and experienced a mean (SD) decline of −2.9 (2.5) mm in lesion severity compared with a decline of −0.6 (2.1) mm in the placebo group (P = .02).

CONCLUSIONS AND RELEVANCE Antimycobacterial therapy may result in significant reductions in chronic cutaneous sarcoidosis lesion diameter compared with placebo. These observed reductions, associated with a clinically significant improvement in symptoms, were present at the 180-day follow-up period. Transcriptome analysis of sarcoidosis CD4+ T cells revealed reversal of pathways associated with disease severity and enhanced T-cell function following T-cell receptor stimulation.

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Antimycobacterial Therapy in Sarcoidosis

C utaneous sarcoidosis is one of the most common extrapulmonary manifestations of pulmonary sarcoidosis in the United States. It is difficult to treat. Current treatment algorithms for cutaneous sarcoidosis support the use of corticosteroids, tumor necrosis factor inhibitors, antimarialirs, and antimetabolites. Despite universal acceptance as standard care, the aforementioned treatments often result in an incomplete clinical response or unacceptable adverse events. In such situations, more innovative treatment options may be investigated. A growing body of literature supports the immunomodulatory effects of antimicrobial therapy, such as quinolones increasing interleukin 2 (IL-2) production in monocytes and macrolides decreasing neutrophil chemotaxis. Case reports show improvement of cutaneous sarcoidosis lesions with antibiotic therapy, such as tetracyclines.

Numerous agents have been attributed to sarcoidosis pathogenesis, such as serum amyloid A, propionibacteria, and mycobacteria. Because of the possible association between sarcoidosis and mycobacterial antigens, we postulated that broad-spectrum antimycobacterial therapy could lead to clinical improvement of chronic cutaneous sarcoidosis lesions through immunomodulation of T-cell function. Independent molecular and immunologic investigations strengthen the association between mycobacterial antigens and sarcoidosis pathogenesis. Molecular analysis of sarcoidosis granulomas has revealed the presence of mycobacterial DNA and proteins that are significantly absent from granulomatous controls. Mycobacterial DNA has been detected in cutaneous sarcoidosis lesions, in addition to systemic immune responses against mycobacterial antigens. We investigated the safety and efficacy of concomitant levofloxacin, ethambutol, azithromycin, and rifampin (CLEAR) therapy among patients with chronic cutaneous sarcoidosis, with a change in lesion diameter from baseline to completion of 8 weeks of therapy as the primary end point; we assessed for decreases in granuloma burden., if granulomas were evident on histologic examination. Change in the modified Sarcoidosis Activity Severity Index (SASI) was the secondary end point.

Methods

Protocol
This study was a randomized, single-masked, placebo-controlled trial of the effectiveness of adding antimycobacterial therapy or placebo to a standard regimen among patients with chronic cutaneous sarcoidosis. Patients with cutaneous sarcoidosis were enrolled, regardless of whether they had received therapy, if they were 18 years or older and had clinically active chronic lesions. Patients with clinically active sarcoidosis had active disease, as evidenced by expansion of the existing lesion, progressive induration, erythema, or desquamation of the existing lesion or the development of new lesions in previously unaffected areas. Sarcoidosis was defined according to the statement by the American Thoracic Society, European Respiratory Society, and World Association for Sarcoidosis and Other Granulomatous Disorders. Volunteers were ineligible if they were allergic to or had potential interactions with one of the antibiotics in the CLEAR regimen or had had a change in their immunosuppressive regimen within the past 3 months. The inclusion and exclusion criteria are provided in the clinicaltrials.gov registration of the study (http://clinicaltrials.gov/ct2/show/NCT01074554). The study protocol was approved by the institutional review board at the Vanderbilt University Human Research Protection Program. Declaration of Helsinki protocols were followed, and all patients provided written informed consent before enrolling in the study. Participants completed 3 study visits. At baseline, the index lesion was originally selected by the lead dermatologist (L.E.K.) and confirmed by the patient that this lesion had been identified by his or her private dermatologist as cutaneous sarcoidosis. The index lesion was biopsied immediately at study enrollment and again following completion of the 8-week regimen. Direct measurement in a region unaffected by biopsy and photography of the lesion were obtained at baseline and at 4 and 8 weeks, before each biopsy. Characterization of lesion severity was conducted independently by 2 authors (L.E.K. and W.P.D.) using the modified SASI, measuring erythema, induration, and desquamation at baseline and at 4 and 8 weeks. The modification was that the same scale was applied to any part of the body, instead of the face alone, as originally described. At weeks 4 and 8, participants had complete blood count and complete metabolic profile tests to assess for toxicity and were interviewed regarding adverse events, medication diary review, and pill count to determine compliance with the regimen. The participants were followed up for 180 days from baseline. Histologic analysis of the biopsy specimens for granulomas was conducted by the lead pathologist (A.S.B.). All investigators (A.S.B., L.E.K., and W.P.D.) were masked to the treatment arm. All data were collected at Vanderbilt University Medical Center.

Enrolled participants were randomized in a 1:1 ratio by the biostatistician (C.Y.) to receive either placebo or 8 weeks of oral antimycobacterial therapy, in addition to their standard therapy. The treatment assignment and individual subject identification number were assigned at the time of enrollment via a computer-generated randomization process. Medications were dispensed by the Vanderbilt Investigation Drug Pharmacy. Antibiotic dosages were based on guidelines from the American Thoracic Society and Infectious Diseases Society of America for the treatment of atypical mycobacterial infection. The 8-week regimen consisted of levofloxacin,750 mg on day 1, followed by 500 mg/d; ethambutol, 25 mg/kg/d up to a maximum dosage of 1200 mg/d; azithromycin, 500 mg on day 1, followed by 250 mg/d; and rifampin, 10 mg/kg/d up to a maximum dosage of 300 mg/d. The placebo regimen consisted of rifobavillin (in place of rifampin) and lactose (in place of the other 3 drugs). Treatment with study drugs was stopped upon reaching one of the following conditions: drug toxicity, pill burden, failure to maintain study visits, or completion of the 8-week regimen.

Outcomes
The primary outcome was change in lesion diameter after the completion of therapy as a continuous end point. If there was evidence of granuloma formation on histologic examination,
reduction in granuloma burden was also assessed. A secondary end point was change in modified SASI. A 3-point or greater decline in SASI at the completion of therapy was considered clinically significant, due to the observation of reduced symptoms. With a sample size of 32 patients and a 5% significance level, the study was expected to have a 92% probability of detecting a therapeutic benefit if the true difference in benefit was 60% (from 10% to 70%) with antimycobacterial therapy.

Molecular Microarray Analysis
RNA Isolation
Samples were obtained from patients at baseline and after receiving 8 weeks of antibiotics. Total RNA was extracted from CD4+ and CD8+ sorted T cells using the miRNeasy Mini Kit (QIAGEN). The quantity and quality were checked on the NanoDrop and Bioanalyzer (both from Agilent Technologies), respectively.

Gene Expression Microarrays
RNA was labeled and hybridized to Agilent 8X60K whole human genome microarrays and analyzed as previously described. The complete microarray data have been deposited in the Gene Expression Omnibus (GSE39606).

T-Cell Function Assay
For T-cell receptor stimulation, $2 \times 10^5$ CD4+ T cells were stimulated through their receptor by plate-bound anti-CD3 and soluble anti-CD28 antibodies. Extracellular cytokine expression and T-cell proliferation were determined as previously described.

Statistical Analysis
We performed intention-to-treat analyses. An analysis of differences in lesion size at baseline and 8 weeks in individual subjects between treatment groups, as well as between-group comparisons on a continuous end point, was conducted using the Wilcoxon rank sum test. Continuous variables were also compared between baseline and week 8 within treatment arm using the Wilcoxon signed rank test. Laboratory data are presented as means and standard deviations, unless otherwise stated. All tests are 2-tailed. Statistical analyses were performed using the statistical package SAS for Windows (version 9; SAS Institute) and the statistical software R (www.r-project.org).

Results
Characteristics and Randomization of the Study Population
The 30 patients enrolled in the cutaneous sarcoidosis study had clinical or histologic evidence of chronic sarcoidosis previously documented. Twenty-two patients had biopsy-proven cutaneous disease and 8 had biopsy-proven pulmonary disease with dermatologic lesions consistent with cutaneous sarcoidosis. The histologic diagnosis of cutaneous sarcoidosis was confirmed in 7 of 8 patients; 1 patient did not have lesions consistent with cutaneous sarcoidosis at study enrollment. En-

| Table 1. Baseline Characteristics of the Cutaneous Sarcoidosis Study Population |
|-------------------------------|-----------------|-----------------|
| Characteristic                | Total (N = 29)  | CLEAR (n = 14)  | Placebo (n = 15) |
| Age, y                        | 51              | 50              | 52              |
| Median (IQR)                  | 53 (42-58)      | 55 (41-60)      | 53 (42-57)      |
| Range                         | 23-72           | 23-63           | 33-72           |
| Sex, No. (%)                  |                 |                 |                 |
| Male                          | 10 (34)         | 5 (36)          | 5 (33)          |
| Female                        | 19 (66)         | 9 (64)          | 10 (67)         |
| Race, No. (%)                 |                 |                 |                 |
| White                         | 18 (62)         | 9 (64)          | 9 (60)          |
| African American              | 11 (38)         | 5 (36)          | 6 (40)          |
| Years since histologic diagnosis | (N = 22)      | (n = 12)        | (n = 10)        |
| Mean                          | 8.3             | 9.2             | 7.2             |
| Median (IQR)                  | 5 (3-9)         | 4 (3-10)        | 6 (3-9)         |
| Range                         | 1-40            | 1-40            | 1-18            |
| Concomitant therapy, No. (%)  |                 |                 |                 |
| Corticosteroids only          | 1 (3)           | 1 (7)           | 0               |
| Immunomodulators only         | 8 (28)          | 2 (14)          | 6 (40)          |
| Corticosteroids + immunomodulators | 3 (11)       | 1 (7)           | 2 (13)          |
| Corticosteroid dose (daily prednisone dose), mg | | | |
| Mean (range)                  | 11 (1.5-40)     | 12.5 (5-20)     | 15 (1.5-40)     |
| Lesion diameter, mm           |                 |                 |                 |
| Mean                          | 22.4            | 17.1            |                 |
| Median (IQR)                  | 12.5 (7-14)     | 10 (7-23)       |                 |
| Range                         | 2-97            | 4-60            |                 |
| SASI                          |                 |                 |                 |
| Mean                          | 4.9             | 4.6             |                 |
| Median (IQR)                  | 5 (3-7)         | 4 (3-6)         |                 |
| Range                         | 1-8             | 1-10            |                 |

Abbreviations: CLEAR, concomitant levofloxacin, ethambutol, azithromycin, and rifampin; IQR, interquartile range; SASI, Sarcoidosis Activity Severity Index.
Enrollment occurred from February 10, 2010, through October 20, 2010, in Nashville. Of the 30 patients, the mean age was 51 years, 19 of 30 were women, and 19 of 30 were white; 10 of 30 were using immunosuppressants. With regard to immunosuppression, 8 of 15 patients randomized to placebo were using immunosuppressants compared with 4 of 15 randomized to the CLEAR regimen (Table 1). The immunosuppressants used among the 8 patients randomized to placebo were methotrexate (n = 1), pentoxifylline (n = 1), hydroxychloroquine (n = 3), remicade (n = 1), or prednisone and methotrexate (n = 2). The immunosuppressant regimen of the 4 patients randomized to the CLEAR regimen was prednisone (n = 1), methotrexate (n = 1), hydroxychloroquine (n = 1), and prednisone and hydroxychloroquine (n = 1). The mean time in years since their histologic diagnosis was 7 years. There were no significant differences between the placebo and treatment groups with respect to age, sex, ethnicity, immunosuppression use, or years since histologic diagnosis (Table 1).

The clinical diagnosis of the 15 patients randomized to the CLEAR regimen was as follows: macular sarcoidosis (n = 2), annular sarcoidosis (n = 3), erythematous papular sarcoidosis (n = 6), plaque stage sarcoidosis (n = 1), subcutaneous granulomatous sarcoidosis (n = 1), and ulcerative sarcoidosis (n = 1). One patient who was randomized to the CLEAR regimen did not have lesions compatible with sarcoidosis at baseline. The lesions were believed to be more consistent with chronic inflammatory changes from repeated cutaneous injections. Because sarcoidosis is a diagnosis of exclusion, we did not proceed with study participation in this patient. The clinical diagnoses of 15 participants randomized to placebo were as follows: annular sarcoidosis (n = 3), erythematous papular sarcoidosis (n = 3), subcutaneous granulomatous sarcoidosis (n = 5), plaque stage sarcoidosis (n = 1), ulcerative sarcoidosis (n = 1), and lupus pernio (n = 2). There was no significant difference in any form of cutaneous sarcoidosis between the CLEAR regimen and placebo cohort. Assessment for latent tuberculosis by skin testing was performed at the discretion of the primary care physician. Fourteen patients in the placebo-treated group and 14 patients in the CLEAR-treated group had been tested previously. All were negative for the purified protein derivative of tuberculin. All 30 patients denied a history of tuberculosis or a known tuberculosis exposure.

Thirty patients were randomized to either the CLEAR regimen (n = 15) or placebo (n = 15), with 11 of 15 patients (73%) in each group completing 8 weeks of therapy. Reasons for study discontinuation are outlined in Figure 1.

**Effects of Treatment on Lesion Diameter and SASI**

Of the patients randomized to the CLEAR regimen, 10 had evidence of granulomas on biopsy of the index lesion, with 7 of 10 having a reduction in lesion diameter and granulomas. The remaining 3 patients did not complete the 8-week regimen. Seven of the 15 patients in the placebo cohort had granulomas on biopsy; none had a reduction in lesion diameter and granuloma burden. For the primary end point of change in lesion size, as evidenced by a reduction in lesion diameter and number of granulomas, 7 of 10 patients randomized to the CLEAR regimen had a reduction in lesion size and granuloma burden.
CLEAR regimen met this benchmark compared with 0 of 7 randomized to placebo (P = .01, Fisher exact test).

In the intention-to-treat analysis, the 14 patients randomized to the CLEAR regimen demonstrated a mean (SD) decrease in index lesion size from 22.4 (24.9) mm to 14.0 (25.8) mm, reflecting a decrease of −8.4 (14.0) mm (P = .008). Of the 15 patients randomized to placebo, the index lesion diameter changed from a mean (SD) of 17.1 (16.0) mm to 17.9 (16.0) mm, reflecting an increase of 0.07 (3.2) mm (P = .87). While there was no significant difference in lesion diameter between the 2 groups at baseline (P = .62), there was a significant difference in the absolute change in lesion diameter after 8 weeks of therapy. The CLEAR-treated group had a mean (SD) absolute difference of −8.4 (14.0) mm compared with an increase of 0.07 (3.2) mm in the placebo-treated group (P = .05). In the per-protocol analysis, there were 11 patients in each group. Nine of 11 patients in the CLEAR-treated group had reductions in lesion diameter compared with 2 of 11 patients in the placebo group (P = .009, Fisher exact test). After 8 weeks of the CLEAR regimen or placebo, the index lesion diameter decreased from a mean (SD) of 25.8 (27.1) mm to 15.2 (29.1) mm (P = .008) in 11 patients receiving the CLEAR regimen and from 15.5 (11.9) mm to 15.6 (12.9) mm (P = .87) among the 11 patients in the placebo-treated group (Table 2 and Table 3). While there was no significant difference in lesion diameter between the 2 groups at baseline (P = .40), there was a significant difference in the absolute difference in lesion diameter after 8 weeks of therapy. The CLEAR-treated cohort had a mean (SD) absolute difference of −10.6 (15.1) mm compared with 0.1 (3.7) mm in the placebo-treated group (P = .04). Five patients randomized to the CLEAR regimen had complete resolution of their lesions compared with 1 in the placebo-treated group (Figure 2A); there was no recurrence of lesions at the 180-day follow-up.

Table 2. Intention-to-Treat Analysis of the Effects of the CLEAR Regimen on Index Lesion Diameter, SASI, and ALT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CLEAR (n = 14)</th>
<th>Placebo (n = 15)</th>
<th>Absolute Difference</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Absolute Difference</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion diameter, mm</td>
<td>22.4 (24.9)</td>
<td>14.0 (25.8)</td>
<td>−8.4 (14.0)</td>
<td>.05</td>
<td>.008</td>
<td>17.1 (16.0)</td>
<td>17.9 (16.0)</td>
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<tr>
<td>SASI</td>
<td>4.9 (2.1)</td>
<td>2.0 (2.2)</td>
<td>−2.9 (2.5)</td>
<td>.02</td>
<td>.002</td>
<td>4.6 (2.3)</td>
<td>4.0 (2.2)</td>
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<tr>
<td>ALT</td>
<td>24.6 (17.4)</td>
<td>21.3 (8.9)</td>
<td>10.6</td>
<td>.71</td>
<td>.17</td>
<td>27.1 (14.8)</td>
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</table>

Abbreviations: ALT, alanine aminotransferase; CLEAR, concomitant levofloxacin, ethambutol, azithromycin, and rifampin; SASI, Sarcoidosis Activity Severity Index.

<sup>a</sup> Statistical difference between 8 weeks of CLEAR therapy or placebo.

<sup>b</sup> Statistical difference in lesion diameter and severity within treatment group after completion of therapy.

Table 3. Per-Protocol Analysis of the Effects of the CLEAR Regimen on Index Lesion Diameter, SASI, and ALT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CLEAR (n = 11)</th>
<th>Placebo (n = 11)</th>
<th>Absolute Difference</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Absolute Difference</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Lesion diameter, mm</td>
<td>25.8 (27.1)</td>
<td>15.2 (29.1)</td>
<td>−10.6 (15.1)</td>
<td>.04</td>
<td>.008</td>
<td>15.5 (11.9)</td>
<td>15.6 (12.9)</td>
</tr>
<tr>
<td>SASI</td>
<td>5.7 (1.5)</td>
<td>2.0 (2.4)</td>
<td>−3.7 (2.2)</td>
<td>.02</td>
<td>.002</td>
<td>4.5 (2.5)</td>
<td>3.7 (2.3)</td>
</tr>
<tr>
<td>ALT</td>
<td>24.6 (17.4)</td>
<td>21.3 (8.9)</td>
<td>10.6</td>
<td>.90</td>
<td>.17</td>
<td>27.6 (15.4)</td>
<td>22.5 (6.1)</td>
</tr>
</tbody>
</table>

Abbreviations: See Table 2.

<sup>a</sup> Statistical difference between 8 weeks of CLEAR therapy or placebo.

<sup>b</sup> Statistical difference in lesion diameter and severity within treatment group after completion of therapy.

Figure 2. Change in Lesion Diameter and Sarcoidosis Activity Severity Index (SASI) From Baseline to 8 Weeks

A, Change in lesion diameter was apparent in those randomized to the concomitant levofloxacin, ethambutol, azithromycin, and rifampin (CLEAR) regimen compared with the cohort taking placebo, with complete resolution of the cutaneous lesions among 5 patients in the CLEAR group. B, A significant decline in the SASI was observed in the CLEAR group; minimal change was observed in the placebo group. Each colored bar represents an individual study participant.
This reduction in lesion diameter was associated with a decrease in symptoms. A secondary end point was change in lesion severity, as determined by the SASI, which measures erythema, induration, and desquamation. In the intention-to-treat analysis, 9 of 14 patients in the CLEAR-treated group had more than a 3-point drop in SASI compared with 3 of 15 placebo-treated patients (P = .03, 2-tailed Fisher exact test). The index lesion severity decreased from a mean (SD) of 4.9 (2.1) mm to 2.0 (2.2) mm (P = .002) in the CLEAR-treated group and from 4.6 (2.3) mm to 4.0 (2.2) mm (P = .34) in the placebo-treated group (Table 2 and Figure 2B). While there was no significant difference in lesion severity between the placebo-treated and CLEAR-treated patients at baseline (P = .53), there was a significant difference in the lesion severity between the 2 cohorts at 8 weeks (P = .03). The CLEAR-treated group experienced a mean (SD) decline of -2.9 (2.5) mm in lesion severity compared with a decline of -0.6 (2.1) mm among patients randomized to placebo (P = .02). In the per-protocol analysis, 9 of 11 CLEAR-treated patients experienced more than a 3-point decline in SASI compared with 3 of 11 patients in the placebo group (P = .03, 2-tailed Fisher exact test). The reduction in lesion diameter and SASI seen with the CLEAR regimen was associated with reductions in granulomatous inflammation per the lead pathologist (A.S.B.), who reviewed all slides of each participant while masked to the treatment arm. A photograph demonstrating the observed clinical improvement is provided (Figure 3).

Transcriptional Signatures Associated With Cutaneous Resolution

To determine the mechanisms associated with clinical improvement following the CLEAR regimen, we conducted transcriptome analysis of CD4+ and CD8+ T cells of patients randomized to the CLEAR or placebo regimen at baseline compared with completion at 8 weeks. No changes in gene expression were detected in the patients who were administered the placebo regimen. Significant distinctions in expression were noted in those randomized to the CLEAR regimen. On comparing baseline transcriptome expression with transcriptome expression after completion of the CLEAR regimen, 443 and 352 genes were differentially expressed (Figure 4A and B). Relevant functional categories in both data sets were statistically significant, including antigen presentation and immune cell responses (Figure 4C and D). Enriched pathways included normalization of those previously reported to contribute to sarcoidosis pathogenesis, such as Wingless and integrase-1 (wnt/β-catenin) signaling and the Jak-Stat pathway (Figure 4 and Table 4).

In many chronic cutaneous diseases, a fundamental contributor to disease progression is reduced IL-2 production and T-cell proliferation. We postulated that alterations in gene expression seen with the CLEAR regimen would be associated with improvement in CD4+ T-cell biological function, exhibited by increased interferon γ (IFN-γ) expression and cellular proliferation. Measurement of IL-2 and IFN-γ production following T-cell receptor stimulation revealed an increase in expression after completion of the CLEAR regimen. The increase in IL-2 expression was not significant (P = .07); however, the increase in IFN-γ production was significant (P = .04) (Figure 5A). There was not a significant increase in IL-2 or IFN-γ expression among the patients with sarcoidosis who were randomized to the placebo regimen. In addition, we measured CD4+ T-cell proliferation following T-cell receptor stimulation. We noted a significant increase in proliferative capacity among those randomized to the CLEAR regimen (P = 2 × 10^{-7}) but not among those randomized to placebo (P = .95) (Figure 5B and C).

Adverse Events

Six of the 30 patients with chronic cutaneous sarcoidosis (3 in the CLEAR-treated group and 3 in the placebo-treated group) experienced an adverse event that resulted in study withdrawal. Among those randomized to the CLEAR regimen, 1 developed diarrhea, 1 had joint pain, and 1 had insomnia. A fourth patient was asked to withdraw because, upon examination, cutaneous lesions were not consistent with sarcoidosis. Among those randomized to placebo, 1 developed diarrhea, 1 had joint pain, and 1 withdrew because of pill burden. One patient denied any adverse events but did not maintain any study visits after enrollment. Review of baseline and week 8 alanine aminotransferase of the patients as a cohort did not reveal any significant differences (Table 2).

Discussion

Cutaneous sarcoidosis is a chronic granulomatous disease characterized by genetic susceptibility and CD4+ T-cell predominance. To our knowledge, this phase I study is the first ran-
Figure 4. Gene Expression Profile Changes Following Concomitant Levofloxacin, Ethambutol, Azithromycin, and Rifampin (CLEAR) Treatment

Table 4. List of Enriched Pathways in CD4+ and CD8+ Sorted Cells

<table>
<thead>
<tr>
<th>Pathway</th>
<th>P Value</th>
<th>Differentially Expressed Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon signaling</td>
<td>&lt;.01</td>
<td>BAX, IFNA21, and PIAS1</td>
</tr>
<tr>
<td>NOD-like receptor signaling</td>
<td>&lt;.01</td>
<td>NLRRC4, CCL13, MAPK12, MAPK9, RIPK2, and NAIP</td>
</tr>
<tr>
<td>Arginase and proline metabolism</td>
<td>.06</td>
<td>NOS1, GATM, PRODH, and CKB</td>
</tr>
<tr>
<td>Endocytosis</td>
<td>.10</td>
<td>RET, RABSC, ACAP1, HLA-A, RABSA5, IQSEC3, and SH3GL2</td>
</tr>
<tr>
<td>Jak-Stat signaling</td>
<td>&lt;.01</td>
<td>PIAS1 and PIAS4</td>
</tr>
<tr>
<td>MAPK</td>
<td>&lt;.01</td>
<td>UBB, CCKBR, MAPK9, RPS6KA3, ITPKB, RIPK2, TDALK, RET, and MAPK12</td>
</tr>
<tr>
<td>MIF regulation of innate immunity</td>
<td>&lt;.01</td>
<td>MAPK9 and MAPK12</td>
</tr>
</tbody>
</table>

Abbreviations: MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; NOD, nitric oxide synthase.

domized, placebo-controlled, masked study to demonstrate that antimycobacterial therapy reduces lesion diameter and disease severity among patients with chronic cutaneous sarcoidosis. Decreased severity was defined as reductions in erythema, induration, or desquamation after completion of therapy. Clinical resolution was associated with alterations of the sarcoidosis CD4+ immunologic transcriptome. The major benefit was reduction in lesion diameter, as well as severity. All enrolled patients had recalcitrant disease that was clinically active, whether receiving therapy or not. Notably, clinical improvement was observed despite the variant of chronic cutaneous sarcoidosis. The observed improvement with the 8-week CLEAR regimen was still present 180 days after baseline among all but 1 patient, despite no changes in their immunosuppressant regimen.

In defining the molecular mechanisms associated with clinical improvement, we conducted a gene expression microarray of participants at baseline and following completion of their regimen. No significant alterations in gene expression were observed among those randomized to placebo, even if they were using immunosuppressant therapy. Among patients randomized to the CLEAR regimen, analysis of the immunologic transcriptome identified enrichment in terms of pathways associated with innate and adaptive immune response, cytokine expression, whether or not they were using immunosuppressant therapy. A significant portion of the genes identified were involved in the innate and adaptive immune response, such as the interferon signaling pathway (P < .01), with genes such as BAX (OMIM 600040), IFNA21 (OMIM 147584), and PIAS1 (OMIM 603566); Jak-Stat signaling (P < .01); and mitogen-activated protein kinase (P < .01) (Table 3). All of these categories contain genes involved in the immune response, which are important in diseases such as psoriasis. Additional investigations support the role of the Jak-Stat pathway in sarcoidosis specimens. Microarray analysis has confirmed reductions in Wnt/β-catenin expression among patients improving with CLEAR therapy (Figure 4). Increases in Wnt expression also have been seen with sarcoidosis progression. Antibiotics are known to exert effects on the immune transcriptome. Levofloxacin affects intracellular cyclic adenosine monophosphate, phosphodiesterases, nuclear factor-κB, and activator protein 1, as well as IL-2 production by monocytes. Azithromycin decreases neutrophil chemotaxis and reduces tumor necrosis factor production in lipopolysaccharide-stimulated...
monocytes by modifying p38 signaling pathways. Rifampin inhibits monocyte secretion of IL-1β and tumor necrosis factor and increases secretion of IL-6 and IL-10. The presence of amelioration of the transcriptome supports that immunoregulation is a mechanism by which the CLEAR regimen has efficacy. Because no viable infectious agent has been associated with sarcoidosis pathogenesis, it is unclear whether the regimen works through antimicrobial effects; however, further investigation into this possibility is warranted.

We also investigated whether the observed alterations in the T-cell transcriptome carried functional significance. Consistent with observations of enhanced CD4+ T-cell function and clinical resolution following antimicrobial therapy, we noted enhanced Th1 cytokine expression and proliferative capacity in the CD4+ T cells of patients with sarcoidosis experiencing clinical improvement.

Despite the observed clinical benefit, there are some limitations to this trial. First, there were only 30 participants. We chose this number because it was statistically predicted to show a benefit. We were reluctant to needlessly expose a larger number of patients to a regimen with known toxic effects. Second, the dropout rate was 4 of 15 in both treatment arms. This adherence rate is consistent with prior reports of nonadherence ranges of 17.5% to 50% among patients using a 4-drug antimycobacterial regimen. The same factors associated with nonadherence in those trials, including number of pills and toxicity profile, contributed to our dropout rate. In future trials, we will consider continued participation with 3 of the 4 drugs if an enrollee has a toxic reaction to a single agent. We noted no evidence of rifampin-induced hepatotoxicity.

A third study limitation is that 8 of 15 patients in the placebo-treated group were using immunosuppressant therapy compared with 4 of 15 on the CLEAR regimen (Table 1). Although each patient was randomized at the time of presentation, this could imply that the control group had more difficult-to-treat disease. We did not observe distinctions in the lesion severity as measured by the SASI; in fact, the SASI was higher in the CLEAR-treated group, suggesting that they had more severe manifestations of disease, possibly because fewer patients were using immunosuppressant therapy. Future studies will involve a crossover arm to assess the effects of the regimen regardless of the form of cutaneous sarcoidosis.
Great efforts are needed to identify therapeutics that will effectively alleviate chronic cutaneous sarcoidosis. The CLEAR regimen leads to lesion regression and severity of symptoms in a cohort of patients with chronic cutaneous sarcoidosis. A multicenter trial of a larger cohort should be conducted to confirm these findings, further delineate the adverse effect profile, and determine optimal dosing and patient selection.

REFERENCES


**NOTABLE NOTES**

### Hippocrates on Ulcers

Faisal R. Ali, MA, MRCP; James Fox, MA, PhD; Alexander E. T. Finlayson, BMedsSci, MRCP

The father of medicine, Hippocrates, is arguably the most renowned and enduring of all physicians. He constructed the eponym on which all physicians swear allegiance. Residing in the Greek island of Kos from 460 BC to 370 BC, Hippocrates was a gifted orator and philosopher. In the later years of his career, he devoted time to establishing a practice of medicine that sought to describe diseases in a natural, scientific way, in contrast to the sorcery and superstition that dominated Greek medicine at that time. In the fourth century BC, the Corpus Hippocraticum was compiled by his followers and comprises a heterogeneous collection of essays relating to various bodily systems.

One chapter is devoted to the care of ulcers and contains a plethora of suggested remedies and medicaments to be deployed in various circumstances (we cannot do justice to all of them herein). For ulcers, he claimed that the principal aim was to simulate normality, with dry ulcers being nearer to the sound and wet to the unsound.2

For inflamed swellings and ulcers requiring cleansing, Hippocrates recommended boiled mullein plant, raw leaves of the trefoil, and boiled leaves of the epipetrum and polye, or, alternatively, boiled leaves of the fig tree, olive, and horehound. In cases in which there was a risk of infection overriding the ulcer, he proposed that linseed with leaves of a wood or the juice of strychnos be applied as a cataplasm. For clean ulcers with inflamed surrounding skin, he recommended finely ground lentils boiled in wine.

Certain body parts were afforded dedicated treatments. Wounds of the head, ears, and penis could be treated with a combination of dried ox bile, honey, white wine, lotus shavings, frankincense, and myrrh. As an emollient in winter, Hippocrates recommended goose and pig fat blended with squill and oil.

Lessons from this work remain pertinent to today’s physician, including the elevation of bleeding limbs to assuage exsanguination. While visual descriptions of ulcers are scant, the advice to rest and avoid standing remains relevant advice for current patients with varicose ulcers. Contemporary dermatologists may be accused of overlooking the Hellenistic advice that ulcers benefit from a spare diet, the summer season, and purging of the bowels.

Above all, Hippocrates’ work underlines the perpetual need for physicians to use evidence-based treatments, their responsibility to contribute to that evidence base, and, where necessary, to challenge the prevailing dogma in pursuit of clinical excellence.

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