

# Cibinetide Improves Corneal Nerve Fiber Abundance in Patients With Sarcoidosis-Associated Small Nerve Fiber Loss and Neuropathic Pain

Daniel A. Culver,<sup>1</sup> Albert Dahan,<sup>2</sup> Daiva Bajorunas,<sup>3</sup> Maria Jeziorska,<sup>4</sup> Monique van Velzen,<sup>2</sup> Leon P. H. J. Aarts,<sup>2</sup> Jinny Tavee,<sup>5</sup> Martijn R. Tannemaat,<sup>6</sup> Ann N. Dunne,<sup>3</sup> Rita I. Kirk,<sup>3</sup> Ioannis N. Petropoulos,<sup>7</sup> Anthony Cerami,<sup>3</sup> Rayaz A. Malik,<sup>7</sup> and Michael Brines<sup>3</sup>

<sup>1</sup>Pulmonary Medicine, Cleveland Clinic, Cleveland, Ohio, United States

<sup>2</sup>Anesthesiology, Leiden University Medical Center, Leiden, The Netherlands

<sup>3</sup>Araim Pharmaceuticals, Tarrytown, New York, United States

<sup>4</sup>Division of Cardiovascular Sciences, University of Manchester, Manchester, United Kingdom

<sup>5</sup>Neuromuscular Center, Cleveland Clinic, Cleveland, Ohio, United States

<sup>6</sup>Neurology, Leiden University Medical Center, Leiden, The Netherlands

<sup>7</sup>Weill Cornell Medicine-Qatar, Doha, Qatar

Correspondence: Daniel A. Culver, Department of Pulmonary Medicine and Department of Pathobiology, Respiratory Institute, Cleveland Clinic, 9500 Euclid Avenue, Desk A90, Cleveland OH 44195, USA; culverd@ccf.org.

Submitted: December 13, 2016

Accepted: March 27, 2017

Citation: Culver DA, Dahan A, Bajorunas D, et al. Cibinetide improves corneal nerve fiber abundance in patients with sarcoidosis-associated small nerve fiber loss and neuropathic pain. *Invest Ophthalmol Vis Sci*. 2017;58: BIO52-BIO60. DOI:10.1167/ iovs.16-21291

**PURPOSE.** Sarcoidosis frequently is complicated by small nerve fiber loss (SNFL), which can be quantified using corneal confocal microscopy (CCM). Prior studies suggest that the innate repair receptor agonist cibinetide reverses corneal nerve loss. This phase 2b, 28-day, randomized trial of 64 subjects with sarcoid-associated SNFL and neuropathic pain assessed the effect of cibinetide on corneal nerve fiber area (CNFA) and regenerating intraepidermal fibers (GAP-43<sup>+</sup>) as surrogate endpoints for disease modification, pain severity, and functional capacity (6-minute walk test [6MWT]).

**METHODS.** Cibinetide (1, 4, or 8 mg/day) was compared to placebo. The primary study endpoint was a change in CNFA at 28 days.

**RESULTS.** The placebo-corrected mean change from baseline CNFA ( $\mu\text{m}^2$ ) at day 28 was 109 (95% confidence interval [CI], -429, 647), 697 (159, 1236;  $P = 0.012$ ), and 431 (-130, 992) in the 1, 4, and 8 mg groups, respectively. Intraepidermal GAP-43<sup>+</sup> fibers increased in the 4 mg group ( $P = 0.035$ ). Further, changes in CNFA correlated with changes in GAP-43<sup>+</sup> ( $\rho = 0.575$ ;  $P = 0.025$ ) and 6MWT ( $\rho = 0.645$ ;  $P = 0.009$ ). Pain improved significantly in all groups, with subjects having moderate-severe pain reporting a clinically meaningful placebo-corrected decrease in pain intensity in the 4 mg group ( $P = 0.157$ ).

**CONCLUSIONS.** Cibinetide significantly increased small nerve fiber abundance in the cornea and skin, consistent with a disease modifying effect. The relationships between CNFA and other clinical measures of disease support its use as a surrogate endpoint to assess potential disease modifying therapies for neuropathy.

Keywords: neuropathy, inflammation, tissue protection, ARA 290, helix B surface peptide

Small, unmyelinated C and thinly myelinated A $\delta$  nerve fibers mediate autonomic function, nociception, and thermal sensitivity. These nerve fibers are either selectively reduced or predate large fiber involvement in many diseases,<sup>1</sup> often resulting in significant, disabling neuropathic symptoms, especially pain. A common etiology underlying small nerve fiber loss (SNFL) may be chronic inflammation.<sup>2</sup> Sarcoidosis is an orphan disease of immune dysregulation and sustained inflammation characterized by pulmonary, skin, joint, nervous system, and eye involvement.<sup>3</sup> Although sarcoidosis often resolves spontaneously, many patients have persistent disease, and the majority of these suffer a painful neuropathy associated with SNFL.<sup>4</sup> Currently, to our knowledge there are no approved therapies to treat the underlying pathophysiology of SNFL and its symptoms remain poorly managed with analgesic, anticonvulsant, or antidepressant therapies.<sup>1</sup>

Inflammation occurs via a cascade of self-amplifying proinflammatory cytokines and chemokines, which drive

progressive tissue damage. Recently, a counter-regulatory mechanism, that limits damage while simultaneously activating tissue repair has been identified. This system is activated by the innate repair receptor (IRR) comprised of  $\beta$  common (CD131) and erythropoietin receptor subunits, which signals via janus kinase-2 and multiple downstream intracellular molecular pathways.<sup>5</sup> IRR activation promotes tissue protection and repair in a wide variety of preclinical models,<sup>5</sup> including neuropathy.<sup>6-8</sup>

Cibinetide (ARA 290; helix B surface peptide) is a novel 11 amino acid peptide with high affinity and selectivity for the IRR.<sup>9</sup> Despite a short plasma half-life, cibinetide triggers sustained biological effects when concentrations exceed the low nanomolar affinity of the receptor.<sup>5</sup> Notably, in a mouse model of diabetic SNFL, daily administration of cibinetide reversed neuronal dystrophy.<sup>7</sup> In neuropathic states the transient receptor potential vanilloid-1 (TRPV1) ion channel, a key integrator of nociception and neurogenic inflammation,



undergoes upregulation and sensitization in peripheral small nerve fibers and central pain pathways. Recent data demonstrate that cibinetide antagonizes the TRPV1 channel of small nerve fibers and relieves mechanical hypersensitivity.<sup>10</sup>

Three prior clinical studies have shown that cibinetide reduces neuropathic symptoms<sup>11</sup> and may promote nerve fiber repair<sup>12,13</sup> in patients with sarcoidosis or diabetes-related SNFL. Nerve fiber regeneration in two studies was evaluated using corneal confocal microscopy (CCM), an in vivo ophthalmic imaging modality that noninvasively visualizes nerve fibers within the cornea, including the small fibers of the sub-basal layer. CCM-assessed sub-basal corneal nerve status correlates with sensory nerve function and lower extremity intraepidermal nerve fiber density (IENFD).<sup>14</sup>

The present phase 2b clinical trial evaluated safety, efficacy, and dose range of 28-day subcutaneous (SC) administration of cibinetide on corneal nerve fiber area (CNFA) compared to placebo in subjects with sarcoidosis and painful SNFL. Secondary goals were to evaluate the effect of cibinetide on other objective cutaneous markers of SNFL, including intraepidermal nerve fiber density (IENFD), growth-associated protein-43 (GAP-43) positive nerve fiber length, neuropathic symptoms, and functional (6-minute walk test [6MWT]) endpoints. Additionally, potential relationships between CNFA and clinical endpoints were explored to provide information concerning the suitability of CNFA as a surrogate endpoint for SNFL.

## METHODS

### Study Design

The study was a double-blind, randomized, placebo-controlled, two-center assessment of the effects of daily SC administration of 3 doses of cibinetide or placebo for 28 days in sarcoidosis subjects with painful SNFL. The trial was registered (EudraCT [2013-003016-45]; Clinicaltrials.gov [NCT02039687]) and conducted in compliance with all regulatory requirements, including the tenets of the Declaration of Helsinki. Following approval of the Ethics Committees of Leiden University Medical Center and The Cleveland Clinic, all subjects provided informed consent. The trial commenced on January 24, 2014 and was completed on February 3, 2015.

After a  $\leq 28$ -day screening period, the subjects were randomized (1:1:1:1) to 4 treatment groups: 1, 4, or 8 mg cibinetide or placebo daily from a block randomization scheme generated by a unit independent from the study sites. On the basis of preclinical and human pharmacokinetic/pharmacodynamic data, 1 mg was expected to be an ineffective dose. Additionally, the 8 mg dose was expected to be similar to 4 mg, as preclinical neuropathy data suggest that higher doses of cibinetide do not further decrease pain behaviors if a threshold dose is exceeded.<sup>6</sup> The first subcutaneous administration of study drug occurred at the research site and thereafter it was self-administered daily for an additional 27 days. Subjects returned at weeks 2 and 4 for protocol-specified testing and safety assessment. Vials were returned to the pharmacy to determine study drug compliance.

### Study Population

Subjects were eligible for study participation if they were between 18 and 70 years of age with a diagnosis of sarcoidosis using established criteria<sup>15</sup> and SNFL based on: (1) a score of  $\geq 4$  on Brief Pain Inventory (BPI) "pain now" or "average pain" questions and (2) distal leg pain characterized by at least one of the following: dysesthesia, burning/painful feet worsening at

night, or intolerance of sheets/clothes touching the legs or feet. Additionally, subjects were required to meet either of the following two screening criteria: CNFA or IENFD from a distal leg biopsy obtained within the prior 2 years greater than 2 SD below the mean of a normative population.<sup>16,17</sup> Exclusion criteria included body mass index  $\geq 40$ , pregnant or breastfeeding females, a history of a serious malignancy, or use of biological anti-inflammatory agents or erythropoietin within 3 months before enrollment. Other medical conditions known to be associated with SNFL (except for diabetes in good control) were grounds for exclusion. An ophthalmologic history was obtained to identify corneal pathology that could potentially confound CCM-derived data. Patients carrying a formal diagnosis of dry eye ( $n = 2$ ) were not excluded from the study, since loss of corneal nerve fibers is itself associated with dry eye<sup>18</sup> and patients with sarcoidosis-associated SNFL frequently complain of dry eye.<sup>19</sup> As the use of contact lenses has not been associated with a reduction in corneal nerve fibers as assessed by CCM,<sup>20,21</sup> this was not a cause for study exclusion.

### Outcome Measures

The primary study endpoint was a change in corneal nerve fiber abundance at day 28 as determined by CCM. It currently is uncertain which single CCM parameter might best characterize change in corneal nerve fibers and, therefore, serve as a surrogate endpoint for disease modification. Quantification of CCM-derived corneal nerve morphology within the sub-basal plexus can be characterized using a variety of features, including fiber density, length, width (reflecting the variable number of C-fibers within each bundle), branching, tortuosity, and beading frequency. The area of the corneal nerve net, that is, the extent of the cornea surface area covered by sub-basal nerve fibers (CNFA), reflects a combination of these variables. Because corneal nerve fiber density is expressed as integer values normalized per unit area, and is characterized by a discrete distribution with relatively few numerical possibilities, it is relatively insensitive to small changes in the nerve fiber net. In contrast, corneal nerve fiber length (CNFL) and CNFA are calculated from a large number of pixels overlying the nerves, which approximates a continuous distribution and is more sensitive to changes within the nerve net. Although CNFL has been suggested to be an optimal single parameter for assessment of small nerve fiber damage,<sup>22</sup> it is a one-dimensional measure. Nerve fiber regeneration arises not only from elongation, but also branching, and widening of existing corneal nerve fibers, that is, it is a two-dimensional process, which will be better detected by quantification of CNFA. Notably, in a comparison of CCM variables obtained from a group of healthy subjects and patients with a wide range of neuropathic severity due to diabetes, CNFA most closely paralleled CNFL ( $r = 0.93$ ;  $P < 0.0001$ ;  $n = 101$ ; Supplementary Figs. S1, S2).

Corneal nerves were imaged as described previously,<sup>13</sup> using the Rostock Corneal Module of the Heidelberg Retinal Tomograph III (Heidelberg Engineering, Heidelberg, Germany) by one of three experienced investigators. Six to eight images of sub-basal corneal nerves of each eye were selected on the basis of image quality by a single analyst blinded to treatment (MB).<sup>23</sup> CNFA was quantified using a custom-developed macro for FIJI (<https://imagej.net/Fiji/downloads>), a public-domain image analysis program, version 1.47e. Corneal nerve fiber density (CNFD), corneal nerve fiber branch density (CNBD), and CNFL were calculated in a post hoc assessment using the automated program ACCmetrics (See Supplementary Materials for details). Analysis of the image data set from the present study confirmed the hypothesis that CNFA was a sensitive

assessment of longitudinal change of the corneal nerve fiber net and, therefore, appears to be a reasonable surrogate endpoint for disease modification in small fiber neuropathy (Supplementary Fig. S3).

Standard dermatologic skin biopsies of 3 mm diameter were obtained 10 cm above the lateral malleolus at baseline and on day 28 for IENFD quantification according to international guidelines.<sup>24</sup> Four randomly selected sections were stained in a free-floating protocol with anti-protein gene product 9.5, a pan-axonal marker. The sections were analyzed in a blinded fashion by an expert (MJ). GAP-43 is a specific marker for new or recently regenerated nerve fibers.<sup>25</sup> Changes in intraepidermal GAP-43 positivity were assessed using 50- $\mu$ m thick sections adjacent to those used to determine IENFD. These were immunostained for GAP-43 and Z-stacked images (Axiovision 4.8.1; Carl Zeiss Microimaging, Jena, Germany) were used to manually trace the extent of nerve fibers visualized within the epidermis using a novel measurement introduced by one of the authors (MJ). Total length was normalized per mm and mm<sup>2</sup> to provide data with units that could be compared to IENFD (per mm) or CNFA (per mm<sup>2</sup>), respectively.

Additional endpoints, obtained at baseline and day 28, were the 6MWT, performed according to American Thoracic Society guidelines,<sup>26</sup> and self-administered questionnaires (RAND 36-item health survey [RAND-36], Small Fiber Neuropathy Screening List [SFNSL],<sup>19</sup> BPI, Fatigue Assessment Scale [FAS],<sup>27</sup> and Neuropathic Pain Symptom Inventory [NPSI]<sup>28</sup>). Safety endpoints, including serious adverse events (SAEs), also were obtained.

### Statistical Analysis

The safety population (intent-to-treat [ITT]) included all subjects who received  $\geq 1$  dose of study drug. The modified ITT (MITT) population consisted of all randomized subjects who received  $\geq 1$  dose of study drug and had a baseline and at least one postbaseline assessment of CNFA. As per the prespecified Statistical Analysis Plan, the primary efficacy endpoint was CNFA at day 28 compared to baseline. Between-group significance was estimated using a Mixed Model Repeated Measures (MMRM) analysis of CNFA change from baseline at endpoint, which included treatment, visit, and treatment by visit interaction as fixed effects, the baseline density as a covariate, and study site as a random effect using the Kenward-Roger degree of freedom estimate and including data from all 4 treatment groups. Within-group significance was analyzed using a paired *t*-test at the  $P = 0.05$  (2-sided) significance level.

IENFD, 6MWT, BPI, RAND-36, SFNSL, FAS, and NPSI change from baseline at day 28 were analyzed using a paired *t*-test analysis. Between-group differences for IENFD were determined using MMRM as for CNFA, and for 6MWT, GAP-43, and BPI pain intensity by analysis of covariance (ANCOVA) with the treatment and baseline value as factors. For correlation analyses Pearson or Spearman Rank Order tests were performed depending upon whether the data were normally distributed.

Based on previous studies,<sup>11,13</sup> a sample size of 16 for each group was estimated to provide 90% statistical power to detect a change of 8.0 points from baseline to day 28 in the secondary endpoint of SFNSL at the 2-sided 0.05 level.

### RESULTS

The disposition of the study subjects is shown in Figure 1. Although 64 subjects were randomized, treatment assignment could not be confirmed for two subjects in the 8 mg treatment

group due to pharmacy error. One patient withdrew voluntarily from the 4 mg group. Baseline characteristics of the MITT groups are summarized in Table 1. Subjects in the groups were similar in age, CNFA, severity of SNFL, pain and other symptom scores, and functional capacity. The mean age of the study population was 50.8 years. The median time since diagnosis of sarcoidosis was 8 years (interquartile range [IR], 5.8–12), while the median duration of presumptive SNFL was 6 years (IR, 3–8.3). Five patients had well-controlled diabetes (2 in the placebo, 2 in the 1 mg, and 1 in the 4 mg groups).

Following 28 days of dosing, subjects in the 1 mg and placebo groups exhibited a mean decrease in CNFA ( $\mu\text{m}^2$ ) from baseline of  $-64.3$  ( $P = 0.748$ ) and  $-170.0$  ( $P = 0.32$ ), respectively. In contrast, CNFA in the 4 and 8 mg groups increased by  $533.8$  ( $P = 0.084$ ) and  $203.8$  ( $P = 0.274$ ), respectively. For the primary efficacy endpoint, MMRM analysis for treatment group differences demonstrated a significant increase in CNFA compared to placebo for the 4 mg group ( $697.4 \mu\text{m}^2$ ;  $P = 0.012$ ; Table 2).

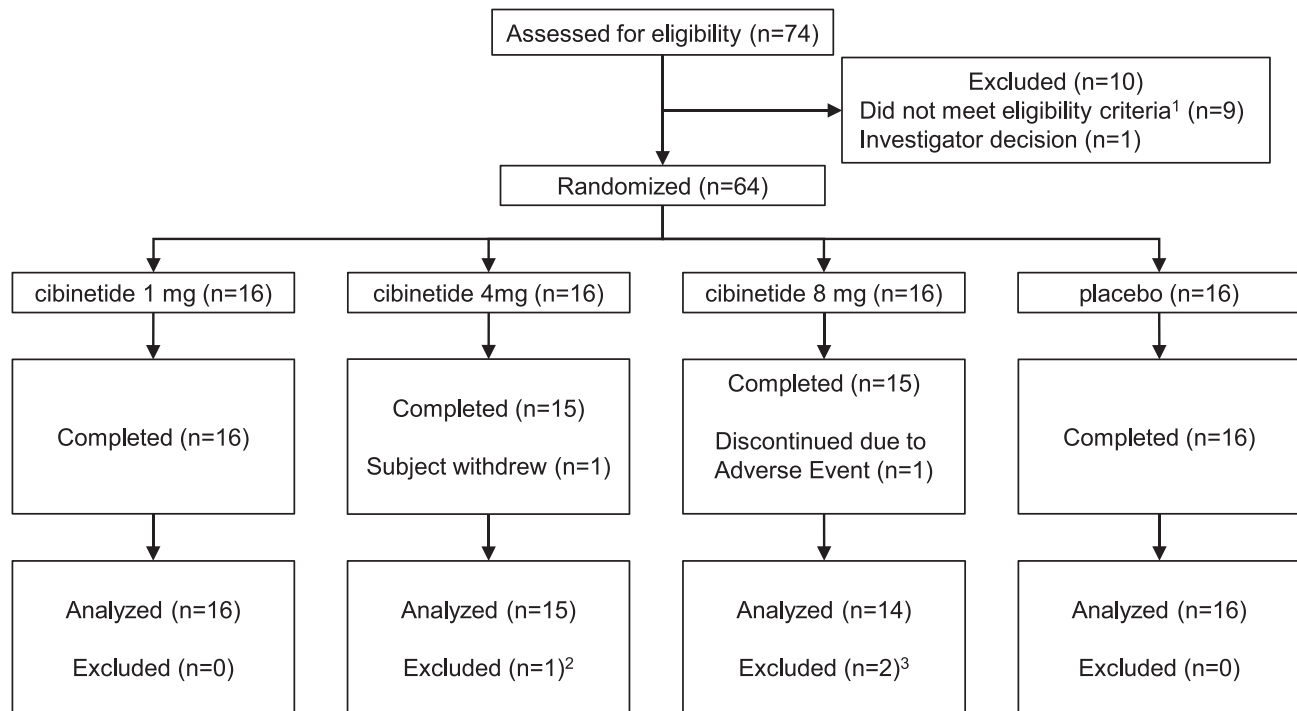
Following discontinuation of drug administration, CNFA trended back toward baseline. A sensitivity analysis performed by excluding the few diabetic patients in this study did not change the observed outcome for CNFA as an endpoint (data not shown). Assessment of the change in the corneal nerve fiber net using ACCmetrics-derived variables showed similar treatment group response patterns (see Supplementary Material).

A representative CCM image for a study subject (Figs. 2A–C) demonstrated decreased CNFA at baseline typical of sarcoidosis-associated SNFL compared to a healthy control, with clear improvement noted following 28 days of cibinetide administration.

In contrast to the significant increase in CNFA observed after 4 mg cibinetide dosing, no treatment group differences were observed for IENFD changes as determined by MMRM, with the 1, 4, and 8 mg groups exhibiting least squares (LS) mean changes of  $0.45 \pm 0.62$  (SEM),  $0.43 \pm 0.64$ , and  $-0.55 \pm 0.63$  nerve fibers/mm, compared to  $0.75 \pm 0.60$  in the placebo group. However, the cibinetide 4 mg group showed a significant increase in the LS mean GAP-43<sup>+</sup> fiber length of  $1730.8 \pm 658 \mu\text{m}/\text{mm}^2$  (corresponding to  $\sim 23\%$  increase from baseline) compared to a decrease of  $-333.5 \pm 645 \mu\text{m}/\text{mm}^2$  for the placebo group ( $P = 0.035$ ). Additionally, the 1 and 8 mg groups had LS mean values of  $1181.1 \pm 638$  and  $-344.4 \pm 713 \mu\text{m}/\text{mm}^2$ , respectively. As an example, Figures 2D and 2E illustrate representative change in GAP-43<sup>+</sup> fibers following 4 mg of cibinetide. Changes in CNFA and skin biopsy intraepidermal GAP-43<sup>+</sup> fiber length were significantly correlated (Spearman  $\rho = 0.5750$ ;  $P = 0.025$ ; Fig. 3A), suggesting that the effects of cibinetide were consistent across two anatomic sites and small nerve fiber assessment methodologies.

When expressed as length of GAP-43<sup>+</sup> fibers per mm of epidermis, the 4 mg dose group was increased 44% above baseline and was significant ( $P = 0.03$ ) when compared to placebo ( $181.7 \pm 48.2$  vs.  $29.9 \pm 48.6 \mu\text{m}/\text{mm}$ ). Change in fiber length in the 1 and 8 mg dose groups was intermediate at  $88.8 \pm 48.2$  and  $73.8 \pm 53.8 \mu\text{m}/\text{mm}$ , respectively. When normalized for the number of nerve fibers, the change in GAP-43<sup>+</sup> fiber length in the 4 mg group was a mean of  $49.5 \mu\text{m}/\text{fiber}$  over 28 days, or approximately  $1.8 \mu\text{m}/\text{fiber}/\text{day}$ .

The baseline mean 6MWT distance for all subjects at baseline was reduced (Table 1) compared to predicted normative values.<sup>29</sup> Additionally, baseline 6MWT distance was inversely related to the level of self-reported pain interference ( $r = -0.384$ ;  $P = 0.002$ ) as determined by the BPI. At day 28, the cibinetide 1, 4, and 8 mg groups exhibited an increase in the total distance walked by 19.3, 17.7, and 18.2 m, compared to 1.2 m in the placebo group ( $P = 0.11$  for the 4 mg vs. placebo



<sup>1</sup> Reason for ineligibility: Corneal confocal microscopy score (n=4), lab abnormality (n=2), Brief Pain Inventory score (n=1), concomitant medication (n=1), non-compliance (n=1)

<sup>2</sup> Reason for exclusion: withdrew without primary endpoint follow-up (n=1)

<sup>3</sup> Reason for exclusion: treatment allocation could not be verified due to discrepancy in pharmacy records (n=2)

FIGURE 1. Disposition of study subjects.

groups). Further, there was a significant correlation between the change in CNFA and change in the 6MWT for the cibinetide 4 mg group (Spearman  $\rho = 0.645$ ;  $P = 0.009$ ; Fig. 3B) which was not observed in the placebo group.

At baseline, subjects reported similar symptom scores (moderate pain) as measured by self-administered questionnaire instruments (Table 1). Patient self-reported outcomes exhibited strong placebo effects following dosing, with significant increases from baseline noted for all treatment groups for the SFNSL, BPI, and NPSI (Table 3). The RAND-36 Item Health Survey showed significant increases from baseline at day 28 for the cibinetide 1 mg group in health change; in the cibinetide 4 mg group for physical functioning, social functioning, pain, and health change; and in the placebo group for emotional well-being and pain (data not shown). No group exhibited a significant change from baseline in the FAS. For subjects with moderate-to-severe pain (baseline BPI score  $\geq 5$ ,  $n = 44$ ) the largest placebo-corrected treatment effect in pain reduction was seen in the cibinetide 4 mg group (LS mean difference,  $-1.01$ ; 95% confidence interval [CI],  $-2.42, 0.41$ ;  $P = 0.157$ ; Table 3).

The occurrence of adverse events is summarized in Table 4. No relationship to cibinetide dosing was evident, with the lowest incidence across all four treatment groups occurring in the 4 mg group. The most frequent adverse events were injection site pain, diarrhea, fatigue, headache, and nausea. Serious adverse events occurred in 2 subjects in the 1 mg group (syncope, headache, enteritis), considered unrelated to the study drug with no change in study drug administration, and one subject in the 8 mg group (suicidal ideation), considered possibly related.

## DISCUSSION

The study results show a significant, placebo-corrected increase in CNFA after 28 days of cibinetide administration, which corroborates a similar increase observed in a prior phase 2a trial<sup>15</sup> and provides clinical validation of preclinical data demonstrating neuroprotective and neurotrophic effects of cibinetide.<sup>5</sup> Although parallel assessments of lower limb distal IENFD did not show a similar change, quantification of new nerve fiber growth within the epidermis using GAP-43 immunostaining also supported a cibinetide-related increase in small nerve fiber regeneration. This observation is strengthened further by the significant positive correlation noted between changes in CNFA and GAP-43<sup>+</sup> fiber length. As expected, the 1 mg dose does not appear to be effective. Although the 8 mg dose group exhibited a trend for improvement similar to the 4 mg group, the loss of 2 patients in this group may have reduced discriminatory power in this small trial.

The results of the present study are unique in that a therapeutic benefit of a pharmacologic therapy was demonstrated after what is an exceedingly short treatment course for chronic neuropathy. Notably, evaluation of normal subjects has shown that the corneal nerve fiber net is highly dynamic, undergoing significant morphologic change over a few days.<sup>30</sup> In contrast, IENFD is stable over at least 20 days.<sup>31</sup> This observation depends, in part, on the method of calculating IENFD. Specifically, density counts do not take into account the extent of nerve fiber arborization. Since elongation and branching of existing nerve fibers is the major mechanism underlying regrowth,<sup>32</sup> IENFD is an insensitive measure of change. In contrast, quantification of new nerve fiber length

TABLE 1. Baseline Characteristics of MITT Population

Variable	Placebo	Cibinetide 1 mg	Cibinetide 4 mg	Cibinetide 8 mg
<i>n</i>	16	16	15	14
Male	9	8	8	6
Age	47.8 (10.7)*	51.4 (7.5)	52.0 (11.0)	52.2 (9.2)
CNFA, $\mu\text{m}$ , see note	2958.2 (886.0)	2941.1 (1050.6)	2954.8 (629.1)	3191.2 (853.2)
IENFD, no./mm	4.7 (2.8)	5.2 (2.4)	6.3 (3.0)	5.1 (2.7)
GAP-43, $\mu\text{m}/\text{mm}$	353 (225)	419(246)	418 (237)	438 (340)
GAP-43, $\mu\text{m}/\text{mm}$	5958 (3825)	7349 (4299)	7413 (4299)	7811 (6293)
BPI severity†	5.7 (1.6)	6.1 (1.9)	6.2 (1.4)	5.8 (1.2)
BPI interference‡	5.6 (2.4)	6.1 (2.5)	5.8 (2.3)	6.1 (2.1)
NPSI, total score§	48.9 (20.3)	50.1 (20.0)	58.3 (20.8)	50.3 (17.1)
SFNSL	38.0 (11.0)	41.4 (15.1)	42.5 (17.8)	41.2 (14.6)
FAS¶	32.6 (5.6)	33.4 (6.8)	31.8 (6.0)	32.9 (5.1)
6MWT, m**	468 (121)	495 (109)	445 (88)	416 (110)

Note: To convert CNFA  $\mu\text{m}^2$  to CNFA  $\mu\text{m}^2/\text{mm}^2$ , multiply by 6.32.

\* Standard deviation.

† Average of 4 pain questions for subjects with baseline  $\geq 5$ ; maximum score 10.

‡ Average of 7 interference questions; maximum score 10.

§ Maximum score 100.

|| Maximum score 84.

¶ Maximum score 50.

\*\* Mean predicted<sup>29</sup> 6MWT distance was  $676 \pm 7.0$  m.

using GAP-43 as a marker directly captures alterations in the cutaneous nerve fiber net.

Whether the observed effect of cibinetide takes place at the level of the nerve fiber terminals or cell bodies currently is unclear. Based on a preclinical model of neuropathy,<sup>33</sup> a major target for cibinetide could be at the level of the trigeminal ganglion where the sensory neuronal cell bodies reside. It currently is unknown to what extent cibinetide may be secreted into tears and thereby exert a local effect. However, the low nanomolar peak plasma concentrations of cibinetide at the doses administered, coupled with its short circulating half-life, make this possibility unlikely.

The underlying plasticity of corneal nerve fibers likely contributes to the demonstration of a significant nerve fiber regenerative effect of cibinetide over the short treatment period. This further is supported by the increase in epidermal GAP-43<sup>+</sup> nerve fiber length, suggesting that skin nerve fibers also possess cibinetide-induced regenerative capacity. The approximately 2  $\mu\text{m}/\text{fiber}/\text{day}$  rate of cutaneous regrowth as estimated by the GAP-43 analysis is of the same order of magnitude as what has been observed following intracutane-

ous axotomy, but a longer treatment duration will be required to assess the true magnitude of the effect.

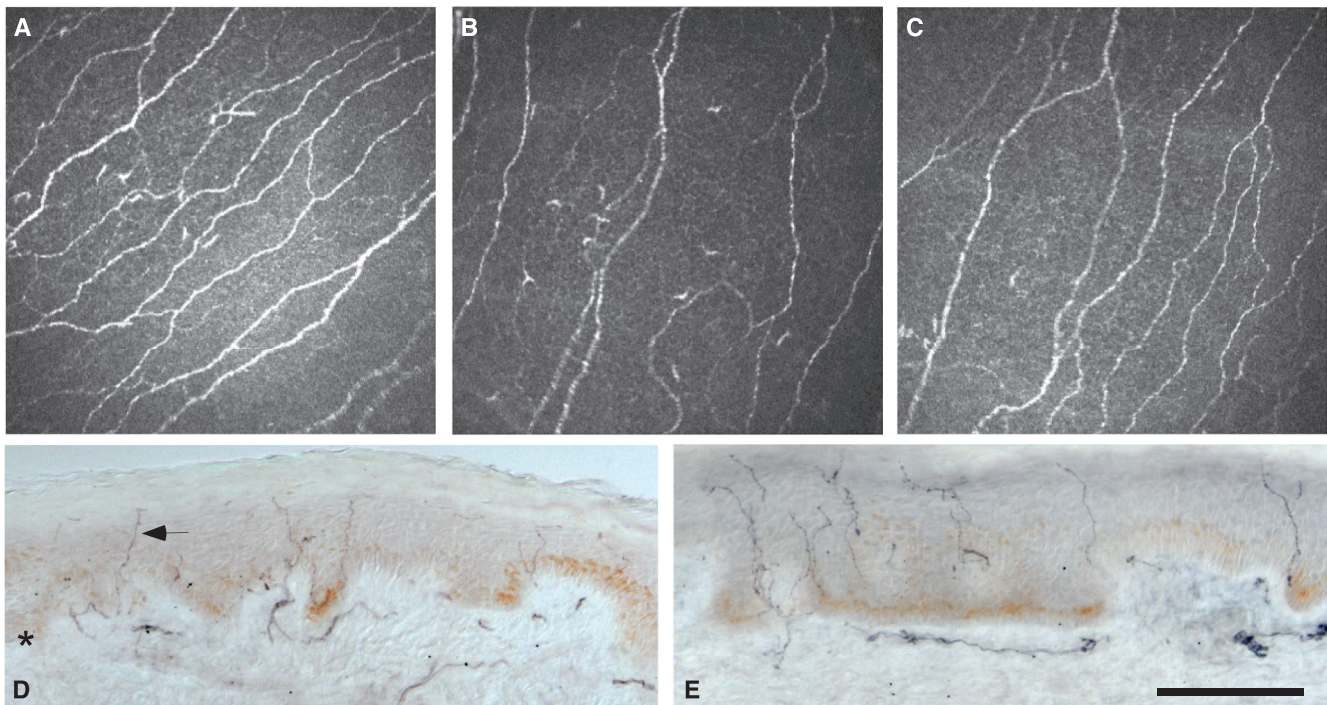
Effective pharmacologic interventions have long been sought for patients with peripheral neuropathies. Unfortunately, disease modification strategies for these debilitating and prevalent disorders have not yielded any approved drug therapies to date, as long-term clinical trials have failed to demonstrate significant treatment effects when using currently accepted end points.<sup>34-37</sup> In this regard, it is notable that a significant increase in CNBD was demonstrated in type 1 diabetic patients 6 months after successful kidney-pancreas transplantation, with a significant improvement in CNFL and CNFD at 12 months, without a significant improvement in quantitative sensory testing, nerve conduction studies, or IENFD.<sup>38</sup>

An increasing body of evidence supports the use of CCM to reliably detect reduced nerve fibers in idiopathic SFN, hereditary sensory neuropathies, Fabry disease,<sup>39</sup> and after chemotherapy.<sup>40</sup> This technique has high reproducibility and repeatability, with good sensitivity and specificity for diagnosing diabetic neuropathy (DPN).<sup>41</sup> A recent meta-analysis of CCM in DPN (13 studies, 1680 participants) confirmed its use

TABLE 2. Mean Change in CNFA ( $\mu\text{m}^2$ ) From Baseline to Day 28 and Baseline to Day 58

	Cibinetide 1 mg	Cibinetide 4 mg	Cibinetide 8 mg	Placebo
<i>N</i>	16	15	14	16
Change from baseline to day 28				
LS mean (SE)	-77.17 (191.4)	511.1 (191.7)	244.6 (206.0)	-186.30 (191.4)
95% CI of LS mean	-476.5, 322.1	111.1, 911.0	-180.9, 670.1	-585.7, 213.1
LS mean (SE) difference, treatment - placebo	109.13 (268.5)	697.4 (268.6)	430.94 (279.9)	
95% CI of LS mean difference, treatment - placebo	-429.1, 647.4	158.8, 1235.9	-130.3, 992.1	
Two-sided <i>P</i> value of LS mean difference, treatment - placebo	0.686	0.012	0.130	
Change from baseline to day 56				
LS mean (SE)	-10.07 (200.6)	134.58 (207.3)	32.62 (216.3)	-26.79 (200.5)
95% CI of LS mean	-423.3, 403.2	-291.0, 560.2	-410.0, 475.2	-440.0, 386.4
LS mean (SE) difference, treatment - placebo	16.73 (281.7)	161.38 (286.5)	59.42 (293.5)	
95% CI of LS mean difference, treatment - placebo	-547.6, 581.0	-412.6, 735.4	-528.4, 647.3	
Two-sided <i>P</i> value of LS mean difference, treatment - placebo	0.953	0.576	0.840	

*P* values and CIs are obtained from the MMRM model, which includes treatment, visit, and treatment by visit interaction as fixed effects, the baseline density is a covariate, and center as a random effect. All post-baseline visits are included in the analysis.

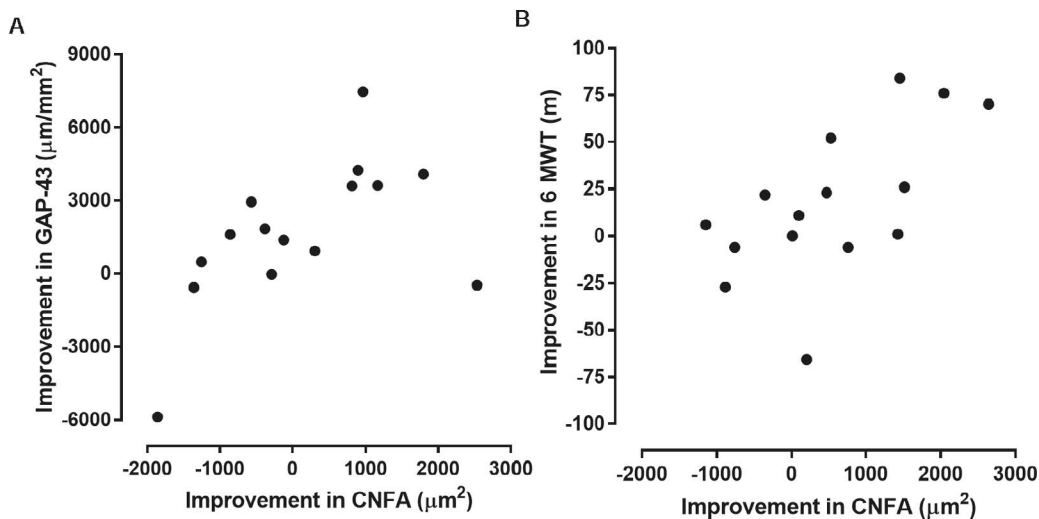


**FIGURE 2.** Examples of corneal small nerve fiber change following 28 doses of cibinetide. Small nerve fibers visualized in the sub-basal layer of the cornea using in vivo confocal microscopy (*top*) and GAP-43 immunoreactivity within the epidermis of biopsies of distal lower limb (*bottom*), exhibit increases following daily administration of 4 mg of cibinetide. The distribution of corneal nerve fibers is shown for a healthy volunteer (A), compared to that of a subject having sarcoidosis with SNFL enrolled in the current study at baseline (B) and after 28 days of cibinetide (4 mg) treatment (C). Skin biopsies immunostained for GAP-43 reveal intraepidermal nerve fibers at baseline (D); *arrow*), which become more numerous with branching following cibinetide (4 mg; [E]). \*Dermal-epidermal junction. Skin biopsy scale bar: 100  $\mu\text{m}$ .

in detecting and assessing early nerve damage.<sup>42</sup> Two recent longitudinal studies have shown that corneal nerve fiber loss predicts the development of DPN<sup>43</sup> more accurately than traditional measures of neuropathy, such as quantitative sensory testing and nerve conduction studies,<sup>44</sup> and corneal nerve loss has been related to elevated hemoglobin A1c (HbA1c) and reduced high density lipoprotein.<sup>45</sup> Our findings further support the use of CCM as a surrogate endpoint to

assess small nerve fiber damage and repair in peripheral neuropathies.

In addition to enhanced nerve fiber growth, the current results, along with those of a prior sarcoidosis study,<sup>13</sup> suggested that cibinetide therapy may improve functional capacity as assessed by the 6MWT. The 6MWT is a well-established outcome assessment for a variety of diseases, as it provides an integrated global measure of ambulatory function.



**FIGURE 3.** Change in CNFA is correlated with changes in biomarker and functional indices following cibinetide administration. (A) At day 28, change in CNFA was positively correlated with change in GAP-43<sup>+</sup> nerve fiber staining as assessed by Spearman's rank-order correlation for the cibinetide 4 mg group ( $\rho = 0.575$ ;  $P = 0.025$ ). (B) Change in CNFA also was positively correlated with change in 6MWT (Spearman's  $\rho = 0.645$ ;  $P = 0.009$ ).

TABLE 3. Change From Baseline at Day 28 in Patient-Reported Symptom Scores

Instrument	Cibinetide 1 mg	Cibinetide 4 mg	Cibinetide 8 mg	Placebo
SFNSL				
Mean (SD)	-4.9 (8.9)	-8.7 (14.1)	-5.5 (7.1)	-7.3 (9.5)
<i>P</i> value	0.04	0.03	0.02	0.01
BPI severity				
Mean (SD)	-0.59 (1.67)	-1.17 (1.69)	-1.11 (1.71)	-0.92 (1.44)
<i>P</i> value	0.18	0.02	0.04	0.02
BPI interference				
Mean (SD)	-1.19 (1.95)	-1.66 (1.65)	-1.31 (2.44)	-1.73 (2.22)
<i>P</i> value	0.03	0.002	0.08	0.007
NPSI				
Mean (SD)	-12.3 (16.1)	-14.5 (17.9)	-8.4 (12.7)	-12.9 (13.8)
<i>P</i> value	0.01	0.01	0.03	0.002
Placebo-corrected BPI average pain intensity*				
LS mean difference (95% CI)	-0.39 (-1.80, 1.03)	-1.01 (-2.42, 0.41)	-0.77 (-2.17, 0.63)	
<i>P</i> value	0.584	0.157	0.275	

Change from baseline obtained by paired *t*-test analysis. For BPI average pain intensity, *P* values and CI obtained by ANCOVA model.

\* In subjects with BPI average pain intensity  $\geq 5$ .

Recently, the 6MWT was shown to correlate with CNFL in patients with DPN<sup>46</sup> as well as with the severity of pain in a population with chronic pain.<sup>47</sup> The strong, significant correlation between an increase in CNFA and an increase in 6MWT noted in the current study provides support for the clinical relevance of the early corneal nerve fiber improvements seen with cibinetide. It remains unclear whether the improvements in the 6MWT are due to a reduction of pain, improvement of dysautonomia, or other factors.

In contrast to previous trials of short-term cibinetide administration in painful SNFL, our results did not demonstrate between-group differences using patient-reported outcome instruments. The design of the trial, without a run-in period and only limited exposure to cibinetide, may help explain this finding, as a strong placebo effect was noted for these parameters. This phenomenon has been noted with increasing frequency in painful neuropathy clinical trials, with the magnitude of placebo group improvement ranging from 0% to 60% of subjects experiencing pain reductions of  $\geq 50\%$  from baseline.<sup>48</sup> However, in patients with moderate pain at baseline, the cibinetide 4 mg treatment arm demonstrated a placebo-corrected improvement in pain at day 28 with an

effect size comparable to results noted for approved/first-line neuropathic pain medications.<sup>48</sup>

One theoretical concern is whether newly regenerated nerve fibers could adversely affect neuropathic symptoms, as preclinical models show that new nerve fiber regrowth often is accompanied by the development of allodynia and/or hyperalgesia.<sup>49</sup> Therefore, it is reassuring to note that, in addition to demonstrable pain relief, preclinical data with cibinetide in capsaicin-induced pain have shown antagonism of TRPV1-mediated hyperalgesia,<sup>10</sup> and improved thermal sensory thresholds mediated by C-fibers, following 28 days of cibinetide administration in subjects with sarcoidosis and SNFL.<sup>13</sup> Nevertheless, additional studies will be needed to assess the potential long-term effects of cibinetide with respect to the development of allodynia.

This study has limitations. First, only patients having pain were included whereas some patients have painless SNFL.<sup>4</sup> It currently is unknown how these individuals would respond to cibinetide, particularly with respect to potential symptoms related to nerve fiber regrowth. Second, the treatment period was only for 28 days, which is likely not long enough to predict the full effects of cibinetide, nor offset placebo responses.

TABLE 4. Overall Summary of Treatment Emergent Adverse Events, Safety Population

	Cibinetide, No. (%) of Patients			Placebo, N = 16 No. (%) of Patients
	1 mg, N = 16	4 mg, N = 16	8 mg, N = 14	
Patients reporting at least one TEAE, N(%)	14 (87.5)	11 (68.8)	12 (85.7)	12 (75.0)
Patients reporting at least one serious TEAE	2 (12.5)	0	1 (7.1)	0
Patients reporting at least one TEAE leading to study drug withdrawal	0	0	1 (7.1)	0
Patients reporting at least one TEAE leading to death	0	0	0	0
Maximum severity				
Mild	8 (50.0)	5 (31.3)	7 (50.0)	8 (50.0)
Moderate	5 (31.3)	5 (31.3)	4 (28.6)	4 (25.0)
Severe	1 (6.3)	1 (6.3)	1 (7.1)	0
Closest relationship to study drug				
Related*	11 (68.8)	9 (56.3)	9 (64.3)	10 (62.5)
Not related	3 (18.8)	2 (12.5)	3 (21.4)	2 (12.5)

TEAE, treatment emergent adverse event.

\* Includes all events reported as "Possibly," "Probably," "Definitely," or missing relationship to study drug.

Third, the study was powered to detect a change from baseline in the SFNSL in a within-group analysis, and, thus, may have been underpowered to be able to detect significant treatment effects for other patient-reported outcomes. Fourth, the number of patients included is small and the loss of the patients in the 8 mg group may have prevented a clearer assessment as to whether the trend toward improvement noted for this dosage was real. Finally, we did not formally mandate ophthalmologic assessment for ocular sarcoidosis during the screening period. Although the subjects typically had been screened previously for ocular disease, it is possible that occult ocular inflammation may have been present, which also may have led to reduced corneal nerve fiber in a subset of patients. However, the correlation between CCM and markers of extraocular neuropathy (i.e., GAP-43 staining) suggested that it is more likely that the CNFA is representative of SFN rather than an effect of ocular sarcoidosis.

Although the present study focused on sarcoidosis, SNFL is increasingly recognized as commonly associated with a broad spectrum of disease processes.<sup>1</sup> Therefore, cibinetide may exhibit restorative function in other diseases complicated by SNFL, and indeed, data obtained from a group of type 2 diabetic patients with painful SNFL have been encouraging.<sup>12</sup> Based on the promising effect on objective (nerve regrowth assessed by two different measures of nerve fiber regeneration: CNFA and GAP 43<sup>+</sup> IENF immunostaining), functional (6MWT), and symptomatic (pain) parameters, cibinetide warrants continued assessment as a potential disease modifying therapy for SNFL.

### Acknowledgments

The authors thank study personnel, including Tani Martin, Karla Pearson, and Michelle Ferrari. They also thank Andrew Atkinson who prepared and stained the skin biopsy specimens.

Supported in part by a grant from the Dutch government to the Netherlands Institute for Regenerative Medicine (NIRM; Grant No. FES0908).

Disclosure: **D.A. Culver**, Araim Pharmaceuticals (R); **A. Dahan**, Araim Pharmaceuticals (R); **D. Bajorunas**, Araim Pharmaceuticals (C); **M. Jeziorska**, None; **M. van Velzen**, None; **L.P.H.J. Aarts**, None; **J. Tavee**, Araim Pharmaceuticals (R); **M.R. Tannemaat**, None; **A.N. Dunne**, Araim Pharmaceuticals (E); **R.I. Kirk**, Araim Pharmaceuticals (E); **I.N. Petropoulos**, None; **A. Cerami**, Araim Pharmaceuticals (E), P; **R.A. Malik**, Araim Pharmaceuticals (R); **M. Brines**, Araim Pharmaceuticals (E), P

### References

- Tavee J, Zhou L. Small fiber neuropathy: a burning problem. *Cleve Clin J Med*. 2009;76:297-305.
- Uceyler N, Kafke W, Riediger N, et al. Elevated proinflammatory cytokine expression in affected skin in small fiber neuropathy. *Neurology*. 2010;74:1806-1813.
- Chen ES, Moller DR. Sarcoidosis—scientific progress and clinical challenges. *Nat Rev Rheumatol*. 2011;7:457-467.
- Bakkers M, Merkies IS, Lauria G, et al. Intraepidermal nerve fiber density and its application in sarcoidosis. *Neurology*. 2009;73:1142-1148.
- Collino M, Thiernemann C, Cerami A, Brines M. Flipping the molecular switch for innate protection and repair of tissues: long-lasting effects of a non-erythropoietic small peptide engineered from erythropoietin. *Pharmacol Ther*. 2015;151:32-40.
- Pulman KG, Smith M, Mengozzi M, Ghezzi P, Dille A. The erythropoietin-derived peptide ARA290 reverses mechanical allodynia in the neuritis model. *Neuroscience*. 2013;233:174-183.
- Schmidt RE, Feng D, Wang Q, et al. Effect of insulin and an erythropoietin-derived peptide (ARA290) on established neuritic dystrophy and neuronopathy in Akita (Ins2 Akita) diabetic mouse sympathetic ganglia. *Exp Neurol*. 2011;232:126-135.
- Swartjes M, Morariu A, Niesters M, et al. ARA290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain: an experimental study in rats and beta-common receptor knockout mice. *Anesthesiology*. 2011;115:1084-1092.
- Brines M, Patel NS, Villa P, et al. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. *Proc Natl Acad Sci U S A*. 2008;105:10925-10930.
- Zhang W, Yu G, Zhang M. ARA 290 relieves pathophysiological pain by targeting TRPV1 channel: integration between immune system and nociception. *Peptides*. 2016;76:73-79.
- Heij L, Niesters M, Swartjes M, et al. Safety and efficacy of ARA 290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double-blind pilot study. *Mol Med*. 2012;18:1430-1436.
- Brines M, Dunne AN, van Velzen M, et al. ARA 290, a nonerythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes. *Mol Med*. 2014;20:658-666.
- Dahan A, Dunne A, Swartjes M, et al. ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density. *Mol Med*. 2013;19:334-345.
- Quattrini C, Tavakoli M, Jeziorska M, et al. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes*. 2007;56:2148-2154.
- Hunninghake GW, Costabel U, Ando M, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis*. 1999;16:149-173.
- Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *J Peripher Nerv Syst*. 2010;15:202-207.
- Brines M, Swartjes M, Tannemaat M, et al. Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. *Technology*. 2013;1:1-7.
- Labbe A, Liang Q, Wang Z, et al. Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. *Invest Ophthalmol Vis Sci*. 2013;54:5144-5150.
- Hoitsma E, De Vries J, Drent M. The small fiber neuropathy screening list: construction and cross-validation in sarcoidosis. *Respir Med*. 2011;105:95-100.
- Oliveira-Soto L, Efron N. Morphology of corneal nerves in soft contact lens wear. A comparative study using confocal microscopy. *Ophthalmic Physiol Opt*. 2003;23:163-174.
- Patel SV, McLaren JW, Hodge DO, Bourne WM. Confocal microscopy in vivo in corneas of long-term contact lens wearers. *Invest Ophthalmol Vis Sci*. 2002;43:995-1003.
- Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Fully automated, semiautomated, and manual morphometric analysis of corneal subbasal nerve plexus in individuals with and without diabetes. *Cornea*. 2014;33:696-702.
- Chen X, Graham J, Dabbah M, Petropoulos I, Tavakoli M, Malik R. An automatic tool for quantification of nerve fibres in corneal confocal microscopy images. *IEEE Trans Biomed Eng*. 2017;64:786-794.
- Lauria G, Hsieh ST, Johansson O, et al. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on



- the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *Eur J Neurol*. 2010;17:903-912, e944-e909.
25. Denny JB. Molecular mechanisms, biological actions, and neuropharmacology of the growth-associated protein GAP-43. *Curr Neuropharmacol*. 2006;4:293-304.
  26. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166:111-117.
  27. De Vries J, Michielsen H, Van Heck GL, Drent M. Measuring fatigue in sarcoidosis: the Fatigue Assessment Scale (FAS). *Br J Health Psychol*. 2004;9:279-291.
  28. Bouhassira D, Attal N, Fermanian J, et al. Development and validation of the Neuropathic Pain Symptom Inventory. *Pain*. 2004;108:248-257.
  29. Camarri B, Eastwood PR, Ciccini NM, Thompson PJ, Jenkins S. Six minute walk distance in healthy subjects aged 55-75 years. *Respir Med*. 2006;100:658-665.
  30. Patel DV, McGhee CN. In vivo laser scanning confocal microscopy confirms that the human corneal sub-basal nerve plexus is a highly dynamic structure. *Invest Ophthalmol Vis Sci*. 2008;49:3409-3412.
  31. Lauria G, Dacci P, Lombardi R, et al. Side and time variability of intraepidermal nerve fiber density. *Neurology*. 2015;84:2368-2371.
  32. Rajan B, Polydefkis M, Hauer P, Griffin JW, McArthur JC. Epidermal reinnervation after intracutaneous axotomy in man. *J Comp Neurol*. 2003;457:24-36.
  33. Swartjes M, van Velzen M, Niesters M, et al. ARA 290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain coupled with suppression of the spinal microglia response. *Mol Pain*. 2014;10:13.
  34. Coelho T, Maia LF, Martins da Silva A, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology*. 2012;79:785-792.
  35. Tesfaye S, Tandan R, Bastyr EJ III, Kles KA, Skljarevski V, Price KL. Factors that impact symptomatic diabetic peripheral neuropathy in placebo-administered patients from two 1-year clinical trials. *Diabetes Care*. 2007;30:2626-2632.
  36. Wahren J, Foyt H, Daniels M, Arezzo JC. Long-acting C-peptide and neuropathy in type 1 diabetes: a 12-month clinical trial. *Diabetes Care*. 2016;39:596-602.
  37. Ziegler D, Low PA, Litchy WJ, et al. Efficacy and safety of antioxidant treatment with alpha-lipoic acid over 4 years in diabetic polyneuropathy: the NATHAN 1 trial. *Diabetes Care*. 2011;34:2054-2060.
  38. Tavakoli M, Mitu-Pretorian M, Petropoulos IN, et al. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes*. 2013;62:254-260.
  39. Tavakoli M, Petropoulos IN, Malik RA. Corneal confocal microscopy to assess diabetic neuropathy: an eye on the foot. *J Diabetes Sci Technol*. 2013;7:1179-1189.
  40. Ferdousi M, Azmi S, Petropoulos IN, et al. Corneal confocal microscopy detects small fibre neuropathy in patients with upper gastrointestinal cancer and nerve regeneration in chemotherapy induced peripheral neuropathy. *PLoS One*. 2015;10:e0139394.
  41. Petropoulos IN, Alam U, Fadavi H, et al. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care*. 2013;36:3646-3651.
  42. Jiang MS, Yuan Y, Gu ZX, Zhuang SL. Corneal confocal microscopy for assessment of diabetic peripheral neuropathy: a meta-analysis. *Br J Ophthalmol*. 2016;100:9-14.
  43. Pritchard N, Edwards K, Russell AW, Perkins BA, Malik RA, Efron N. Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care*. 2015;38:671-675.
  44. Lovblom LE, Halpern EM, Wu T, et al. In vivo corneal confocal microscopy and prediction of future incident neuropathy in type 1 diabetes: a preliminary longitudinal analysis. *Can J Diabetes*. 2015;39:390-397.
  45. Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, Efron N. Risk factors associated with corneal nerve alteration in type 1 diabetes in the absence of neuropathy: a longitudinal in vivo corneal confocal microscopy study. *Cornea*. 2016;35:847-852.
  46. Smith AG, Kowalsky G, Hauer P, Aperghis A, Singleton J. The diagnostic performance and clinical relevance of corneal confocal microscopy (CCM) in patients with diabetic peripheral neuropathy. *Neurology*. 2016;86: S44.001.
  47. Peppin JF, Marcum S, Kirsh KL. The chronic pain patient and functional assessment: use of the 6-Minute Walk Test in a multidisciplinary pain clinic. *Curr Med Res Opin*. 2014;30:361-365.
  48. Tuttle AH, Tohyama S, Ramsay T, et al. Increasing placebo responses over time in U.S. clinical trials of neuropathic pain. *Pain*. 2015;156:2616-2626.
  49. Cobiauchi S, de Cruz J, Navarro X. Assessment of sensory thresholds and nociceptive fiber growth after sciatic nerve injury reveals the differential contribution of collateral reinnervation and nerve regeneration to neuropathic pain. *Exp Neurol*. 2014;255:1-11.