ARA290

a Novel Treatment for Neuropathic Pain in Sarcoidosis

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CHAPTER 1

Introduction

Chapter 1. Introduction

Neuropathic pain

The International Association for the Study of Pain (IASP) defines neuropathic pain as *Pain caused by a lesion of the somatosensory nervous system* (www.iasp-pain.org/Taxonomy). While this definition seems simple, one needs to realize -as mentioned by the IASP- that neuropathic pain is a clinical description and not a diagnosis. A demonstrable lesion and/or an underlying disease that produces a series of symptoms that together with positive diagnostic tests is required to diagnose a neurological state that is best described by neuropathic pain. The term somatosensory relates to the sensory system that receives information from the (inner) body (rather than from the outside world, such as seeing or hearing) that includes all organs (from the skin to the viscera; from the peripheral to the central nervous system). Neuropathic pain is different from nociceptive pain, both in cause (nociceptive pain is related to non-neural tissue damage and is due to activation of nociceptors with an intact somatosensory system) and symptoms. However, both conditions may occur simultaneously (often referred to as mixed pain). Neuropathic pain is divided into central neuropathic pain and peripheral neuropathic pain. While in the former pain arises from a lesion within the central nervous system (brain and/or spinal cord), the later relates to a lesion of the peripheral nervous system. Peripheral neuropathy may be due to damage to just one nerve (*eg.* due to trauma or surgery), multiple nerves or may be diffuse (polyneuropathy).

Small Fiber Neuropathy

One form of peripheral neuropathy is small-fiber neuropathy (SFN). SFN is produced by lesions of predominantly small nerve fibers: unmyelinated C-fibers and thinly myelinated Aδ-fibers. The small nerve fibers are involved in autonomic functions, nociception and heat perception.[1] Small fibers contrast the large nerve fibers of the peripheral nervous system that are involved in motor functions, touch and vibration sense. Typical, so-called “positive” symptoms of SFN are allodynia (sheet intolerance), deep aching, burning, lancinating, squeezing or contact or thermal hyperalgesia and prickling. Additionally there are “negative” sensory symptoms and autonomic complaints. Negative symptoms can include thermal sensory loss, loss of pinprick sensation, numbness and tight feeling. Autonomic dysfunction may lead patients to complaints of dry eyes, dry mouth, orthostatic dizziness, bowel dysfunction, micturation disturbances, sweating (see Chapter 2 for a more complete overview of symptoms). In several diseases SFN seems restricted to the distal parts of the body (*ie.* length-dependent SFN or LD-SFN). Patients with LD-SFN usually start to develop symptoms in toes and feet, and symptoms gradually progress to involve distal legs, fingertips, and hands. Uncommon is non-length-dependent SFN (NLD-SFN) and those patients develop signs and symptoms in a ‘patchy’ distribution. This can include the face, upper limbs, or trunk before lower limbs.

Various conditions are associated with small fiber neuropathy. They can be classified in different groups: metabolic (incl. diabetes mellitus and sarcoidosis), immune-mediated, drug-related, toxic, infectious (incl. leprosy and HIV), hereditary and idiopathic. The underlying pathophysiology of the degeneration of the loss of small fibers remains unknown. It is suggested that increased levels of pro-inflammatory cytokines, such as TNF-α and IL-1, play a mechanistic role in the development and persistence of pain in neuropathy. TNF-α expression in human Schwann cells is upregulated in painful peripheral neuropathies.[3,4] Nerve biopsies from patients with neuropathies revealed higher TNF-α immunoreactivities in myelating Schwann cells when the neuropathy was painful, and serum soluble TNF-α-receptor 1 levels were higher in patients with a centrally mediated allodynia.[4] These data suggest an involvement of TNF-α in neuropathic pain at both central and peripheral sites. Indeed, it is currently accepted that the release of pro-inflammatory cytokines together with other mediators such

as interleukins, nerve growth factor, chemokines and interferons interact with downstream signaling mechanisms to cause neuropathic pain-like symptoms.[5] TNF-α and other pro-inflammatory cytokines are released in local tissue in response to injury, hypoxia or metabolic stress.[6,7] This innate immune response is curtailed by the local release of the protective cytokine erythropoietin (EPO).[6,7] EPO, through its actions at the innate repair receptor (IRR, a receptor hetero-complex that becomes upregulated in response to TNF-α release and tissue damage) triggers anti-inflammatory and anti-apoptotic processes that limit injury and promote repair.[2,6,7] In this sense EPO is the opposite of TNF-α, and assuming that the mechanism of neuropathic pain is related to central or peripheral release of TNF-α, one may assume that treatment with EPO counteracts neuropathic pain and restores damage to neuronal tissue, including peripheral nerves.[8] While it seems attractive to treat neuropathic pain patients with EPO, its side effect profile related to hematopoiesis (hypertension, thrombosis, myocardial infarction) prevents such therapies.

ARA290

Several EPO analogs have been developed that do not stimulate hematopoiesis but do activate the IRR and cause tissue protection. One such molecule developed by ARAIM Pharmaceuticals is ARA290.[6,7] ARA290 mimics the spatial configuration of EPO that interacts with the IRR. Animal studies (see the thesis of Maarten Swartjes, Ref. 2) showed the efficacy of ARA290 to reduce allodynia from peripheral nerve damage due activation of anti-inflammatory pathways via the IRR. ARA290 is an 11-amino-acid peptide with sequence: Pyr-Glu-Gln-Leu-Glu-Arg-Ala-Leu-Asn-Ser-Ser-OH (Pyr is pyroglutamatic acid) that displayed no toxic reactions in human and animal studies. So far, ARA290 has been tested in human volunteers and patients with diabetes mellitus and sarcoidosis, all without major side effects.[2,9]

Sarcoidosis

Sarcoidosis is a systemic (multi-organ), chronic, inflammatory disease that causes granulomas in local tissue, most frequently in lungs and lymphatic tissue. Other organ involvements include the heart, nervous system, eyes, kidney and skin. The presentation of the disease varies from patients with an absence of symptoms to patients with an insidious course followed by a chronic form, or patients that have an acute onset of disease. The etiology of sarcoidosis remains unknown. Pain in sarcoidosis is often related to SFN. Treatment of neuropathic pain in sarcoidosis is difficult and often unsuccessful. Limited treatment efficacy is a common observation in neuropathic pain from all etiologies. In general, pain relief is restricted to 30-50% of patients with just 30-50% of reduction of pain scores.[10,11] Current treatments of neuropathic pain include anticonvulsants, antidepressants, opioids and topical applications (capsaicin, lidocaine). Hence, there is an immediate need for novel treatment options. One such novel approach could be to treat sarcoidosis patients with moderate to severe neuropathic pain with ARA290.

Aim and outline of the thesis

The general aim of this thesis is to investigate small fiber neuropathy in sarcoidosis patients and to assess whether ARA290 is a possible new agent to treat the neuropathic complaints in the sarcoidosis population.

In Section 1 (**Chapters 2 and 3**) symptoms from SFN in sarcoidosis patients are examined and discussed. **Chapter 2** gives an overview of pain- and autonomic function-related symptoms with special emphasis on SFN and current treatment options. In **Chapter 3** the sensory profiles of SFN in sarcoidosis patients are examined by Quantitative Sensory Testing (QST), skin biopsies and Cornea Confocal Microscopy (CCM). QST employs non-invasive tests to examine the different aspects of the somatosensory nervous system such as tests to define the cold and warm detection and pain thresholds. The QST battery developed by the German Research Network on Neuropathic Pain is highly sensitive to diagnose SFN.[12] Skin biopsies are currently the gold standard for detection of SFN and visualize intra-epidermal nerve fiber densities by immunofluorescence technique. Finally, CCM is a

novel approach to quantify C-fiber length and density in the cornea.[13] It is currently still considered a surrogate measure of the small fiber state of the body. Yet, CCM gains rapidly in popularity as it allows repetitive and very sensitive testing of small nerve fibers in patients with SFN such as diabetes and sarcoidosis patients.

In Section 2 (**Chapters 4-6)** the effect of ARA290 in sarcoidosis neuropathy is examined. In **Chapter 4** the pharmacokinetics of ARA290 is studied. **Chapter 5** describes the first double blind, placebo-controlled (phase 2) study on the safety and efficacy of ARA290 in sarcoidosis SFN (NARA trial). Twenty-two patients received either ARA290 or placebo for 4 weeks and pain symptoms, QST and SFN specific questionnaires (small fiber neuropathy screening list or SFNSL) were obtained. **Chapter 6** describes a next randomized controlled (phase 2) trial on ARA290 in sarcoidosis patients with SFN (NERVARA trial). Outcome parameters included pain score using specialized questionnaires (brief pain inventory or BPI and SFNSL), QST, skin biopsies, CCM and the 6-min walk as surrogate measure of vitality and functionality.

Section 3 (**Chapter 7**) gives a general discussion and expert opinion on the efficacy of ARA290 on various end-points measured during and following treatment in patients with sarcoidosis-related SFN.

Chapter 8 gives a summary of the thesis and discusses future developments.

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CHAPTER 2

Sarcoidosis and pain caused by small fiber neuropathy

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Review Article

Sarcoidosis and Pain Caused by Small-Fiber Neuropathy

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Sarcoidosis is a chronic inflammatory illness and small-fiber neuropathy (SFN) is one of the disabling and often chronic manifestations of the disease. SFN presents with peripheral pain and symptoms of autonomic dysfunction. The character of the pain can be burning or shooting. Besides, allodynia and hyperesthesia can exist. Diagnosis is usually made on the basis of clinical features, in combination with abnormal specialized tests. The aim of treatment is often to reduce pain; however, total pain relieve is seldom achieved. The role of TNF-α in the pathogenesis of SFN in sarcoidosis appears interesting to explore. Novel therapeutic agents such as ARA 290, a nonhematopoietic erythropoietin analogue with potent anti-inflammatory and tissue protective properties, are interesting to explore in the treatment of SFN in sarcoidosis.

1. Sarcoidosis

Sarcoidosis has been known for more than 100 years and has been first described by the dermatologist Hutchinson and several years later by two other dermatologists, Besnier and Boeck. It is a multiorgan inflammatory disorder that is characterized by noncaseating granuloma (Figure 1). The exact etiology remains unknown. It is suspected that exposure to one or more extrinsic antigens in a genetically susceptible individual leads to the overactivation of inflammatory pathways that promote the formation of sarcoid granuloma [1]. Granuloma formation is regulated by a complex interaction between T-helper lymphocytes and macrophages, in which cytokines such as tumor necrosis factor (TNF)-α play an important role.

The clinical course of sarcoidosis is highly variable and depends on ethnicity, duration of illness, site and extension of organ involvement, and activity of the granulomatous process, which shows a tendency to wax and wane. Mode of presentation varies from asymptomatic, to an "acute onset" presenting as Lofgren's syndrome and to a chronic course, frequently accompanied with pain and fatigue. Practically every organ can be involved. However, most commonly (>90%) the lungs are affected [2, 3]. Often patients suffer from symptoms a long time before the diagnosis sarcoidosis is confirmed. Due to the manifold presentation of the disease, it is a challenge to recognize in an early phase. The acute stage of disease usually presents itself with erythema nodosum, arthritis, fever, and fatigue with a good prognosis. Spontaneous remission usually occurs within two years, while chronic sarcoidosis mostly has an insidious onset with often relapses, resolution being less likely. In some of the cases, the disease is progressive. Development of lung fibrosis, cardiac sarcoidosis, and neurosarcoidosis is related to worse prognosis. Factors that trigger the formation of fibrosis in sarcoidosis are poorly understood. Up to 5% will eventually die from sarcoidosis.

In chronic sarcoidosis, pain and fatigue are important symptoms, even when sarcoidosis is clinically in remission fatigue and pain may persist and become a chronic complaint. These complaints often result in a severe reduction in quality of life. Although a lot of research has been done, the exact mechanism behind this "postsarcoidosis chronic fatigue syndrome" remains unsolved.

Recently, it has been shown that pain in patients with sarcoidosis is often related to neuropathy of small fibers of the peripheral nervous system [4–7].

2. Small Fiber Neuropathy

Small-fiber neuropathy (SFN) is a peripheral nerve disorder that selectively affects thinly myelinated Aδ fibers

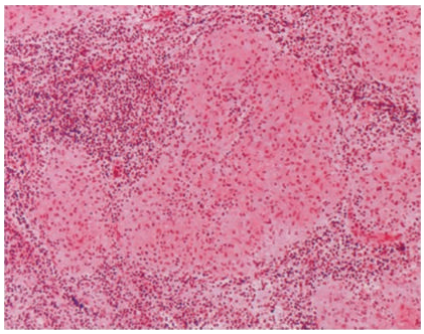

Figure 1: A microscopical section of mediastinal lymph node with HE stain, ×40. Multiple granulomas with various sizes from 0,2 to 0,8 mm in diameter are observed in the lymph node. These granulomas consist of histiocytes, which have large cytoplasm and partly connect to each other but lack a necrotic region.

Table 1: Symptoms of small fiber neuropathy.

Sensory symptoms	Pain*
	Paraesthesias
	Sheet intolerance
	Restless legs syndrome**
Symptoms of autonomic dysfunction	Hypo- or hyperhidrosis
	Diarrhoea or constipation
	Urinary incontinence or -retention
	Gastroparesis
	Sicca syndrome
	Blurry vision
	Facial flushes
	Orthostatic intolerance
	Sexual dysfunction

*Pain in small fiber neuropathy is often burning, tingling, shooting, or prickling in character.
**Restless legs syndrome is a disorder characterized by disagreeable leg sensations that usually occur prior to sleep onset and that cause an almost irresistible urge to move.

and unmyelinated C fibers. Small nerve fibers are involved in both somatic and autonomic function [8]. As a result, patients with SFN may present with symptoms of neuropathic pain (NP) and autonomic dysfunction [5].

Damage to or loss of small somatic nerve fibers results in burning pain, tingling, or numbness that typically affects the limbs in a distal to proximal gradient. Symptoms are usually worse at night and often affect sleep. People sometimes sleep with the feet uncovered because they can not bear the touch of the sheets. Besides, walking may be difficult due to pain by the pressure on the floor. When autonomic fibers are affected, patients may experience dry eyes, dry mouth, orthostatic dizziness, constipation, bladder incontinence, sexual dysfunction, hyperhidrosis or hypohidrosis, or red or white skin discoloration. Finally restless legs syndrome may be present, characterized by disagreeable leg sensations that usually occur prior to sleep onset and cause an almost irresistible urge to move (Table 1).

Most patients suffer from length-dependent small-fiber neuropathy (LD-SFSN): symptoms and signs start to develop in the toes and feet, symptoms gradually progress to involve distal legs, fingertips, and hands. Non-length-dependent small-fiber neuropathy (NLD-SFSN) is not as common as LD-SFSN and patients develop complaints in a patchydistribution. This can include face, upper limbs, or trunk before the lower limbs are affected. The NLD-SFSN is more seen in women and presents at a younger age [9, 10].

2.1. Diagnosis of Small Fiber Neuropathy. Nerve conduction studies, which are the key in evaluation of other (large fiber) neuropathies, are generally normal in SFN. Therefore, the syndrome of SFN has been an enigma to practitioners because of the unexplained contrast between severe pain and a paucity of neurological and electrophysiological findings. Recent advantages in diagnostic techniques facilitate objective confirmation of clinical diagnosis and characterization of fiber involvement. However, a golden standard for the diagnosis of SFN is not available yet. Diagnosis is usually made on the basis of clinical features, in combination with abnormal specialized tests, which include among others, assessment of intraepidermal nerve fiber density (IENFD) in skin biopsy, temperature sensation tests, and sudomotor and cardiovagal testing for autonomic fibers [4, 6, 11]. However, all tests have their limitations.

Quantitive sensory testing (QST) includes temperature threshold testing. Thermal (cold and warm) and mechanical (tactile and vibration) detection thresholds assess small-fiber function (including the central pathways). The cold detection threshold (CDT) examines the A-delta-fiber function, while assessment of C-fibre function is examined by the warm detection threshold (WDT). The major limitation of QST is its psychophysical character. As a consequence, malingering and other nonorganic factors can influence test results [12].

To objectively test small nerve fibers, laser-evoked potentials (LEP) and contact heat-evoked potentials (CHEPs), have been developed. It is well established that both laser and contact heat stimulation activate thermo-nociceptive cutaneous nerves. Even though attention and other cognitive processes influence the amplitude of Laser Evoked Potentials (LEPs) and Contact Heat Evoked Potentials (CHEPs) these tests carry up relevant information on the functional state of nociceptive terminals.

For the CHEPs, a thermofoil thermode stimulator is used to reach a temperature of 53°C at a rate of 70°C/s. It has been shown that patients with sensory neuropathy have lower-amplitude CHEPs, which correlates with other SFN tests [13].

Multiple studies have emphasized the importance of intraepidermal nerve fiber density (IENFD) assessment using PGP-9.5 immunofluorescent staining in skin biopsy in the evaluation SFN [14]. Epidermal nerves are the distal terminals of small dorsal root ganglia neurons that pierce

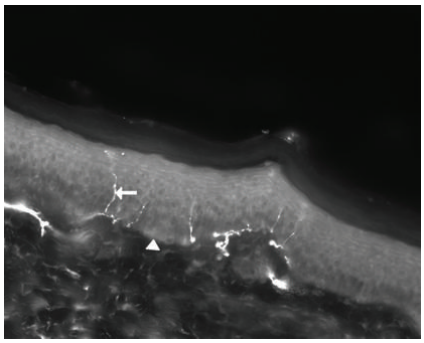

Figure 2: Magnification 200x. Punch skin biopsy from a healthy control showing intraepidermal nerve fibers. Arrow: intraepidermal nerve fiber. Arrowhead: basal membrane (above the basal membrane the epidermis is shown, under the basal membrane the dermis is shown).

the dermal-epidermal basement membrane and penetrate the epidermis. The discovery of the antibody to the neuropeptide protein gene product (PGP) 9.5 made it possible to effectively stain nerve fibers (Figure 2). PGP 9.5 is a ubiquitin C-terminal hydrolase and is enriched in epidermal nerve fibers [14]. A punch biopsy is performed following established procedures, mostly 10 cm above the lateral malleolus after local anesthesia with 1% lidocaine. A limitation of skin biopsies is that they are available in only a few academic centers. The histological technique is moderately complicated and time consuming, and before implementing it, a relatively large subset of healthy controls should be studied as the normative range is wide. Finally skin biopsy appears to have a high specificity but low sensitivity in sarcoidosis: Bakkers in 2009 showed that 32,8% of sarcoidosis patients with symptoms of SFN had a reduced IENFD score in the skin biopsy, and 14,3% in patients without SFN symptoms had a reduced IENFD [4]. The rule "physicians, not tests make diagnosis" appears especially applicable for SFN. Examination often reveals allodynia, hyperalgesia, or reduced pinprick and thermal sensation in the affected area. Motor strength en proprioception, however, are (as functions of the large fibers) preserved.

2.2. Etiology of Small Fiber Neuropathy. In 50% of the cases presenting with SFN no underlying disease is found: "idiopathic SFN" [15]. Recent studies have shown gain of function mutations in sodium channel Na(V)1.7 in a subset (28.6%) of those patients with idiopathic SFN [16]. The exact role of these mutations is unresolved yet.

In 50% of the cases presenting with SFN, an underlying disease is present, including diabetes, sarcoidosis, and amyloidosis among others (Table 2) [6]. It is remarkable that SFN appears frequent in several immune-mediated diseases. This leads to the hypothesis that there might be a common pathway in immune-mediated diseases resulting in SFN. The idea of an immune-mediated mechanism as the cause of SFN has also been reported by others [17–19].

Table 2: Causes of small fiber neuropathy [6].

Idiopathic	
Inherited	Familial amyloidosis
	Autosomal recessive hereditary neuropathy
	Hereditary sensory and autonomic neuropathy
	Fabry's disease
	Ross syndrome
	Friedreich's ataxia
	Tangier disease
Acquired	Diabetes mellitus
	Impaired glucose tolerance
	Alcoholism
	Systemic amyloidosis
	Vasculitis
	Sarcoidosis
	Sjögren's disease
	Systemic lupus erythematosus
	Guillain-Barre syndrome
	Antecedent viral infection
	HIV
	Antisulfatide antibodies
	Hyperlipidemia
	Complex regional pain syndrome
	Paraneoplastic syndrome
	Neurotoxic medication

The pathogenetic role of oxidative stress, inflammatory cytokines such as TNF-α, and neuropeptides such as substance P (SP) are interesting to explore as a common final pathway in SFN in several immune-mediated inflammatory diseases. We described a patient with severe SFN who showed spectacular improvement after treatment with anti-TNF-α therapy [20]. This case supports the idea that TNF-α may be a crucial cytokine in the pathogenesis of SFN related to sarcoidosis and presumably in SFN related to other immune-mediated inflammatory diseases as well. Theoretical support for the effect of anti-TNF-α therapy on SFN may be found in the following. First, TNF-α plays an important role in immune-mediated neuropathies such as Guillain-Barré syndrome, in which small nerve fibers are also involved. Elevated serum concentration of TNF-α shows a positive correlation with neuropathy severity in patients with Guillain-Barré syndrome. Furthermore, the decrease in serum TNF-α and increase in serum soluble TNF receptors show a positive correlation with neuropathy recovery in those patients. Second, pharmacological and physiological studies report that proinflammatory cytokines such as TNF-α are strongly involved in the generation and maintenance of neuropathic pain [21–25].

Table 3: Drugs for pain control in small fiber neuropathy.

Drug	Dosage (per day)	Common side effects
Antidepressants		
Amitriptyline (Elavil)	20–150 mg	Sedation, weight gain, anticholinergic effects, sexual dysfunction, arrhythmia (side effects most prominent
Nortriptyline (Aventyl)	20–150 mg	Sedation, weight gain, anticholinergic effects, sexual dysfunction, arrhythmia (side effects most prominent with amitriptyline)
Desipramine (Norpramin)	20–200 mg	
Duloxetine (Cymbalta)	60–120 mg	
Anticonvulsants		
Gabapentin (Neurontin)	600–3,600 mg	Sedation, dizziness, peripheral edema, weight gain
Pregabalin (Lyrica)	150–600 mg	Similar to gabapentin
Topiramate (Topamax)	25–400 mg	Weight loss, sedation, cognitive slowing, renal stones, paresthesias
Lamotrigine (Lamictal)	25–400 mg	Stevens-Johnson syndrome, rash, dizziness, nausea, sedation
Carbamazepine (Tegretol)	200–1,200 mg	Dizziness, sedation, ataxia, aplastic anemia, liver enzyme elevation
Oxcarbazepine (Trileptal)	600–2,400 mg	Dizziness, nausea, fatigue, leukopenia
Topical anesthetics		
5% Lidocaine patch (Lidoderm)	Every 12 hours	Local edema, burning, erythema
0.075% Capsaicin patch	Three or four times a day	Burning
Opioids, opioid agonists		
Tramadol (Ultram)	100–400 mg	Sedation, dizziness, seizures, nausea, constipation
Oxycodone (Oxycontin)	10–100 mg	Sedation, constipation, nausea; potential for addiction and abuse

3. Treatment

Although alternatives to corticosteroids have been frequently administered in this disease, corticosteroids remain the mainstay of treatment in sarcoidosis. Immunosuppressive agents (chlorambucil, cyclophosphamide, methotrexate, cyclosporine, azathioprine), anticytokine agents (thalidomide, pentoxifylline), antimalarials (chloroquine, hydroxychloroquine), melatonin, and monoclonal antibody (infliximab) have been used in chronic resistant sarcoidosis [26].

Usual treatments in sarcoidosis such as prednisone and methotrexate do not appear beneficial in sarcoidosis-related SFN (personal experience). SFN is disabling for patients and the pain is often difficult to treat. SFN has a high impact on the quality of life and often invalidates the patient. Case reports mention beneficial effects of intravenous immunoglobulin [19] and anti-TNF-alpha therapy [20]. The exact potency of these drugs needs further study, however.

Symptomatic neuropathic pain treatment in sarcoidosis patients is not different from treatment of neuropathic pain from other causes and consists of antidepressants, anticonvulsants and prolonged-release opioids (Table 3). However, in common with their effects in other neuropathic pain states, these agents provide limited pain relief in just 30–60% of patients, at the cost of considerable side effects. These data indicate that there is an imminent need for analgesic agents with high efficacy in neuropathic pain patients without causing debilitating side effects.

4. Directions for Future Studies

As the role of TNF-α in the pathogenesis of SFN in sarcoidosis appears interesting to explore, anti-TNF therapy might be beneficial in the treatment of SFN in sarcoidosis. A recent therapeutic development has been the availability of agents that directly inactivate the proinflammatory cytokine TNF-α. Those are expensive drugs with possible severe side effects including opportunistic infection.

Recently, we initiated a program aimed at the treatment of neuropathic pain in patients with sarcoidosis with a novel therapeutic agent, ARA 290. ARA 290 is a nonhematopoietic erythropoietin analogue with potent anti-inflammatory and tissue protective properties, acting at the innate repair receptor [27–29]. In recent years, an endogenous system has been identified that antagonizes the production and action of proinflammatory cytokines that are involved in promoting tissue injury, while simultaneously activating repair processes. The primary mediator of this system is hypoglycosylated erythropoietin (EPO) that acts through a unique receptor isoform, the innate repair receptor (IRR), which is a combination of EPO and beta common receptor subunits. Many diverse preclinical models of tissue injury have demonstrated the efficacy of EPO as an effective cytoprotectant and activator of healing and repair. For example, EPO acting through the IRR has been shown to improve recovery and function from nerve injury in a variety of preclinical models, including the small-fiber neuropathy caused by uncontrolled

diabetes [28]. Because the IRR has a lower affinity for EPO than the receptor utilized in hematopoiesis (~2·0–20·0 nM versus 0·2 nM resp.), larger doses of erythropoietin must be administered to activate the IRR. Since EPO interacts with both of these receptors, translation of this knowledge into clinical use has been hindered by the presence of unavoidable hematopoietic side effects triggered by the hematopoietic receptor. For example, clinical studies evaluating use of EPO for tissue protection have consistently revealed increased rates of serious thrombosis [28]. To circumvent this problem, a number of IRR-specific ligands have been engineered.

One novel approach is pyroglutamate helix B surface peptide (ARA 290). This peptide mimics the spatial configuration of EPO that is believed to interact with the IRR. In spite of having a plasma half life of less than 2 minutes, ARA 290 is as efficacious as EPO in a wide variety of models of tissue injury. Additionally, preclinical toxicology studies of ARA 290 and single- and multiple-ascending repeated dosing of human volunteers and patients with kidney disease, diabetes mellitus, or sarcoidosis have raised no safety issues (unpublished data, Araim Pharmaceuticals).

First studies in animals (with nerve-damage induced neuropathic pain) and in patients with chronic neuropathic pain from sarcoidosis and diabetes mellitus indicated that ARA 290 is highly effective in causing pain relief in these neuropathic pain states. This compound appears potential for this chronic inflammatory disease and further investigation has been started.

Abbreviations

SFN:	Small-fiber neuropathy
TNF-α:	Tumor necrosis factor-α
NP:	Neuropathic pain
LD-SFSN:	length dependent small-fiber neuropathy
NLD-SFSN:	Non-length-dependent small-fiber neuropathy
IENFD:	Intra-epidermal nerve fiber density
QST:	Quantitive sensory testing
CDT:	Cold detection threshold
WDT:	Warm detection threshold
PHS:	Paradoxical heat sensation
MDT:	Mechanical detection threshold
VDT:	Vibration detection threshold
CPT:	Cold pain threshold
HPT:	Heat pain threshold
PPT:	Pain pressure threshold
MPT:	Mechanical pain threshold
LEP:	Laser evoked potential
CHEPs:	Contact heat evoked potentials.

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CHAPTER 3

Assessing sensory profiles in sarcoidosis patients with neuropathic pain.

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Assessing sensory profiles in sarcoidosis patients with neuropathic pain

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ABSTRACT Chronic neuropathic pain is a debilitating complication of sarcoidosis. Various testing modalities have been designed to characterize nerve fiber dysfunction in affected patients. In the current study, we characterized the sensory phenotype of sarcoidosis patients with neuropathy using quantitative sensory testing, skin biopsy, and cornea confocal microscopy. On average, patients displayed sensory abnormalities and decreased nerve fiber densities. However, some cases are discussed that show distinct disease characteristics. Discrepancies between used methods indicate that combinatory techniques should be used to link disease features with symptom expression. The construction of sensory profiles or subgroups aids in the identification of commonalities in neuropathy phenotypes and will guide personalized, more successful therapy.

SAMENVATTING Chronische neuropathische pijn is een invaliderende complicatie bij sarcoïdose. Om zenuwfunctie te meten in aangedane patiënten zijn verschillende technieken ontwikkeld. In de huidige studie hebben we het sensorisch fenotype van sarcoïdose patiënten gekarakteriseerd door kwantitatieve sensorische testen, huid biopten en cornea confocale microscopie. Gemiddeld hadden deze patiënten een afwijkend sensorisch profiel en verlaagde zenuwvezeldichtheid. Echter, sommige cases worden getoond met specifieke onderscheidende afwijkingen. Ook laten we discrepanties zien tussen de gebruikte technieken, waardoor wij pleiten voor gecombineerd gebruik van meetmethoden om ziekte karakteristieken te kunnen verbinden aan symptomen. De definitie van subgroepen zal gemeenschappelijkheid in fenotypes kunnen aantonen en gepersonaliseerde, meer succesvolle therapie waarborgen.

Key words: Cornea confocal microscopy, neuropathy, pain, sarcoidosis, sensory profiles, skin biopsy.

Introduction

Chronic neuropathic pain is a debilitating complication of several systemic illnesses, decreasing the patient's quality of life, and providing continuous psychological and pharmacological challenges to patients and treating physicians. Neuropathic pain is a well-recognized complication of diabetes mellitus, but it is also the most commonly reported symptom in patients with sarcoidosis [1, 2]. It is the result of damage or destruction of nerve fibers of the sensory and autonomic nervous system. Although the underlying pathophysiology in various diseases may be different, the outcome of peripheral neuropathy is often similar and typified by spontaneous pain, allodynia, hyperalgesia, numbness, or autonomic symptoms such as gastrointestinal complaints, orthostatic hypotension, dry eyes, or blurry vision [1]. Both small and large sensory nerve fibers can be affected, leading to a wide range of clinical signs and symptoms that may be present in patients.

Various testing modalities have been developed to characterize nerve fiber dysfunction in affected patients. These tests can be invasive and non-invasive, and range from patient-reported subjective outcomes in the form of validated questionnaires to more objectified measurements of nerve fiber quantity or quality. In our research we use Quantitative Sensory Testing (QST), skin biopsies and Corneal Confocal Microscopy (CCM) to assess the sensory profiles of patients with painful neuropathy.

QST. Wide ranges of sensory testing modalities were developed to assess nerve fiber deficits in patients with neuropathy. The German Research Network on Neuropathic Pain implemented a standardized QST protocol including a defined testing order [3]. The tests are non-invasive and aim at examining different aspects of the somatosensory nervous system, including thermal and mechanical detection thresholds, mechanical allodynia, and vibration and pressure thresholds [3, 4]. A major advantage of this approach is that both loss and gain of nerve function can be assessed, the method is relatively fast (approx. 60-90 minutes per patient) and extensive information on the distribution of fiber dysfunction can be acquired. The availability of gender-, age- and testing location reference values allows normalization of testing results and direct comparison of these results with a healthy control population [3, 4].

Skin biopsy. The currently accepted measure for the diagnosis of small fiber neuropathy (SFN) is a skin biopsy. It is commonly performed using a 3-mm diameter punch, after topical anesthesia with lidocaine [5]. The standard location for these skin biopsies is 10-cm above the lateral malleolus, as density of nerve fibers differ between anatomic locations [6]. In these skin biopsies, intra-epidermal nerve fiber densities, determined by immunofluorescence technique, can be compared to gender- and age-related reference values [5, 6], to reliably and efficiently confirm the clinical diagnosis of SFN. A recent study demonstrated that more neuropathy-related symptoms were present in patients with abnormal nerve fiber densities in skin biopsies, compared to patients with normal skin biopsies [7].

CCM. Recently, a novel technique has been developed for the quantification of nerve fibers in the cornea [8]. CCM examines the densely innervated cornea as a surrogate for small nerve fiber state, and hence can serve as a quantitative and qualitative measure of neuropathy [9, 10]. Nerve fiber counts in the cornea appear highly correlated with skin biopsies, and correlate with clinical symptoms of neuropathy [9]. Hitherto, CCM appears superior compared to skin biopsy for the detection, quantification and repeated assessment of SFN in diabetes patients [8, 9, 11-13]. For CCM, normative reference values have been published to allow comparison of nerve fiber quantifications in diseased vs. non-diseased individuals [9, 14-16].

Here, we describe the sensory phenotype of sarcoidosis patients as measured by QST of face, hand and foot, skin biopsy, and CCM of both eyes. We highlight several cases with typical and distinct disease characteristics, all of which are sarcoidosis patients with chronic painful neuropathy. We discuss the outcomes and relevance of the used techniques in the assessment of their disease phenotype.

Cases

1 Patient 1, a 65-year old male, was diagnosed with sarcoidosis and neuropathic pain in 2010. His pain symptoms are primarily present in both legs, with a spontaneous pain score of 7 (numerical rating scale NRS, possible range 0-10). He suffers from abnormal temperature sensations in his legs and feet, and his hands and feet are tingling intermittently.

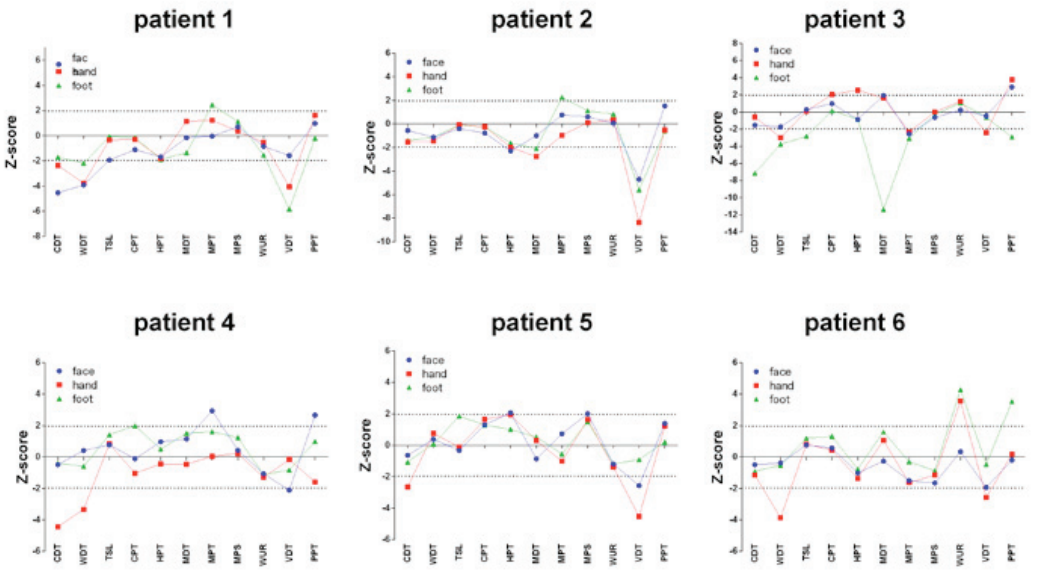
- *QST.* This test was performed on face, hand and foot. At all locations abnormal thermal detection thresholds were observed. Hand and foot showed a loss of vibration detection. Moreover, severe allodynia was detected on the foot. All other tests were within the 95% confidence interval of the mean corrected for gender, age and tested location (see Figure 1).
- *Skin biopsy.* Nerve fiber density in his biopsy was significantly decreased (0.19 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 1739 μm^2 (see Figure 2), which is significantly decreased compared to the average of normal controls [9, 14].

Conclusion. This patient reports symptoms of paresthesia in legs and feet. Thermal sensation is normal in the foot. However, abnormal thermal detection thresholds were observed on the face and hand location, indicative for generalized, subclinical SFN in these locations. SFN was detected by skin biopsy and CCM. The patient did not report allodynia symptoms, but did score positive by QST for mechanical allodynia. The loss of vibration sensation is indicative for a large fiber defect as well.

2 Patient 2, 38-year old female, was diagnosed with sarcoidosis in 2010 following a lung biopsy and chest X-ray; neuropathy was diagnosed in 2011 after a skin biopsy. Her average daily pain score is 7, and symptoms present as cramping muscles, abnormal cold sensation in hands and feet, allodynia and tingling hands and feet.

- *QST.* Face, hand and foot were tested according to standardized QST procedures. Hand and foot showed abnormally increased mechanical

Figure 1. Quantitative sensory testing profiles of the discussed cases.



Quantitative sensory testing results of six discussed patients. Data are represented as Z-scores, which are corrected for age, gender and testing location according to published reference values [3]. Z-scores below -1.96 or above 1.96 (two broken lines) indicate loss or gain of function, respectively. CDT, cold detection threshold; WDT, warm detection threshold; TSL, thermal sensory limen; PHS, paradoxical heat sensations; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; ALL, dynamic mechanical allodynia; WUR, wind-up ratio; VDT, vibration detection threshold; PPT, pressure pain threshold.

detection thresholds, and mechanical hyperalgesia was detected on the foot. Most strikingly, a loss of vibration detection was observed on face, hand and foot site. All other tested parameters were normal (see Figure 1).

- *Skin biopsy.* Nerve fiber density in this biopsy was significantly decreased (5.80 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 941 μm^2, which is significantly decreased compared to the average of normal controls [9, 14].

Conclusion. The neuropathic pain complaints of this patient consist of cramping pain, paresthesia, and allodynia in hands and feet. QST did not show alterations indicative for SFN, although nerve fiber densities in skin biopsy and cornea were both abnormally decreased. A profound loss of vibration sensation indicates large fiber dysfunction, and correlates with clinical symptoms of cramping pain. This patient suffers from symptomatic, generalized large fiber neuropathy with SFN as well.

3 Patient 3 is a 50-year old female, who was diagnosed with sarcoidosis in 2008 after a lung biopsy and chest X-ray. Neuropathy was never diagnosed formally, but was treated symptomatically. Pain symptoms are present in hands and arms, allodynia on the legs, and abnormal temperature sensations in the feet. Her average daily pain score is 8.

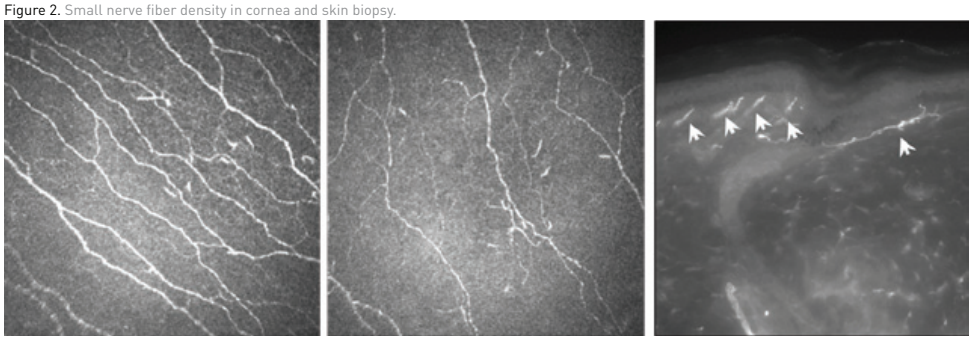
- *QST.* This test demonstrated most prominent abnormalities on the foot. However, thermal detection thresholds and vibration sensations were also abnormal on the hand. A profound loss of function of the mechanical detection threshold and an increased pressure pain threshold was detected on the foot. Moreover, paradoxical heat sensations were present on the foot (see Figure 1).
- *Skin biopsy.* Nerve fiber density in this biopsy was within the normal range (5.30 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 1340 μm^2, which is significantly decreased compared to the average of normal controls [9, 14].

Conclusion. This case has paresthesia and allodynia, which is reflected by abnormal thermal detection thresholds on the hand and foot. Moreover, the foot is affected as evidenced by paradoxical heat sensations and mechanical hypoalgesia. CCM identified loss of small nerve fibers, which was not confirmed by skin biopsy. This patient has a predominant SFN phenotype.

4 Patient 4 is a 53-year old male who was diagnosed after a chest X-ray with sarcoidosis in 2003. A neurologist diagnosed neuropathy in 2007 after sensory testing. This patient presents with pain symptoms bilaterally in hands and feet (average daily pain score of 7), paresthesia, hyperalgesia and allodynia.

- *QST.* Tests were performed on face, hand and foot. With the exception of mild hyperalgesia in the face, tested parameters on both face and hand location were within the normal range for age and gender. Thermal detection thresholds were clearly abnormal, and paradoxical heat sensations and mechanical allodynia

Figure 2. Small nerve fiber density in cornea and skin biopsy.



Cornea confocal microscopy (CCM) in a normal individual (left panel) shows high nerve fiber density. Middle panel shows CCM from a sarcoidosis patient with neuropathy (patient 1), demonstrating decreased nerve fiber counts. Right panel is a representative immune-fluorescent image from a stained skin biopsy, obtained from a sarcoidosis patient with painful neuropathy (patient 6), illustrating decreased intra-epidermal nerve fiber density. Nerve fibers are indicated with arrows.

were present on the foot (see Figure 1).
- *Skin biopsy.* Nerve fiber density in this biopsy was within the normal range (3.88 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 1442 μm², which is significantly lower than the average of normal controls [9, 14].

Conclusion. Clear thermal sensation disturbances were detected on the hand, whereas the type of sensory disturbances on the foot was different (paradoxical heat sensations). Mechanical allodynia was only detected on the foot, although the patient did report complaints of painful hands and arms in response to light touch. SFN was not confirmed by skin biopsy, but corneal nerve fiber density was decreased. Isolated SFN in this patient is present in hands and feet.

5 Patient 5, a 56-year old female, has sarcoidosis since 2008 and neuropathic pain in hands and feet since 2010. Her daily pain score is 5, and hyperalgesia is confined to her face. Her hands and feet are tingling infrequently and her primary symptom is hyperalgesia in these areas.
- *QST.* No abnormalities were detected on the foot. Thermal detection was disturbed on the hand, and vibration detection was also decreased on the hand. Mild vibratory loss and profound dynamic mechanical allodynia were present on the face (see Figure 1).
- *Skin biopsy.* Nerve fiber density in her biopsy was within the normal range (5.01 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 1500 μm², which is significantly decreased compared to the average of normal controls [9, 14].

Conclusion. Face hyperalgesia was confirmed by QST in this patient by the presence of mechanical allodynia. Skin biopsy was normal, but corneal nerve fiber density was decreased. This patient likely has mixed SFN with large fiber involvement as well.

6 Patient 6 is a 37-year old male, who was diagnosed in 2011 after a lung biopsy. He suffers from neuropathic pain since 2000, which is present in hands and feet (average daily pain score of 6). Symptoms consist of allodynia, paresthesia, muscle cramps, and tingling hands and feet.
- *QST.* The tests demonstrated abnormal thermal sensation on the hand. Temporal summation in the form of increased wind-up phenomenon was detected on hand and foot. Vibration sensation was decreased on the hand, and the pressure pain threshold was decreased on the foot. On the foot, cold sensation was disturbed in the form of paradoxical heat sensations (see Figure 1).
- *Skin biopsy.* Nerve fiber density in his skin biopsy (see Figure 2) was significantly decreased (3.57 nerve fibers/mm) compared to age- and gender matched controls [6, 7].
- *CCM.* The corneal nerve fiber area was 1377 μm², which is significantly lower than the average of normal controls [9, 14].

Conclusion. This patient has a distal and symmetrical neuropathic symptomatology, which was not confirmed by QST testing. Abnormalities on the foot included paradoxical heat sensations, increased wind-up phenomenon and pressure hyperalgesia, whereas the hand showed increased warm detection thresholds, increased wind-up and a loss of vibration sensation. SFN was detected by skin biopsy and by CCM. Decreased vibration sensation suggests large fiber involvement in addition to a generalized SFN profile.

Discussion

The main objective of the current study was to characterize sensory profiles of neuropathic pain patients with sarcoidosis and to assess associated symptoms. Neuropathic pain patients display heterogeneity of symptoms, ranging from spontaneous pain and hyperalgesia, to numbness and autonomic dysfunction. Standardized testing of patients is important, and should encompass assessment of thermal and mechanical sensory thresholds, as well as more objectified measurements of nerve fiber density. As neuropathic pain can arise from damage or destruction of small and/or large nerve fibers, ideally sensory testing should include threshold testing for both types of fibers. The standardized QST paradigm includes assessment of small and large fibers,

and multiple anatomic sites [3]. Skin biopsy and quantification of intra-epidermal nerve fiber density is currently accepted as the gold standard for diagnostics of SFN [5], but corneal measurements of small nerve fiber densities are indicative as well [8, 9]. We identified an abnormal albeit heterogeneous sensory phenotype in sarcoidosis patients with neuropathic pain. The majority of patients demonstrate paresthesia, and some patients present with hyperalgesia and allodynia. This is commonly in accordance with their daily symptoms, and is associated with high average daily pain scores. These findings are in line with studies from Hoitsma et al, who first demonstrated the presence of neuropathy in sarcoidosis [2, 17]. It is notable that a number of our patients display disturbed vibration sensation. This test is known to assess large nerve fiber function, and indicates the presence of both small and large fiber neuropathy in sarcoidosis patients [17, 18]. Future examinations, including nerve conduction studies and EMG, should assess the involvement of large fiber dysfunction in sarcoidosis-related neuropathy in more detail.

In several of our above-described cases, a discrepancy between the assessments of nerve fiber density in skin biopsy *vs.* CCM was observed. Although most patients displayed significantly decreased nerve fiber counts in the cornea, only a subset was classified with SFN in skin biopsies (see Figure 2). These results could reflect the differential sensitivity of both techniques to detect paucity of nerve fibers in end-organs but is most likely related to the heterogeneity of the disease. Future studies should address the correlation of the two techniques in their ability to quantify neuropathy in detail. Previous work demonstrated that cornea nerve fiber density was highly predictive for patient-reported pain scores, which was less clear for skin biopsy-assessed nerve density [9]. Hitherto, CCM appears superior compared to skin biopsy for the detection and quantification of neuropathy [8, 9, 11-13]. The recent undertaken effort to provide CCM normative values will aid in the development of specific diagnostic criteria [15]. The sensitivity and specificity of cornea microscopy should be determined and correlated to intra-epidermal nerve densities in a large cohort of neuropathic pain patients.

The cases described in our report highlight some of the discrepancies that can occur between the methods used to examine neuropathy patients. For example, patient 2 had symptoms consistent with SFN, had decreased nerve fiber density in the cornea and in a skin biopsy, but QST results showed thresholds that were within the range of normal controls. This mismatch indicates the complexity of the underlying disease and advocates the detailed examination of neuropathic pain patients with multiple methods. Subepithelial nerve fiber density examination, either by skin biopsy or CCM, allows objective quantification of neuropathy in affected patients.

Recently, sensory profiles of neuropathic pain patients were published that reflected specific signs and symptoms instead of underlying disease etiologies [19]. The authors identified different sensory profiles within each etiological category and could not identify specific pain profiles associated with specific diseases. We observe a range of sensory testing abnormalities in our described cases with a relatively homogeneous pattern of neuropathic pain symptoms (*e.g.* pain scores). Moreover, test results did not always match patients' complaints. While this may reflect the sensitivity and specificity of the sensory tests, it could also be related to the subjective interpretation of the QST by the reporting patient. Our data, together with those of Freeman et al. [19] highlight the importance to perform several types of sensory tests on multiple anatomic locations to acquire a detailed overview of sensory and autonomic deficits within each patient. It is of importance that physicians are aware of the typical neuropathic symptoms associated with his patient for adequate diagnostics and follow-up. Based on observations in post-herpetic neuralgia, it has been suggested that two neuropathic pain phenotypes may exist: one with hyperalgesic symptoms, and one with hypoalgesic symptoms [20, 21]. This is not confirmed in our patient population, where some patients display loss of thermal sensitivity and increased temporal summation (wind-up) even on the same tested location. It is currently unclear if neuropathy phenotypes should be linked to underlying etiological disease, and if these stratifications improve treatment outcomes. The relative heterogeneity of neuropathic pain patients has led to multiple studies aimed to identify subgroups of patients according to their sensory phenotype [22, 23]. Although this stratification could predict patients' response to intervention (including pharmacological treatment), no clinical trials included sensory profile as inclusion or stratification criteria. In addition, the unpredictable course of both sarcoidosis and associated neuropathy highlights the importance of strict monitoring of sarcoidosis activity, and follow-up of neuropathy symptoms. Current pain treatment is symptomatic, and conventional therapy shows limited efficacy [24]. Moreover, the occurrence of severe side effects associated with pain treatment prevents effective treatment at the required dose and decrease patient compliance. To improve outcome, future therapeutic trials should take into account both the heterogeneity of neuropathy symptoms, as well as the sensory profile in which patients could be stratified.

In conclusion, sarcoidosis patient with neuropathy are abnormal in various sensory testing modalities. In some cases, the sensory profiles correspond well with the pattern of complaints, in others it does not. We relate these discrepancies to the heterogeneity of the neuropathic disease, possibly related to different underlying mechanisms of nerve damage despite one systemic disease. It is further unclear whether these patients are different in terms of prognosis or treatment outcomes. In addition, discrepancies between testing modalities support the use of combinatory techniques to identify commonalities in neuropathy phenotypes.

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CHAPTER 4

Population pharmacokinetic analysis of intravenous and subcutaneous ARA290

Subcutaneous ARA 290 administered to healthy volunteers: a safety and pharmacokinetic analysis

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Abstract

Background: ARA290 is an 11-amino acid peptide with tissue protective properties. It is effective in the treatment of neuropathic pain in patients with sarcoidosis and diabetes mellitus type 2. Since ARA290 requires frequent parenteral administrations, various routes of administration are being examined that allow treatment outside the hospital setting with high patient compliance. In this study we determined the safety and pharmacokinetics of subcutaneous (SC) compared to intravenous (IV) ARA290 administration.

Methods: 10 healthy volunteers received on one occasion 2 mg IV ARA 290, and on another occasion 2 mg SC ARA290. On a third occasion, 5 subjects received 4 mg SC and the five others 6 mg SC ARA290. Serial plasma samples were obtained to determine the time-dependent plasma concentrations of ARA290. Safety parameters were obtained; the pharmacokinetics were assessed by non-compartmental analysis.

Results: No safety issues were identified. The mean peak plasma concentration after IV dosing was estimated at 111 ng/mL. After SC administration peak concentrations were observed between 12 and 15 min and were greater than 1.25 ng/mL for all SC doses, the minimum concentration believed to be necessary to trigger the receptor mediating ARA 290 biological effects. The terminal half-life of the IV and SC doses were 1.1 min and 17-26 min, respectively. Based on the area-under the plasma-concentration curve the bioavailability of SC ARA290 ranged from 11 to 25%.

Conclusions: ARA 290 is safe and well tolerated up to 6 mg SC. All three SC doses exhibited peak concentrations greater than the assumed minimum effective concentration.

Introduction

Tissue injury causes an inflammatory response, driven by pro-inflammatory cytokines (most importantly TNF-α) and results in tissue destruction and systemic disease.[1-3] Simultaneous to the pro-inflammatory response an anti-inflammatory reaction is initiated by the release of anti-inflammatory cytokines. Important mediators of the anti-inflammatory response are erythropoietin (EPO) and its receptor, the innate repair receptor (IRR). The IRR is composed of two classical EPO receptors and two β-common receptor subunits (CD131).[1,2] Activation of the IRR by EPO activates multiple anti-inflammatory, tissue protective and regenerative pathways. While it seems attractive to treat patients with an inflammatory disease with EPO, its side effect profile prohibits its (long-term) use.[4] Side effects include thrombotic events, hypertension and myocardial infarction. Recently, a series of non-hematopoietic EPO analogues have been constructed that mimic the anti-inflammatory effects of EPO without any of its deleterious side effects. One such analogue is ARA290, a pyroglutamate helix B surface peptide that mimics the

spatial configuration of the EPO surface that interacts with the IRR.[1-3] Various experimental and clinical studies show that ARA290 effectively reduces symptoms of tissue injury. For example, in sarcoidosis and diabetic mellitus type 2 patients with severe neuropathic pain (pain caused by a lesion or disease of the peripheral somatosensory nervous system) show a prolonged reduction of neuropathic pain symptoms and an improved quality of life.[5-7] ARA290 is administered intravenously and requires frequent (daily to two-three times weekly) injections. Daily intravenous injections are difficult to manage outside the hospital setting. Subcutaneous injections, however, comparable to insulin injections, are easily accomplished by the patient at home. To test the feasibility of subcutaneous ARA290 injections, we performed this phase 1 study in healthy volunteers to assess the safety of ARA290 injections and determine its pharmacokinetics.

Methods

Subjects

This is a single-center, open-label study performed in 10 healthy volunteers. Five male and five female subjects were enrolled in the study after the protocol was approved by the local human ethics committee and after the subjects had given written informed consent. Subjects were included if they were 18 to 65 years (inclusive), had a body mass index of 18-30 kg/m^2 (inclusive) and body weight 50-90 kg (inclusive), and were able to give informed consent. Exclusion criteria included: a history of physical and/or mental disease, hypertension (systolic blood pressure > 150 mmHg and/or diastolic blood pressure > 90 mmHg), a history of substance abuse (incl. alcohol use > 21 units/week (male) or > 14 units/week (female)), positive pregnancy test, use of nicotine containing products within 3 months prior to screening, use of xanthine containing products during the study, presence of allergies to prescription and non-prescription drugs or food, vaccination or immunization within the last month, participation in other trials in the 3 months prior to administration of the initial dose of the study drug or more than 4 times per year, donation or loss of blood (> 500 mL) within 3 months prior to screening, any other condition that in the opinion of the investigator could complicate or compromise the study, or the well-being of the subject.

Study Design

All subjects were submitted to the laboratory on three separate occasions, at least 1 week apart. They received on one occasion 2 mg intravenous (IV) ARA290 (in 6 mL saline, conc. 0.33 mg/mL, administered over 2 min using an infusion pump) and on the other 2 mg subcutaneous (SC) ARA290 (in 0.25 mL saline, conc. 8 mg/mL). On the third occasion, 5 subjects received 4 mg SC ARA290 (in 0.5 mL saline, conc. mg/mL), while the other 5 received 6 mg SC ARA290 (in 0.75 mL saline, conc. 8 mg/mL).

Blood samples were obtained from an IV access line in the left or right cubital vein (opposite to the line used for the intravenous infusion) at t = 1, 2, 3, 4, 6, 8, 10, 14, 18 and 24 min post dosing on occasion 1 and t = 1, 2, 4, 6, 10, 15, 20, 30, 45 and 60 min post dosing on occasions 2 and 3. The volume drawn was 3 mL; less than 30 mL blood was drawn per visit. Blood samples were collected in 6 mL EDTA-coated tubes. Within 30 min after collection, the samples were centrifuged at 3000 rpm for 10 minutes at 4˚C. The collected plasma was transferred to

duplicate transport tubes (approximately 1 mL per tube) and stored at -80˚C. One set of samples was shipped for analysis; the other set stored as backup.

Subjects were monitored for adverse effects at LUMC until 1-2 hours after administration of study drug and collection of the plasma samples.

ARA29

ARA290 (L pyroglutamyl-L glutamyl-L glutaminyl-L leucyl-L glutamyl- L arginyl-L alanyl- L leucyl-L asparaginyl-L seryl-L serine-OH, ARAIM Pharmaceuticals, Tarrytown, NY) is an 11-amino acid, linear peptide with a molecular weight of 1257 Daltons. ARA290 was manufactured by standard F-moc solid phase peptide synthesis, purified by HPLC and ion-exchange chromatography, and stored as a lyophilized powder. The drug was stored by the local pharmacy and manufactured by Bachem Distribution Services GmbH, Weil am Rhein, Germany. The drug was stored in the local pharmacy at -20 °C; the syringes were prepared by the investigators according to pharmacy guidelines.

ARA 290 plasma concentrations were determined by Charles River Laboratories in Halifax, Canada, using a validated high-performance liquid chromatography with tandem mass spectrometric (LC-MS/MS) detection. The lower limit of quantitation was 0.1 ng/mL.

Pharmacokinetic Analysis

A non-compartmental PK analysis was performed using WinNonlin software (version 5.3, Pharsight Corporation, Mountain View, CA). For the single IV infusion the following parameters were estimated: terminal half-life (t½), area-under the plasma-concentration curve ($AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$), total plasma clearance (CL), apparent volume of distribution at steady state (VSS). For the single SC injection the following parameters were estimated: observed maximum ARA 290 plasma concentrations (C_{MAX}), time to maximum ARA 290 plasma concentrations (TMAX), t½ and $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$. The absolute systemic bioavailability was calculated as F = (SC $AUC_{0\text{-}t}$ /IV $AUC_{0\text{-}t}$) × (IV dose/SC dose). Values given are mean ± SD and coefficient of variation (%CV).

Results

The subject's age ranged from 20 to 23 years (mean ± SD 21.3 ± 1.2 years), weight from 51 to 90 kg (70.8 ± 12.1 kg), height 164 to 198 cm (180 ± 10 cm) and body mass index from 18.7 to 26.0 $kg.m^{-2}$ (22.3 ± 2.5 kg/m^2). Since the ARA290 dose was not corrected for body mass, the average doses given were 29.0 ± 5.0 µg/kg for the 2 mg dose, 58.5 ± 4.5 µg/kg for the 4 mg dose and 86.4 ± 21.4 µg/kg for the 6 mg dose.

All subjects completed the study without any adverse events. No local symptoms or signs were observed after either the intravenous or subcutaneous injection sites.

The individual PK data for the 4 treatment levels are given in Figure 1; the mean ± SEM data are given in Figure 2. Following the IV injection, ARA290 plasma concentration peak concentration was at the first ARA290 measurement at t = 1 min post dosing (54 ± 14 ng/mL) followed by a rapid decline in plasma concentration reaching values < lower limit of quantitation after ~10 min post infusion. In contrast, following SC administration, peak concentrations are observed after 8

min. The elimination phase is slower than that observed after the IV injection with mean concentrations > 0.3 ng/mL after 60 min.

IV ARA290. Plasma concentrations of ARA 290 following IV administration declined in a mono-exponential fashion with a mean t½ of ~1.1 min (Table 1). The extrapolated ARA290 plasma concentration at the end of the 2-min infusion was ~111 ng/mL. The mean total plasma clearance was high at 10.6 L/min and this value exceeded organ blood flows and cardiac output. This finding is highly suggestive of ARA 290 being rapidly metabolized or degraded in the circulation. The volume of distribution at steady-state (V_{SS}) was large with a mean value of approximately 21 L.

Table 1. Pharmacokinetic parameter estimates

	2 mg IV	2 mg SC	4 mg SC	6 mg SC
C_{MAX} ng/mL	-	1.8 ± 1.4 (75)	2.9 ± 1.7 (59)	8.3 ± 4.0 (48)
t½ min	1.1 ± 0.1 (13)	20.6 ± 10.1 (49)	17.6 ± 10.8 (61)	16.9 ± 5.6 (33)
CL L/min	10.6 ± 5.9 (55)	*	*	*
V_{SS} L/min	21.1 ± 13.4 (63)	*	*	*
F %	-	22 ± 13 (60)	18 ± 7 (38)	37 ± 24 (66)
AUC_{0-t} ng.min/mL	274 ± 202 (74)	53 ± 36 (68)	82 ± 51 (61)	223 ± 51 (23)
$AUC_{0-\infty}$ ng.min/mL	274 ± 202 (74)	64 ± 48 (76)	95 ± 46 (49)	275 ± 42 (15)

* not estimated; C_{MAX} is the observed maximum ARA290 concentration in plasma; t½ is the terminal half-life; CL is total plasma clearance; V_{SS} is the apparent volume of distribution; F is the absolute systemic bioavailability ; AUC is the area-under-the-curve.

SC ARA290. ARA 290 plasma concentrations, C_{max} and AUC increased with increasing dose of SC ARA 290 (Table 1). The mean peak plasma concentrations occurred at 6 min and were greater than 1.25 ng/mL for all SC dose levels, the minimum concentration believed to be necessary to trigger the receptor mediating ARA 290 biological effects. After reaching maximal ARA 290 plasma concentrations within 12-15 min, ARA 290 plasma concentrations declined in a mono-exponential fashion with a mean t½ of 17 to 21 minutes. The relatively high value of t½ after SC infusion indicates "flip-flop" kinetics, *ie.* the terminal phase of decline of ARA290 does not represent the elimination phase. The slower PK process governing the decline in concentrations is related to the absorption of the drug from the subcutaneous injection site. The mean absolute bioavailability (F%) was estimated as 22%, 18% and 37% for the single 2 mg, 4 mg and 6 mg SC ARA 290, respectively.

Considerable between-subject variability was observed in ARA290 plasma concentrations for both the intravenous and subcutaneous routes of administration (Fig. 1); the %CV ranged from 24% to 138% at any given sampling time point for the IV and SC routes of administration. The %CV in PK parameters was greatest for the exposure parameters of C_{MAX} and AUC ranging from 15% to 82%.

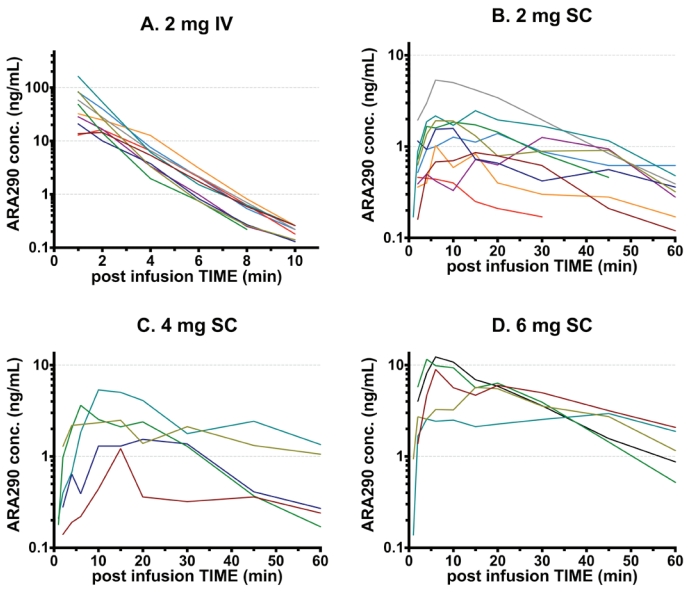

Figure 1. A. Individual plasma concentrations following the administration of 2 mg intravenous (IV) ARA290. **B-D.** Individual plasma concentrations following the administration of 2-6 mg subcutaneous (SC) ARA290.

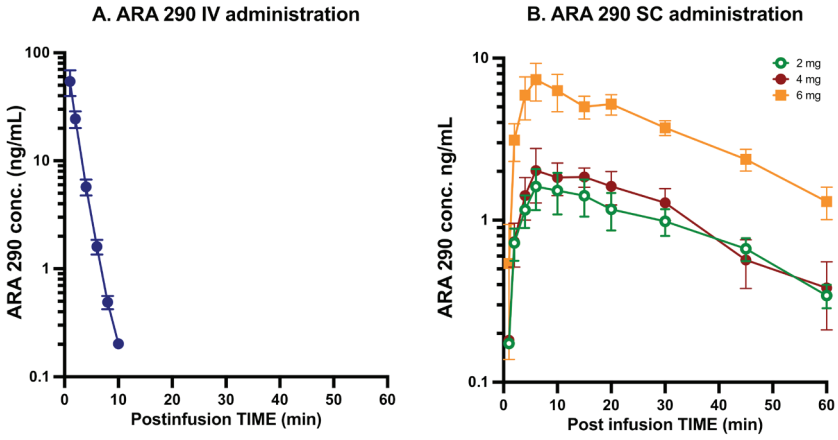

Figure 2. A. Mean ± SEM ARA 290 plasma concentration following 2 mg intravenous administration. **B.** Mean ± SEM ARA 290 plasma concentration following 2, 4 and 6 mg subcutaneous administration.

Discussion

ARA 290 administered in the dosage range of 2-6 mg SC and 2 mg IV was not associated with any adverse events. Specifically, there were no complaints of local pain, redness, or cutaneous sensitivity and no systemic effects reported. On average, all three subcutaneous doses exhibited a C_{MAX} greater than the assumed minimum effective concentration of 1 nM obtained from preclinical studies.

The doses used in the current study were based on previous studies. A preclinical model of neuropathic pain in the rat showed that for a sustained therapeutic effect of ARA290, a minimum dose of 30 $\mu g.kg^{-1}$ twice weekly administered via the intraperitoneal route is required.[8] Results of previous clinical studies of ARA 290 in patients with diabetes or sarcoidosis and small fiber neuropathy have shown that IV dosing (2 mg or approximately 30 $\mu g.kg^{-1}$) administered three days weekly was associated with a significant improvement in pain in a majority of patients, consistent with the preclinical observations.[5-7] Repeated intravenous dosing is, however, not feasible for chronic treatment of disease, as the assistance of a health professional is required. A desirable therapeutic paradigm is one of patient self-administration. An additional consideration of chronic dosing is the frequency required. Although 3 doses of ARA 290 appear to be efficacious on the basis of small, proof of concept trials, in practice, such a dosing paradigm is difficult for patients to attain, as frequently doses are inadvertently omitted. Daily dosing as a routine will likely be associated with a better compliance. Therefore, a subcutaneous formulation of ARA 290 is highly desirable.

The results of *in vitro* studies using human endothelial cells have shown that concentrations in the 1-2 nM range provide a maximum effect of signaling pathway phosphorylation.[9] This corresponds well to minimum plasma concentrations of ARA290 required for therapeutic efficacy, which in a rat model corresponds to 30 $\mu g.kg^{-1}$ (intraperitoneally), which gives a C_{max} of approximately 1-2 nM peak plasma concen-tration. PK studies performed on normal volunteers as well as renal insufficiency/renal failure patients have shown that IV doses ranging from 70 µg to 2000 µg result in peak plasma concentrations in excess of 1-2 nM, but have a very short elimination t½ of about 2 min (ARAIM data on file); PK studies comparing the SC and IV routes were carried out in patients with chronic renal failure at a dose of 1400 µg. ARA 290 is partially cleared by the kidney, so the pharmacokinetics are modified compared to normal subjects. Specifically, a higher peak plasma concentration and a doubling of the terminal phase half-life (3.5 min *versus* 1.8 minutes) was observed in renal failure patients.

Subcutaneous dosing studies carried out in the rhesus macaque and rabbit are in agreement with our current findings (Fig. 3; ARAIM, data on file). A prompt rise to a peak at about 4 minutes and then a rapid decay is apparent. Peak plasma concen-trations for 25-30 $\mu g.kg^{-1}$ (corresponding to 8-10 $\mu g.kg^{-1}$ in humans) are in the 1-5 nM range, thus above the expected minimum effective concentration. Increasing the dose by a factor of 4 for the rabbit (equivalent to 8.4 mg in humans) did not result in excessively higher plasma peak concentrations, but rather an increased duration of plasma levels at or slightly above the expected minimum effective concentration (1-2 nM).

Similar to previous studies performed in healthy volunteers, in the current study ARA290 via the IV route had a short terminal elimination half-life. Based on data obtained in all studies to date,

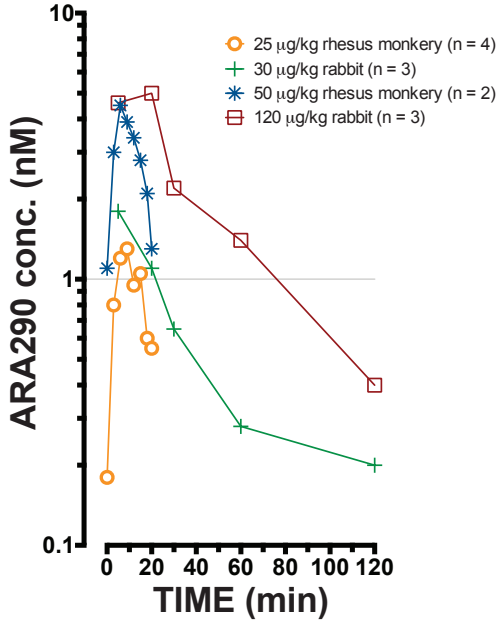

Figure 3. Mean plasma ARA290 concentrations following subcutaneous administration in rhesus monkeys and rabbits (ARAIM, data on file).

the elimination t½ of IV ARA 290 in normal volunteers has ranged from 1.1 to 2.3 minutes (standard fixe dose uncorrected for variations in body mass). Subcutaneous ARA 290 dosing peaked by 12-15 minutes and decreased with a t½ in the range of 17 to 27 minutes. In this study the concentration of ARA 290 in each injection was constant (8 $mg.mL^{-1}$) with variable injection volumes into the lateral thigh. The resulting ratios of the AUCs of the subcutaneous doses were 1:1.3:4.4 for doses of ratios 1:2:3. The observed divergence in the ratio of the observed plasma concentration may be result from, in part, variations of injection volume. Additionally, difference in body mass or other variable not adjusted for in this study could contribute variability in the pharmacokinetic results (Fig. 1). In future studies, dosing base on body weight may result in less variable PK data.

Both for IV and SC administrations the residence time in plasma was short. Still, ARA290 has long-term pharmacodynamic effects in animals and humans. These long-term pharmacodynamic effects suggest that ARA290 initiates a cascade of events involving several steps.[1-3] For neuropathic pain, the site of action of ARA290 is within the central nervous system. Previously we showed that activation of the "Innate Repair Receptor" (a complex of the erythropoietin receptor and the β-common receptor), which is upregulated following tissue injury and inflammation, is a first step that eventually leads to the anti-inflammatory, neuroprotective and healing effects of ARA290.[10] For example, in the rodent, 1-2 weekly doses of ARA290 reduce allodynia following sciatic nerve transection for at least 15 weeks, combined with a reduction of

activated (*ie.* pronociceptive) microglia cells in the spinal cord. Anti-allodynic effects did not occur in rodents that lacked the erythropoietin receptor/β-common receptor complex.

In conclusion, we observed that the administration of SC ARA290 to healthy volunteers is safe. Furthermore, since concentrations following SC injections exceed the minimum effective concentration in the majority of subjects (and in all subjects at 4 and 6 mg SC), SC administration is an effective and reliable method to administer ARA290 to patients with neuropathic pain. The results may be used to design and compare specific dosing paradigms. Future studies should assess whether ARA290's PK obtained in specific patients populations compares to those observed in the current study in healthy volunteers.

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CHAPTER 5

Safety and efficacy of ARA290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double blind, pilot study

Molecular Medicine

Safety and Efficacy of ARA 290 in Sarcoidosis Patients with Symptoms of Small Fiber Neuropathy: A Randomized, Double-Blind Pilot Study

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ARA 290 (a peptide designed to activate the innate repair receptor that arrests injury and initiates cytoprotection, antiinflammation and healing) reduces allodynia in preclinical neuropathy models. We studied the safety and efficacy of ARA 290 to reduce symptoms of small fiber neuropathy (SFN) in patients with sarcoidosis. A total of 22 patients diagnosed with sarcoidosis and symptoms of SFN were enrolled in a double-blind, placebo-controlled exploratory trial consisting of three times weekly intravenous dosing of ARA 290 (2 mg; n = 12) or placebo (n = 10) for 4 wks. Inclusion criteria were a diagnosis of neuropathy and a spontaneous pain score of ≥5 (Brief Pain Inventory (BPI)). Endpoints assessed were changes in pain intensity and the small fiber neuropathy screening list (SFNSL) score, quality of life (SF-36), depressive symptoms (Inventory of Depressive Symptomatology (IDS)) and fatigue (Fatigue Assessment Scale (FAS)). No safety concerns were raised by clinical or laboratory assessments. The ARA 290 group showed significant ($p < 0.05$) improvement at wk 4 in SFNSL score compared with placebo (Δ −11.5 ± 3.04 versus Δ −2.9 ± 3.34 (standard error of the mean)). Additionally, the ARA 290 group showed a significant change from baseline in the pain and physical functioning dimensions of the SF-36 (Δ −23.4 ± 5.5 and Δ −14.6 ± 3.9, respectively). The mean BPI and FAS scores improved significantly but equivalently in both patient groups. No change was observed in the IDS. ARA 290 appears to be safe in patients with sarcoidosis and can reduce neuropathic symptoms.

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INTRODUCTION

Sarcoidosis is an inflammatory disease that targets many tissues. In common with a number of other conditions, for example, Sjogren disease (1), one prominent clinical manifestation is a dysfunction of small nerve fibers that occurs in a patchy, non–length-dependent manner (small fiber neuropathy [SFN]). Pathological investigation of sarcoid SFN has documented a loss of small myelinated (Aδ) and unmyelinated (C) fibers of the sensory and autonomic nervous systems (2), as well as both sensory and motor fibers (3). The clinical sequela of these changes is the development of sharp shock-like or burning pain, characterized by dysesthesia and allodynia, and loss of cutaneous sensation and autonomic function. These symptoms significantly reduce the quality of life and are often disabling and difficult to control (2).

SFN can be diagnosed in patients with neuropathic symptoms by using quantitative sensory testing or quantitative sudomotor axon testing and by performing skin biopsies that show a decreased density of intraepidermal sensory nerve fibers within affected body regions. Additionally, a questionnaire (4) was designed and validated in Dutch patients with sarcoidosis (the small fiber neuropathy screening list [SFNSL]) and is useful in following the clinical course of SFN.

Recent studies have shown that the prevalence of SFN is grossly underestimated. Unlike granulomatous, large neuron involvement of neurosarcoidosis, which has a prevalence of <10% (5), painful SFN is more common, with a

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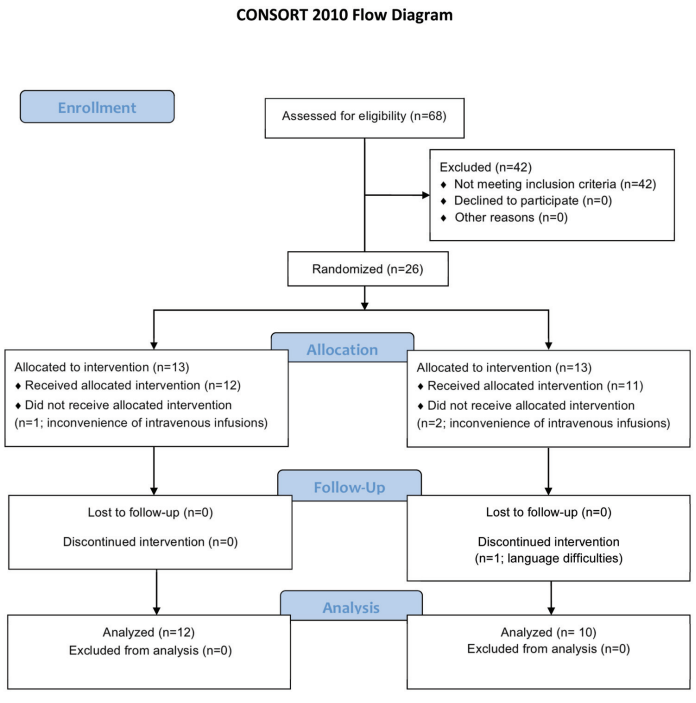

Figure 1. CONSORT flow diagram of the study of ARA 290 in sarcoidosis-associated chronic neuropathic pain.

prevalence of 40% (6) to 60% (7) of patients. The etiology of SFN is unknown, but inflammation is believed to play a prominent role in the generation and maintenance of the symptoms (8). Current therapy of sarcoidosis is primarily via immune suppression, which is generally ineffective for SFN (2).

In recent years, an endogenous system was identified that antagonizes the production and action of proinflammatory cytokines involved in promoting tissue injury, while simultaneously activating repair processes. The primary mediator of this system is locally produced hypoglycosylated erythropoietin (EPO) that acts through a distinct receptor isoform, the innate repair receptor (IRR), which is a combination of EPO receptor and β common receptor subunits (9). EPO acting through the IRR was shown to improve recovery and function after nerve injury in a variety of preclinical models, including SFN caused by uncontrolled diabetes mellitus (10).

ARA 290 is a novel peptide modeled from the three-dimensional structure of EPO that specifically activates anti-inflammation and tissue protection through the innate repair receptor. Preclinical toxicology studies of ARA 290, as well as single and multiple ascending repeated dosing of human volunteers and patients with kidney disease, diabetes mellitus or sarcoidosis have raised no safety issues (11; unpublished data, Araim Pharmaceuticals).

ARA 290 is highly effective in preclinical models of neuropathic pain (12). We hypothesized that patients with symptomatic SFN would benefit from administration of ARA 290. The current trial was undertaken to determine the safety and activity of repeated intravenous dosing of ARA 290 in painful neuropathy.

MATERIALS AND METHODS

Study Design

This study was a single-site, double-blind study carried out at Leiden University Medical Center (LUMC) and is summarized in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram (Figure 1). A total of 26 patients (24 study and 2 alternates) diagnosed with sarcoidosis and as having chronic neuropathic symptoms consistent with SFN were recruited. The diagnosis of sarcoidosis was confirmed as being consistent with the criteria set out in the international guidelines previously reported (13). Only individuals with confirmed sarcoidosis were included. For inclusion, chronic neuropathic symptoms consistent with SFN required at least two of the following: (a) distal symmetrical dys-/paresthesias, (b) burning feet or (c) intolerance of sheets touching the legs or feet. Additionally, a patient's spontaneous pain level was ≥5 (visual analog scale 0–10, with 10 being the worst pain imaginable). Patients also underwent quantitative sensory testing (QST) (Medoc Advanced Medical Systems, Ramat Yishai, Israel) according to the protocol of the German Research Network on Neuropathic Pain (14), with published reference values (15). The results showed a prominent loss of temperature and vibration detection thresholds (Table 1). Additional inclusion criteria were as follows: capable of reading Dutch (n = 1 excluded) and aged between 18 and 65 years, with a body mass index ≤34 kg/m^2, since ARA 290 dosing was not scaled to body size. Women of childbearing potential (n = 1) were required to have a negative pregnancy test and use acceptable contraception for 2 months during the study. Exclusion criteria included the following: receiving a

Table 1. Results of baseline quantitative sensory testing.

Variable	Nerve fibers involved	ARA 290*		Placebo	
		Change	Number of patients (%)	Change	Number of patients (%)
Cold detection threshold	Aδ and C	Decrease	7 (64)	Decrease	9 (90)
Warm detection threshold	Aδ and C	Decrease	7 (64)	Decrease	6 (60)
Thermal sensory limen	Aδ and C	Decrease	3 (27)	Decreased, increased	2 (20), 1 (10)
Paradoxical heat sensation	Aδ	Decrease	1 (9)	—	0
Cold pain threshold	Aδ and C	—	0	Increase	1 (10)
Heat pain threshold	C	Decrease, increase	2 (18), 1 (9)	Decrease, increase	1 (10), 2 (20)
Mechanical detection threshold	Aβ	Decrease, increase	2 (18), 1 (9)	Decrease	1 (10)
Mechanical pain threshold	Aβ	Decrease	2 (18)	Decrease	3 (30)
Mechanical pain sensitivity	Aβ + C	Decrease	1 (9)	Increase	2 (20)
Dynamic mechanical allodynia	Aβ	Increase	1 (9)	Increase	4 (40)
Windup ratio	Aδ and C	—	0	Increase	1 (10)
Vibration detection threshold	Aβ	Decrease	6 (55)	Decrease	6 (60)
Pressure pain threshold	Aδ and C	Increase	2 (18)	Increase	8 (80)

*One patient in the ARA 290 treatment arm refused QST. Patients in both treatment groups showed functional impairment in the function of both small fibers (Aδ and C) as well as larger sensory fibers (Aβ). Data are expressed as either increased or decreased function in those patients deviating 2 or more standard deviations from measurements obtained from a normal population.

vaccination or immunization within the last month; participation in an investigational drug trial in the 3 months before administration of the initial dose of ARA 290 or more than four times per year; major surgery within 6 months before screening; or use of anti–tumor necrosis factor (TNF)-α or EPO or treatment with immunoglobulin drugs 6 months before or during ARA 290 administration and in the follow-up phase. The study was approved and monitored by the Ethics Committee of LUMC and is registered with the International Clinical Trials Registry (NCT 3081), Netherlands Trial Registry (trialregister.nl, NRT 3081) (EudraCT 2010-021518-45). All patients gave written informed consent before entering into the study.

During the study, patients were maintained on a variable regimen of sarcoidosis therapeutics by their physicians, including oral glucocorticoids. Neuropathic symptom-directed agents (for example, tricyclic antidepressants or selective serotonin reuptake inhibitors) were also continued. Patients were randomly assigned (1:1) by the study pharmacist by using a computer-generated randomization code to either ARA 290 or matching placebo (vehicle only). All other study personnel were blinded to the treatment. ARA 290 (pyroglu-glu-gln-leu-glu-arg-ala-leu-asn-ser-ser) was manufactured by Bachem (Bubendorf, Switzerland) by using standard Fmoc solid-phase peptide synthesis. The characteristics of each patient group are summarized in Table 2.

Baseline blood samples were obtained for routine chemistry, high-sensitivity C-reactive protein and hematology determinations. Repeat blood samples were obtained at wk 1 and also just before the last intravenous infusion (d 25). Samples were centrifuged, separated, stored and analyzed according to LUMC Clinical Laboratory protocols.

This study was carried out in the outpatient clinic of the Department of Anesthesiology, Leiden University Medical Center. ARA 290 (2 mg) or placebo was infused intravenously in 6 mL normal saline over 2 min by using a calibrated infusion pump on Monday, Wednesday and Friday for 4 consecutive weeks. Patients were monitored for 60 min after infusion for adverse effects and instructed to contact the research staff if delayed adverse effects were suspected. The endpoints of this exploratory study were change at wk 4 in (a) pain level, as assessed by the BPI and SF-36; (b) neuropathic symptoms, as assessed by the SFNSL (4); and (c) quality of life assessments by the SF-36, Inventory of Depressive Symptomatology (IDS) and the Fatigue Assessment Scale (FAS), which has been validated for sarcoidosis patients (16). These questionnaires were completed at baseline and then weekly for the 4 wks of dosing. Each patient independently completed the weekly questionnaires by using the Project Manager Internet Server maintained by LUMC, which provided a record that could not be modified.

One patient from the ARA 290 treatment group and two from the placebo group withdrew from the study because of the inconvenience of intravenous infusions and were replaced with the two alternates who had been previously recruited as backups for the study. A total of 12 ARA 290 patients and 10 placebo patients completed the study.

The trial sample size was chosen on the basis of the robust efficacy of ARA 290 in a rodent model of neuropathy (12) and from the observations derived from a prior small nonblinded trial of 20 patients with symptoms of SFN who received ARA 290 (2 mg intravenously) for three doses every 2 d over 1 wk (11). Responses

Table 2. Patient characteristics.

Variable	ARA 290	Placebo
n	12	10
M/F	6/6	6/4
Weight (kg)	83.2 ± 3.7	85.5 ± 5.9
Age	48.1 ± 2.7	49.1 ± 2.7
Height (cm)	177.8 ± 2.8	177.4 ± 3.3
Pulmonary involvement	10/12	9/10
Fatigue	12/12	10/10
Use of nonsteroidal antiinflammatory drugs (NSAIDs)	2/12	1/10
Use of psychological drugs	2/12	2/10
Use of oral steroids	4/12	2/10
Use of opioids	1/12	0/10
Use of analgesics	2/12	2/10
Use of anticonvulsants	3/12	3/10
Use of systemic antiinflammatory drug	1/12	2/10
Currently smoking	2/12	2/10
ARA 290 dose (μg/kg)	24.6 ± 1.1	0
ARA 290 dose (μg/m^2)	997.1 ± 26.9	0
C-reactive protein (pretreatment versus posttreatment; NS)	3.0 ± 1 versus 3.1 ± 1.2	3.7 ± 1.5 versus 4.1 ± 1.6
SFNSL score (pretreatment versus posttreatment; $p < 0.05$)	41.0 ± 4.6 versus 29.8 ± 3.5	30.6 ± 4.2 versus 26.2 ± 4.0
BPI mean score (pretreatment versus posttreatment; NS)	7.1 ± 0.2 versus 4.8 ± 0.4	6.2 ± 0.9 versus 4.1 ± 0.3
SF-36 mean score (pretreatment versus posttreatment; NS)	37.6 ± 2.8 versus 50.7 ± 3.1	44.5 ± 2.8 versus 52.3 ± 3.1
FAS (pretreatment versus posttreatment; NS)	37.9 ± 2.6 versus 33.3 ± 2.8	33.6 ± 2.3 versus 29.8 ± 3.3
IDS (pretreatment versus posttreatment; NS)	28.7 ± 4.9 versus 24.7 ± 4.2	24.1 ± 4.3 versus 22.1 ± 4.3

Data are mean ± SEM. BPI score consisted of most pain, average pain and pain now. NS, nonsignificant differences.

from the weekly patient questionnaires were calculated as change from baseline values. Individual missing data points were assigned by using a last observation carried forward approach (number of missing data points summarized below for each variable). Normality of data distribution was assessed and confirmed by using the Kolmogorov-Smirnov test. Statistical significance ($p < 0.05$) of change from baseline value was calculated at wk 4 by using a two-sample *t* test comparing change at wk 4 over baseline (two-tailed distribution). Because no data points were missing from the SFNSL questionnaire, these data were analyzed by using repeated-measures analysis of variance (ANOVA). A cumulative proportional responders graph was constructed according to Farrar *et al.* (17).

RESULTS

There were no documented changes in concomitant drug treatment, including analgesic use, during the 4-wk dosing period. Patients tolerated repeated intravenous infusions of ARA 290 without adverse events noted by study personnel or self-reported. ARA 290 recipients and placebo patients exhibited no significant differences between chemistry and hematology values at baseline and wk 4. Hemoglobin concentrations (mmol/L) of ARA 290 patients compared with placebo were 8.9 ± 0.21 (standard error of the mean [SEM]) versus 8.6 ± 0.26 at baseline and 8.7 ± 0.19 versus 8.7 ± 0.34 at the end of dosing on wk 4.

The SFNSL score (no missing data points) showed a time-dependent, significant difference between treatment groups, reaching a maximum difference by wk 4 (Figure 2). The SFNSL data obtained at the end of dosing at wk 4 are expressed as a cumulative proportion of the responders plot in Figure 3. As illus-

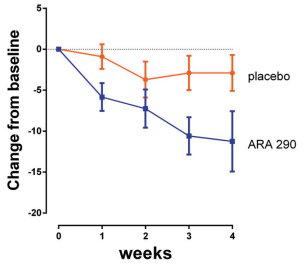

Figure 2. ARA 290 administration is associated with a time-dependent improvement of the SFNSL score. Reduction in SFNSL score from baseline (improvement) in the ARA 290 group occurs within the first week and continues for the whole duration of the dosing period. In contrast, a small, nonsignificant change in placebo patients appeared to reach a plateau. (Curves (mean ± SEM) are significantly different at the $p < 0.05$ level based on repeated-measures ANOVA).

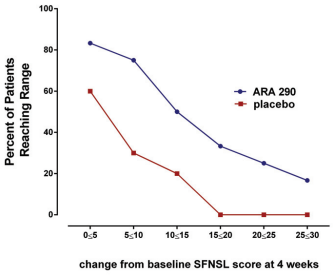

Figure 3. A higher proportion of ARA 290 patients exhibited a superior improvement than placebo patients in the SFNSL score at all levels of response. Cumulative proportion of responder analysis summarizes that across a wide range of score improvements a greater proportion of ARA 290 patients reached a specific level of reduction in the SFNSL score than placebo patients.

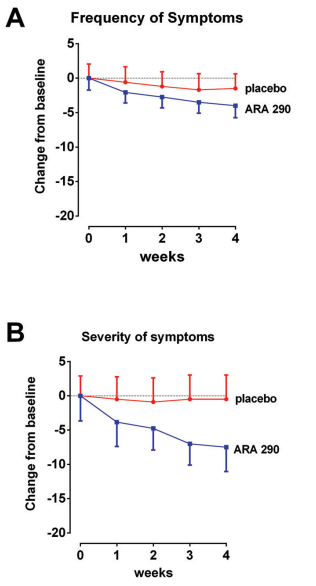

Figure 4. SFNSL subscore analysis. (A) A decrease in frequency of symptoms occurred in both groups over the dosing period, which did not differ significantly. (B) Severity of symptoms remained unchanged in the placebo group, whereas a decrease was noted in the ARA 290 ($p < 0.05$).

trated, 60% of placebo patients experienced an improvement from baseline at wk 4 between 1 and 5 points, versus 83% of ARA 290 patients. In contrast, whereas none of the placebo patients experienced an improvement of 15 points or more, 42% of the ARA 290 recipients did. The SFNSL assesses both the frequency and the severity of symptoms. As shown in Figure 4A, the frequency of SFN symptoms decreased moderately in both groups to a similar extent. In contrast, the severity of neuropathic symptoms remained constant in the placebo group over the dosing period, whereas it significantly improved in the ARA 290 group (Figure 4B). The SFNSL can also be separated into components that assess symptoms referable to autonomic dysfunction (questions 2–5, 9 and 11–16) or to pain

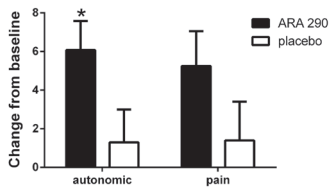

Figure 5. ARA 290 administration was associated with a significant improvement in the autonomic component of the SFNSL. ARA 290 administration was associated with a significant improvement in the autonomic component of the SFNSL at wk 4 compared with baseline (*$p < 0.05$). Although a similar change was noted in the pain subcategory, the magnitude did not differ significantly from that of the placebo group.

(questions 1, 6–8, 10 and 17–21). Patients who received ARA 290 reported a significant improvement in their autonomic symptoms, in contrast to the placebo group (Figure 5). Although a similar improvement was noted for the pain dimension, the magnitude was not significantly different from that observed for the placebo group. Assessing for change from baseline for individual questions showed that significant changes occurred in six questions for the ARA 290 group and one question for the placebo group (Table 3).

Similarly, quality of life, as assessed by the SF-36 (one patient had a total of four data points missing), showed significant improvements from baseline in the active treatment group for pain and physical functioning in contrast to the placebo group (Figure 6). Both groups showed significant improvements from baseline in general health (ARA 290: 35.4 ± 8.3; placebo: 22.7 ± 7.9). There were no significant changes from baseline between active and placebo groups in the remaining dimensions of SF-36. The mean pain score for the BPI (three patients had a total of five data points missing) and FAS (three patients had a total of one data point each missing) decreased to a similar extent for both ARA 290 and the placebo group (Table 2), which were not significantly different from each other. The IDS (four patients had a total of one data point each missing) did not change from baseline for either group (Table 2).

DISCUSSION

This is the first study to demonstrate that ARA 290 appears to be safe when administered repeatedly over a 4-wk period to sarcoidosis patients with symptoms of SFN. During and after dosing, no abnormalities were noted in the laboratory or clinical evaluations, and the patients reported no potentially drug-related adverse effects. Notably, ARA 290 appears to improve symptoms of SFN, as assessed by the SFNSL, as well as on quality of life, as assessed by the pain and physical functioning dimensions of the SF-36. Pain, as assessed by the BPI, decreased significantly, but to the same degree in both patient groups.

Primary treatment of diseases complicated by SFN was reported to variably improve the symptoms of SFN. Sarcoidosis is a disease mediated by a complex

Table 3. Significant changes in individual questions of the SFNSL.

Patient group	Question	Symptom subgroup	Change from baseline (mean ± SEM)	Significance*
ARA 290	6 (muscle cramps)	Frequency	−1.1 ± 0.36	0.012
ARA 290	8 (chest pain)	Frequency	−0.5 ± 0.19	0.026
ARA 290	12 (dry eyes)	Severity	−1.33 ± 0.33	0.002
ARA 290	13 (blurred vision)	Severity	−1.17 ± 0.32	0.004
ARA 290	14 (dizzy when rising)	Severity	−0.83 ± 0.32	0.025
ARA 290	21 (chest pain)	Severity	−0.58 ± 0.19	0.012
Placebo	6 (muscle cramps)	Frequency	−0.7 ± 0.30	0.045

*Determined by two-tailed paired *t* test.

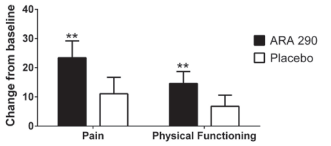

Figure 6. ARA 290 patients demonstrated a significant improvement from baseline in the pain and physical functioning dimensions of the SF-36 quality of life questionnaire. In contrast to the placebo patients, those receiving ARA 290 showed significant improvement from baseline in the dimension of pain and physical functioning at wk 4 (**$p < 0.01$).

interaction involving tissue injury and the responses of immune-competent cells. High-dose glucocorticoids have been the predominant therapeutic approach for all forms of this disease. However, use of glucocorticoids is frequently associated with unacceptable adverse effects and appears to be generally ineffective in improving the symptoms of SFN (2). Substitution of other immunosuppressants, for example, methotrexate, also fails to improve symptomatic SFN. Consequently, sarcoid patients who suffer from chronic symptomatic SFN currently lack effective treatment.

A recent development includes the availability of agents that directly inactivate the proinflammatory cytokine TNFα. Use of anti-TNFα therapy in refractive pulmonary sarcoidosis has been shown to directly reduce systemic proinflammatory cytokine concentrations and has successfully induced a sustained improvement in lung function in some patients (18,19). Case studies have also reported that direct antagonism of proinflammatory cytokines in patients with symptomatic SFN was associated with significant and sustained improvement in neuropathic symptoms, including dysautonomia (20), as well as with cognitive function and fatigue (18,21). The results of these studies directly support the concept that proinflammatory cytokines play a major etiologic role in the development of specific organ involvement in sarcoidosis and that antiproinflammatory cytokine therapy may constitute an effective treatment. However, the high costs and side effects of anti-TNFα therapy currently limit its widespread use. Finally, intravenous immunoglobulin infusions have been effective in a small number of patients with sarcoidosis and SFN (22), as well as in other diseases with associated SFN (23).

Since primary therapy of sarcoidosis does not generally treat SFN, alternative therapies used for for other peripheral neuropathies are emplyed. These include antidepressants, anticonvulsants, topical analgesics and/or opioids. Each of these classes of therapeutic agents has specific adverse effects, sometimes severe, and typically do not adequately treat the symptoms of SFN (24).

ARA 290 is a peptide designed to mitigate inflammation by activating the IRR, which in turn inhibits proinflammatory cytokine production and action. In preclinical models of injury, ARA 290 not only inhibits TNFα, but also other components of the proinflammatory cytokine cascade (25). Although ARA 290 has a short serum half-life of a few minutes (11), the peak plasma concentrations attained after the administration of 2 mg intravenously in normal human volunteers in pharmacokinetic studies reached ~50 ng/mL, exceeding the minimum effective peak concentration of ~1 ng/mL (26). ARA 290 at concentrations exceeding ~1 ng/mL activates the IRR, which functions as a molecular switch to provide long-lasting effects, similar to other effector molecules in the immune response. In this clinical trial, administration every other day for 1 month appears sufficient to produce a significant biological response, similar to the finding in a preclinical rodent model in which treatment every other day with ARA 290 is effective in preventing neuropathic pain (12). ARA 290 therefore constitutes an attractive candidate for treatment of symptomatic SFN.

The most robust effect of ARA 290 observed in this pilot trial was on the SFNSL score, which appeared to be primarily on symptom severity rather than frequency. It is notable that this instrument, which was specifically developed and validated in sarcoidosis patients (4), incorporates prominent symptoms of autonomic dysfunction as well as pain. In the current study, patients receiving ARA 290 appeared to improve specifically with respect to autonomic symptomatology, for example, with respect to dry eyes, blurred vision and orthostatic symptoms. In this regard, it is interesting to note that ARA 290 was observed to reverse neuropathic changes in the sympathetic ganglia of a mouse model of type 2 diabetes (27). Additionally, results of experiments performed on normal volunteers in whom intraepidermal nerve fibers were denervated by the application of capsaicin show that cutaneous autonomic nerve fibers regenerate much faster than sensory nerve fibers (28). This observation could explain, in part, the less robust effects that are noted in the pain dimension, which may require more prolonged or intensive dosing. The results also suggest that future studies in symptomatic SFN that focus on autonomic dysfunction may yield especially informative data.

The primary limitations of this trial are the small sample size, patient variability of neuropathic involvement and lack of skin biopsy or sudomotor testing evidence definitively establishing SFN. Also, a sizeable fraction of the study group also exhibited abnormalities in mechanoreception, as determined by QST in addition to the sensory and autonomic abnormalities attributable to SFN. Therefore, the observed reduction in the severity of symptoms as assessed by the SFNSL cannot be attributed with certainty only to effects of ARA 290 on small fiber function.

CONCLUSION

The acceptable safety profile noted for ARA 290 in this patient group with sarcoidosis, as well as an apparent reduction of symptoms of SFN, encourages a larger study of the potential effects of ARA 290 for this unmet medical need.

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DISCLOSURE

A Dunne, M Brines and A Cerami are employees and officers of Araim Pharmaceuticals and own stock and/or stock options.

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CHAPTER 6

ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density.

Molecular Medicine

ARA 290 Improves Symptoms in Patients with Sarcoidosis-Associated Small Nerve Fiber Loss and Increases Corneal Nerve Fiber Density

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Small nerve fiber loss and damage (SNFLD) is a frequent complication of sarcoidosis that is associated with autonomic dysfunction and sensory abnormalities, including pain syndromes that severely degrade the quality of life. SNFLD is hypothesized to arise from the effects of immune dysregulation, an essential feature of sarcoidosis, on the peripheral and central nervous systems. Current therapy of sarcoidosis-associated SNFLD consists primarily of immune suppression and symptomatic treatment; however, this treatment is typically unsatisfactory. ARA 290 is a small peptide engineered to activate the innate repair receptor that antagonizes inflammatory processes and stimulates tissue repair. Here we show in a blinded, placebo-controlled trial that 28 d of daily subcutaneous administration of ARA 290 in a group of patients with documented SNFLD significantly improves neuropathic symptoms. In addition to improved patient-reported symptom-based outcomes, ARA 290 administration was also associated with a significant increase in corneal small nerve fiber density, changes in cutaneous temperature sensitivity, and an increased exercise capacity as assessed by the 6-minute walk test. On the basis of these results and of prior studies, ARA 290 is a potential disease-modifying agent for treatment of sarcoidosis-associated SNFLD.

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INTRODUCTION

Sarcoidosis is an immune-mediated, inflammatory orphan disease of unclear etiology that can affect virtually any organ of the body (1). In most individuals diagnosed with sarcoidosis, the disease is mild, with pulmonary and hilar lymph node involvement that resolves within several years whether treated by immune suppression or not. However, in about one-third of patients, sarcoidosis evolves into a chronic, progressive disease (2). In these refractory cases, therapy has generally consisted of immune suppression, which has been associated with a variable response rate (3).

Recently, it has become apparent that a significant proportion of patients with chronic sarcoidosis report symptoms that suggest abnormal function of the small nerve fibers of the sensory and autonomic nervous systems (4–8). Clinical evaluation by skin biopsy, typically of the distal leg, has shown that many of these patients have a demonstrable reduction in intraepidermal nerve fibers (6,9). The affected nerves consist of unmyelinated C and lightly myelinated Aδ fibers that comprise the sensory and autonomic peripheral nervous systems. Patients having reduced nerve fiber densities typically complain of pain, numbness and/or dysesthesia, as well as autonomic symptoms that can be extremely variable depending on the organ affected (5). The neuropathic symptoms in these patients are frequently severe and therefore are major contributors to the poor quality of life of those afflicted (10).

The etiology of the loss of small nerve fibers in sarcoidosis has not been definitively identified, but one prevalent hypothesis is that nerve fiber dropout is the end result of systemic and/or local inflammation (11). Neuropathy arising from inflammation can affect both the peripheral nerve endings and the neuronal somata within the dorsal root gan-

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glia of the spinal cord. At the present time, glucocorticoids and other immune suppressants are the principal therapeutic approach to small-fiber neuropathy but are often ineffective (8). In addition to immune modulators as potential disease modifiers, treatment is generally symptomatic, consisting of the analgesics, antiepileptics and antidepressants used for other painful neuropathies (12). Thus, there is a clear need for new therapeutics in sarcoidosis-associated small-fiber neuropathy.

ARA 290 is an 11–amino acid peptide derived from the structure of erythropoietin that possesses potent tissue-protective and tissue-repair activities without stimulating erythropoesis (13). The actions of ARA 290 are mediated through a receptor consisting of a complex formed by the erythropoietin receptor and β common receptor subunits (14), termed the innate repair receptor (IRR). In preclinical models of neuropathic pain, ARA 290 demonstrated beneficial effects that include IRR-dependent prevention of the development of allodynia in a peripheral nerve transection model (15) or in an inflammatory neuritis model (16), as well as attenuation of spinal cord inflammation (15). Also, erythropoietin, and its nonerythropoietic derivatives (for example, ARA 290) have been shown to support the regrowth of intraepidermal nerve fibers in preclinical models of neuropathy arising from toxins (17) or diabetes (18).

An initial open-label study of the effects of three intravenous doses of ARA 290 administered over 1 wk on neuropathic pain of patients with sarcoidosis or diabetes showed a 50% improvement without any safety concerns (19). The results of a follow-up trial of ARA 290 (20) administered intravenously three times weekly for 4 wks to sarcoidosis patients with symptoms of small-fiber neuropathy also appeared to be safe and was associated with a significant improvement in the patient-reported outcomes of the small-fiber neuropathy screening list (SFNSL) (21) and the pain and well-being components of the RAND-36.

On the basis of these observations, we have conducted the present study to assess the effects of ARA 290 on neuropathic symptoms when given as a daily subcutaneous (SC) injection for 28 d. Because of the association of small nerve fiber loss with neuropathic symptoms and the potential for ARA 290 to cause nerve fiber regrowth, we hypothesized that ARA 290 administration will improve symptoms and stimulate the regrowth of small nerve fibers. To evaluate this, the nerve fiber densities in the cornea, proximal thigh and distal leg were assessed. Additionally, cutaneous sensory testing of the face, hand and foot were determined by using quantitative sensory testing (QST), and quality of life was assessed with appropriate patient questionnaires. Finally, functional capacity, which is often reduced in chronic sarcoidosis (22), was assessed by using the 6-minute walk test (6MWT).

MATERIALS AND METHODS

Rationale for Dose Selection

Results of a previous study performed in sarcoidosis patients with painful small-fiber neuropathy showed that 2 mg ARA 290 administered intravenously (IV) three times weekly improved neuropathic symptoms. In the current trial, we sought to assess the potential of SC dosing, as the IV dosing is not practicable in the outpatient setting. Therefore, a crossover pharmacokinetic study was performed using 10 normal volunteers to compare a 2-mg IV dose that was used in the previous study, to 2, 4 or 6 mg ARA 290 administered subcutaneously (19). Results of preclinical and *in vitro* studies have shown that activation of the IRR requires concentrations of ARA 290 greater than or equal to ~1 nmol/L (~1.3 ng/mL) (14). Therefore, the area under the curve (AUC) of the pharmacokinetic data was calculated by using the trapezoidal rule for the period of time in which the plasma concentrations were >1.3 ng/mL. The results of this crossover study showed the following median AUCs: 2 mg IV = 65 ng/mL × min, 2 mg SC = 23 ng/mL × min, 4 mg SC = 59 ng/mL × min and 6 mg = 249 ng/mL × min, with only the 6-mg dose differing significantly from the others ($p < 0.05$; Kruskal-Wallis test). Based on these data, the 4-mg SC group was selected for the daily dosing regimen of this trial.

Study Design

The trial, entitled "Effects of ARA 290 on the regrowth of epidermal nerve fibers in patients with sarcoidosis," was an investigator-initiated, single-site, double-blind, placebo-controlled trial carried out at the Leiden University Medical Center after receiving ethics committee approval. The trial was registered with the International Clinical Trials Registry (NTR3575) and was assigned EudraCT number 2012-001492-37. All study personnel and patients remained blinded to the treatments until the end of the follow-up period (16 wks from the beginning of dosing).

The primary outcomes were as follows: (a) change in epidermal or corneal nerve fiber density at d 28 versus baseline; (b) change in cutaneous sensitivity of d 28 versus baseline by using QST; and (c) change in visual acuity or retinal edema at d 28 versus baseline. Secondary outcomes assessed were as follows: (a) change in the SFNSL score at d 35 versus baseline; (b) change in the Brief Pain Inventory (BPI) at d 35 versus baseline; and (c) change in distance walked in the 6MWT at d 28 versus baseline.

Patients who satisfied the international consensus statement for diagnosis of sarcoidosis (23) and had symptoms suggestive of neuropathy were recruited after referral by sarcoidosis specialists. The Consolidated Standards of Reporting Trial (CONSORT) flow chart corresponding to this trial is illustrated in Figure 1. After obtaining informed consent, a total of 38 patients (18 females, 20 males) of a mean age of 49.5 years (range 28–65) satisfying inclusion criteria were enrolled. These patients had a mean duration since sarcoidosis diagnosis of 8.4 years. The baseline characteristics of these patients with respect to the treatment groups are

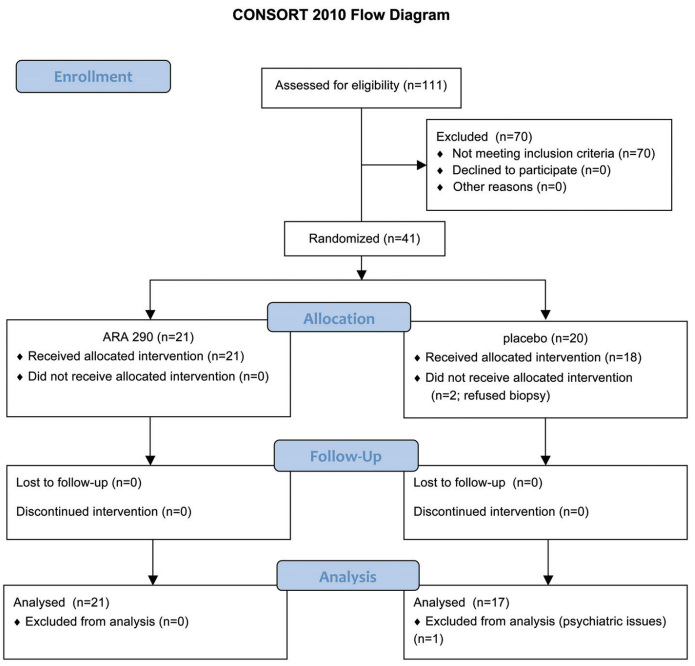

Figure 1. CONSORT flow diagram.

summarized in Table 1. Although all patients were diagnosed as having sarcoidosis, two patients also had Type 2 diabetes mellitus, a condition known to also be associated with small nerve fiber loss and damage (SNFLD) (12).

Study inclusion criteria required meeting three thresholds: (1) spontaneous pain level ("pain now" of the BPI) >5 (scale 0–10); (2) SFNSL score >22 (out of 84 possible), or pain <5 and SFNSL score >37; and (3) pain defined as distal extremity pain plus one of the following: dysesthesia, burning/painful feet worsening at night or intolerance of sheets/clothes touching the legs or feet. Additional inclusion criteria were as follows: age between 18 and 65 years (inclusive), a body mass index (BMI) between 18 and 30 kg/m^2 (inclusive) and the ability to read and understand the written consent form, complete study-related procedures and communicate with the study staff. Exclusion criteria were as follows: abnormal blood pressure, history of alcoholism or illicit drug use, positive pregnancy test, refusal to use acceptable contraception throughout the study period (unless surgically sterilized or postmenopausal), vaccination or surgery within the prior 3 months, or use of anti–tumor necrosis factor (anti-TNF) therapy in the prior 6 months.

Safety was assessed by questioning the patient weekly during ARA 290 administration and throughout the 12-wk follow-up for the occurrence of adverse events. Additionally, the patients were examined at three occasions during the active treatment phase of the study: baseline, 2 wks and 4 wks at the end of dosing. Additionally, blood was drawn for routine hematology and chemistry at these times points. Finally, serum was obtained for determination of possible anti-ARA 290 antibodies.

Patient Questionnaires

Questionnaires were administered at the screening visit and then weekly during the dosing and follow-up period of 3 months (total 16 wks). Questionnaire data were also obtained approximately 6 months after the end of the follow-up period (that is, 9 months from the end of dosing) to assess durability of any effects. The BPI Short Form, consisting of pain intensity and pain interference sections, was administered in the validated Dutch language format. SFNSL is a questionnaire developed specifically for Dutch patients with sarcoidosis to assess pain and autonomic dysfunction consistent with small nerve fiber loss and damage (21). In addition to the total score, the questionnaire was divided into an autonomic component (questions 2–5, 9, 11–16) and a pain component (questions 1, 6–8, 17–21) to assess those dimensions of the patients' neuropathic symptoms.

QST

Small nerve fiber and large fiber cutaneous sensory function was assessed by using QST of the face, hand and foot using a Medoc Advanced Medical Systems TSA-II device (Ramat Yishai, Israel), following the published protocol of the German Research Network on Neuropathic Pain (24). Normative data were obtained from Rolke *et al.* (24). Baseline data for each patient group are summarized in Table 2. To arrive at the percentages shown in Table 2, the three regions tested were pooled (that is, each patient's data were considered abnormal if the results were >2 standard deviations from the normative population mean in at least one of the locations evaluated).

Skin Biopsy

Skin biopsies were obtained at baseline and after 28 d from the proximal thigh (20 cm below the anterior superior iliac spine) and the distal leg (10 cm above the lateral malleolus) by using a disposable punch biopsy (3 mm) and

Table 1. Baseline patient characteristics.

	ARA 290	Placebo
n	21	17
Years since diagnosis of sarcoidosis (mean ± SEM)	7.1 ± 1.2	9.9 ± 2.4
Concomitant medical treatment (n (%))		
NSAIDS	5 (23.8)	8 (47.1)
Neurological/psychological drugs	5 (23.8)	6 (35.3)
Oral corticosteroids	6 (28.6)	7 (41.2)
Opioids	6 (28.6)	2 (11.8)
Systemic immune suppressants (methotrexate or azathioprine)	7 (33.3)	3 (17.7)
Prior TNFα antagonist treatment (n = yes)	2 (9.5)	0
SFNSL		
Total score	43.9 ± 2.9	42.8 ± 3.2
Autonomic component	20.6 ± 2.0	20.8 ± 1.5
Pain component	23.3 ± 1.2	22.9 ± 1.2
BPI		
Mean score (pain now; range 0–10)	5.0 ± 0.4	5.3 ± 0.5
Pain interference (maximum 70)	32.1 ± 1.9	36.5 ± 2.9
6-Min walk		
Test, actual (m)	468 ± 18	479 ± 26
Test, predicted (m)[a]	700 ± 12	683 ± 15
Nerve fiber density		
Corneal nerve fiber area (μm^2)	1,576 ± 94	1,304 ± 104
Normal corneal nerve fiber area[b]	3,134 ± 119	
Ankle IENFD (n/mm)	5.3 ± 0.5	4.6 ± 0.4
Normal sex- and age-adjusted ankle IENFD[c]	9.9 ± 0.3	9.8 ± 0.3
Proximal thigh IENFD (n/mm)	10.8 ± 0.7	11.1 ± 0.9
Normal proximal thigh IENFD[d]	21.1 ± 0.2	21.0 ± 0.1
Laboratory markers		
High sensitivity C-reactive protein (mg/L)	1.5 ± 0.2	2.9 ± 1.1
Angiotensin-converting enzyme[e]	47.4 ± 6.1	53.6 ± 8.0
Number with elevated angiotensin-converting enzyme n(%)	5 (23.8)	6 (35.3)

[a]Predicted 6MWT was calculated by using the formula of Troosters *et al.* (32).
[b]Data calculated from Brines *et al.* (30).
[c]Normal sex- and age-adjusted ankle IENFD is from Lauria *et al.* (26).
[d]Normal proximal thigh IENFD is from Umapathi *et al.* (27).
[e]Normal: 23–67 nmol/min/mL.

processed following established guidelines (25). After fixation of the biopsy specimens, free floating 50-µm-thick sections were cut and stained by using rabbit anti-protein gene product 9.5 antibody (Dako Netherlands BV, Eindhoven, the Netherlands) and visualized using a goat anti-rabbit Alexa fluor 488 antibody (Invitrogen,/Life Technologies, Carlsbad, CA, USA). A minimum of three sections selected from each end and the middle of each biopsy specimen were evaluated by using a Leica M5500 fluorescence microscope (Leica Microsystems, Rijswijk, the Netherlands) at a magnification of 1,000×. The nerve fibers were counted manually. Images of the sections were recorded by using the Leica Application Suite, magnification 400×, and the length of the epidermal-dermal junction measured using ImageJ (National Institutes of Health, Bethesda, MD, USA). Sex- and age-dependent normative data of nerve fiber density used for the distal leg were those of Lauria *et al.* (26) and, for the thigh, Umapathi *et al.* (27). All measurements and counting was performed by the same individual, who was blinded to treatment modality. Technical problems during tissue preparation resulted in the loss of two placebo biopsies of the lower leg, one ARA 290 biopsy of the thigh and three placebo biopsies of the thigh.

Corneal Confocal Microscopy

Corneal nerve fiber density was determined by corneal confocal microscopy carried out by using the Rostock Cornea Module with the Heidelberg Retina Tomograph III using established methodology (28). Briefly, after the application of a topical anesthetic, the sterile objective of the confocal microscope was placed on the apex of the cornea, as determined by the characteristic orientation of the nerve fibers in a superior–inferior direction. By using the automatic scan feature of the device, confocal images of graduated depth in the plane of the cornea were acquired. The field of view of each image was 0.4 × 0.4 mm. Images containing sensory nerve fibers within the subbasal layer between the Bowman layer and the basal epithelium were further analyzed. Collected images were subjected to automated analysis using a custom macro written for Fiji, a public-domain image analysis program, version 1.47e (29). This macro maps all neurites in the image on the basis of their brightness and tubeness. The area covered by the mapping is then expressed as a percentage of total image area. For each patient, the 10 images with the highest nerve fiber density were averaged to generate a representative sample for that patient for that eye. Because the variation between eyes of different patients was similar to the variation between eyes of individual patients (standard deviation of the mean neurite area between patients = 562; standard deviation of the difference between eyes of individual patients = 501), each eye was treated as an independent sample. The automated analysis was validated by comparison of 78 randomly selected images in which total neurite length in each image was determined by manually outlining individual neurites. Linear regression analysis showed an excellent goodness of fit (95% confidence interval of the slope: 0.99–1.19; $R^2 = 0.76$; $p < 0.0001$) between

Table 2. Results of baseline QST.

Variable	Nerve fibers involved	ARA 290 (n = 21)		Placebo (n = 17)	
		Change	Number of patients (%)	Change	Number of patients (%)
Cold detection threshold	Aδ and C	Decrease	19 (91)	Decrease	11 (65)
Warm detection threshold	Aδ and C	Decrease Increase	17 (81) 1 (5)	Decrease	13 (77)
Thermal sensory limen	Aδ and C	Decrease Increase	4 (19) 2 (10)	Decrease	4 (24)
Paradoxical heat sensation	Aδ	Decrease	8 (38)	Decrease	7 (41)
Cold pain threshold	Aδ and C	Increase	3 (14)	—	0
Heat pain threshold	C	Decrease Increase	3 (14) 5 (24)	Decrease	1 (6)
Mechanical detection threshold	Aβ	Decrease	11 (52)	Decrease	10 (59)
Mechanical pain threshold	Aβ	Decrease Increase	11 (52) 4 (19)	Decrease Increase	4 (24) 2 (12)
Mechanical pain sensitivity	Aβ + C	Decrease Increase	2 (10) 5 (24)	Decrease	1 (6)
Dynamic mechanical allodynia	Aβ	Increase	11 (52)	Increase	3 (18)
Windup ratio	Aδ and C	Increase	4 (19)	Increase	2 (12)
Vibration detection threshold	Aβ	Decrease	20 (95)	Decrease	15 (88)
Pressure pain threshold	Aδ and C	Decrease Increase	3 (14) 10 (48)	Decrease Increase	1 (6) 7 (41)

Patients in the ARA 290 and placebo groups showed functional impairment of both small nerve fibers (Aδ and C) as well as larger sensory nerve fibers (Aβ). Data are expressed as number of patients deviating beyond the 95% confidence interval of a sex- and age-matched normal population. Test sites of face, hand and foot are pooled for calculation of percentages. "Decrease" indicates a loss of function; "Increase" indicates a gain in function compared with a normal population. For example, a decreased CDT means that a patient required a lower temperature stimulus than normal to determine that an object was cold (that is, a decrease in sensitivity).

the computer-generated nerve fiber area and the manually measured total nerve fiber length for each image. Both the automated analyses and the manual measurements were performed by a researcher blinded to the treatment modality. The Shapiro-Wilk test showed that, at baseline, the corneal nerve fiber area data were not distributed normally; therefore, nonparametric statistical analysis was performed to determine if a significant treatment effect was observed.

Normative data were calculated from corneal confocal data that were previously reported (30). These data were obtained from 22 healthy volunteers (M/F, 9/13; age 49 ± 2.7 years) by determining the mathematical relationship between corneal nerve fiber area and corneal nerve fiber length. The results showed that a normal corneal nerve fiber area is 3,134 ± 119 μm^2.

6-Minute Walk Test

The 6MWT, the distance in meters walked in 6 min, was conducted following American Thoracic Society guidelines (31). Normal 6MWT values were calculated by Troosters *et al.* (32) by using the regression equation developed from data obtained from a healthy, older normal Dutch population.

Ophthalmologic Tests

To assess for possible retinal edema, optical coherence tomography was carried out to quantitate retinal thickness by using the Zeiss CIRRUS1 system that includes normative values.

Visual acuity was carried out under standard uniform lighting conditions for patients wearing corrective lenses, if any, by using a SLOAN ETDRS chart and scoring system.

Statistical Analysis

Statistical analysis was performed by using JMP version 11.0 (SAS, Cary, NC, USA). Parametric and nonparametric tests, linear modeling and analysis of covariance were carried out where appropriate. p values <0.05 (two-tailed) were considered significant.

RESULTS

Safety

No medically significant deviations were noted in the general blood chemistry or hematology assessments. There was no pain or local irritation surrounding the site of the injection into the upper leg or lower abdomen. No serious adverse events were encountered during the dosing period or within the 12 wks of follow-up. Three adverse events judged to be moderate were noted in the placebo group that resolved spontaneously (diarrhea, irritability, lightheadedness). One patient receiving ARA 290 suffered a moderate adverse event consisting of a long-term weight loss of 14 kg over several months that stabilized thereafter. Verification of the patient's medical history showed that the weight loss began before entering the study. The etiology of the weight loss was undetermined and persisted after administration of ARA 290 ceased. Multiple, mild adverse events were recorded, all of which spontaneously resolved, and none were judged by the investigators as likely to be associated with administration of the study drug. All doses of ARA 290 were administered daily for the full 28-d period. One pla-

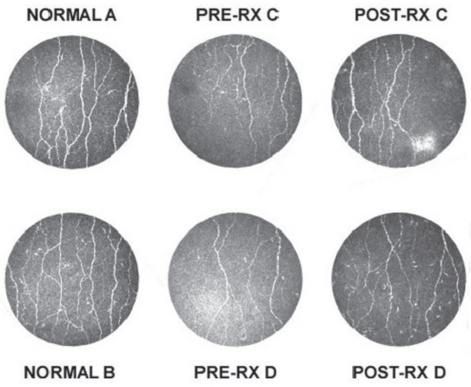

Figure 2. ARA 290 administration is associated with an increase in corneal nerve fiber area. Examples of the distribution and density of corneal nerve fibers obtained via corneal confocal microscopy performed on two normal individuals (A and B) are shown (left panels). Examples of corneal nerve density obtained from two sarcoidosis patients (C and D) show a decreased density at baseline (middle panel: Pre-RX) and an increase when reimaged after 28 d of ARA 290 administration (right panel: Post-RX).

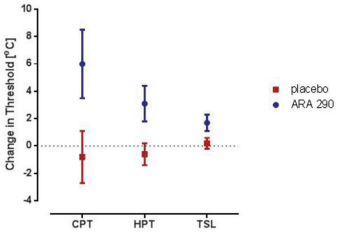

Figure 3. ARA 290 administration increases the threshold for thermal pain and decreases thermal sensitivity in the hand. The CPT, HPT and TSL of most patients were within normal limits at baseline (Table 1). After ARA 290 administration, the mean threshold for determining a painful cold ($p = 0.027$; paired *t* test compared with baseline) or hot ($p = 0.032$) stimulus increased, whereas the placebo group remained unchanged (p = ns). Similarly, the thermal sensory limen (the temperature threshold at which they can discriminate a hot or cold stimulus) increased in the ARA 290 after exposure ($p = 0.008$). This decreased thermal sensitivity could correspond to reduced symptoms of temperature-induced allodynia. After ARA 290 treatment, the CPT, HPT and TSL remained within the normal range. The normative means for CPT, HPT and TSL were 9.7 ± 0.5, 44.8 ± 0.2 and 3.0 ± 0.1°C, respectively. Similar smaller changes were noted for the face, as well as a nonsignificant trend for the foot (data not shown).

cebo patient suffering from diarrhea discontinued dosing for the last week of the study. No anti-ARA 290 antibodies were detected in any of the postexposure serum samples.

Primary Endpoints

Nerve fiber density. *Corneal nerve fibers:* The baseline corneal nerve fiber area showed that the patient population exhibited about a 50% reduction compared with normal controls (Figure 2; Table 1). After 28 d of dosing, the ARA 290 group exhibited a significant increase in the median nerve fiber area over baseline of 14.5%, corresponding to an absolute median increase of 185 μm^2 (p = 0.022; Wilcoxon signed rank test). In contrast, the placebo group had a nonsignificant decrease in median nerve fiber area over baseline of –5.3% and an absolute median decrease of 64 μm^2 (p = 0.462). Figure 2C illustrates the corneal nerve density of two normal individuals compared with two ARA 290 patients who showed the best responses.

Intraepidermal nerve fibers: Similar to the corneal nerve fiber area, at baseline, the mean intraepidermal nerve fiber densities (IENFDs) of the proximal and distal leg were significantly reduced by approximately 50% in both treatment groups, compared with the median of age- and sex-matched normal controls ($p < 0.0001$; Table 1). The mean ratio of IENFD of the proximal thigh to the distal leg was 3.9 ± 1.5 standard error of the mean (SEM), with no patient having a ratio <0.9. The patients in this study, therefore, suffered from a peripheral neuropathy characterized by a length-dependent loss of epidermal nerve fibers. IENFD of the proximal leg was not significantly correlated to that of the distal leg (Pearson correlation coefficient = 0.20; p = 0.22).

After 28 d of dosing, the ARA 290 group exhibited a mean increase in IENFD in the distal leg of 0.38 ± 0.48 fibers/mm (7.2% of baseline; p = ns) compared with the placebo group, which had a mean reduction of nerve fiber density of 0.06 ± 0.42 fibers/mm (1.3% of baseline; p = ns). The thigh IENFD at 28 d showed a mean decrease of 0.49 ± 0.53 fibers/mm for the ARA 290 group (–2.3% of baseline; p = ns), and the placebo group had a mean decrease of 1.24 ± 0.88 fibers/mm (–5.7% of baseline; p = ns).

Cutaneous Sensitivity

Baseline QST data showed that, as a group, the patients with sarcoidosis and painful neuropathy exhibited findings consistent with both small fiber (Aδ and C) and large fiber (Aβ) dysfunction (Table 2). Most patients exhibited a reduced ability to determine cold temperatures (cold detection threshold, 79% of the study group) or warm temperatures (warm detection threshold, 79%) and to detect vibratory stimuli (vibratory stimuli threshold, 92%). A total of 55% of patients also experienced a reduced ability

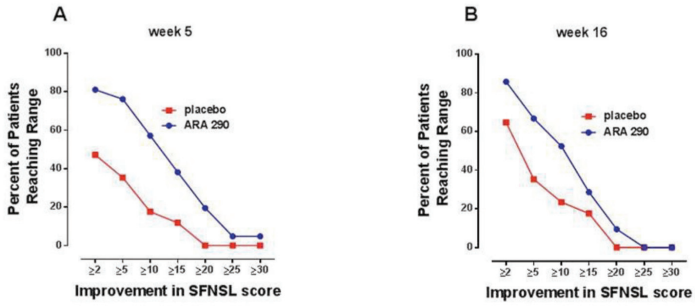

Figure 4. Evaluation of efficacy using the SFNSL shows a sustained improvement in the ARA 290 treatment group compared with placebo. (A) One week after the end of dosing, a larger percentage of patients in the ARA 290 group attained a specified range of score improvement over a broad range of responses. (B) This difference was largely maintained at the end of the 16 wks of follow-up.

to detect graded mechanical stimuli elicited by von Frey fibers (mechanical detection threshold [MDT]) or pain caused by graded pin prick (mechanical pain threshold [MPT]) or to pressure (pressure pain threshold [PPT]). A minority of patients in each treatment group also exhibited abnormalities in a variety of the other sensory modalities tested, as summarized in Table 2.

After 28 d of daily dosing, the cold pain threshold (CPT), heat pain threshold (HPT) and thermal sensory limen (TSL) significantly increased in the ARA 290 group, as illustrated in Figure 3, which summarizes data obtained from the hand testing location. In contrast, there were no changes noted in the placebo group. Although a decreased sensitivity was noted for these sensory modalities, the population means at baseline and after ARA 290 dosing remained within the normal range. Similar but smaller changes were noted in the face test location, whereas a nonsignificant trend was noted at the foot testing site (data not shown). Additionally, the cold detection and warm detection thresholds also decreased (that is, decreased sensitivity) at the hand and face sites, but the changes were not quite large enough to be statistically significant (data not shown). No changes were observed in any other sensory modality within the QST battery.

Retinal Thickness and Visual Acuity

Baseline average thickness of the macula and central macula, and retinal nerve fibers of both eyes, were normal in all patients and did not significantly change over the 28-d observation period (data not shown). Visual acuity at baseline obtained with corrective lenses was normal except for one patient in the ARA 290 group (data not shown). The visual acuity of this patient, and that of all other patients, did not change after ARA 290 exposure.

Secondary Endpoints

SFNSL. Baseline scores of the SFNSL developed specifically for sarcoidosis patients showed that the treatment groups were very symptomatic and well matched, with mean baseline values of 43.9 and 42.8 for the ARA 290 and placebo groups, respectively (not significantly different; *t* test). When evaluated at wk 5 (that is, 1 wk after the end of dosing), the ARA 290 group showed a mean reduction in the SFNSL score of 12.2 ± 1.9 (median 13.0; ~28% reduction from baseline) compared with 3.8 ± 2.1 (median 1.0; ~9% reduction from baseline) for placebo (difference between groups: $p = 0.005$; *t* test). Construction of proportional responders curves (Figure 4A) showed that the percentage of patients receiving ARA 290 having symptomatic improvement in the SFNSL score was greater than the placebo group at each response level. For example, 81% of the ARA 290 patients exhibited at least a two-point improvement in the SFNSL score, compared with only 47% of patients within the placebo group. This response profile was substantially maintained during the 12-wk follow-up period, at which time the mean score reduction from baseline for the ARA 290 group was 9.7 ± 1.8 (median 11.0) and for placebo was 4.1 ± 1.9 (median 3.0; difference between groups: $p = 0.037$; *t* test). The proportional responder curves at 16 wks (12 wks after the end of dosing) were similar to that observed immediately after treatment (Figure 4B). Follow-up at 6 months after the study observation period (that is, 9 months after the termination of dosing) was possible for 19 of 21 of the ARA 290 patients and all of the placebo patients and was notable for a mean SFNSL score of 38.1 ± 3.2 SEM versus 43.7 ± 3.2, respectively. This result represented a significant improvement over baseline for the ARA 290 group (5.2 ± 1.9) compared with the placebo group (–0.9 ± 2.0; $p = 0.036$).

With respect to the autonomic component of the SFNSL, the ARA 290 group demonstrated a significant improvement in the autonomic score when compared with the placebo group with mean improvements of 6.0 ± 1.1 and 1.2 ± 1.3, respectively ($p = 0.009$; *t* test). These improvements correspond to a 29% change from baseline for the ARA 290 group compared with a 6% improvement in the placebo group. A significant difference was observed in the pain component, with the ARA 290 group having a mean improvement of 6.2 ± 1.1 points (27% of baseline) compared with the placebo group, with a mean improvement of 2.6 ± 1.3 points (12% of baseline; $p = 0.032$; *t* test).

BPI. *Pain intensity:* One week after the last injection (that is, on d 35), the aver-

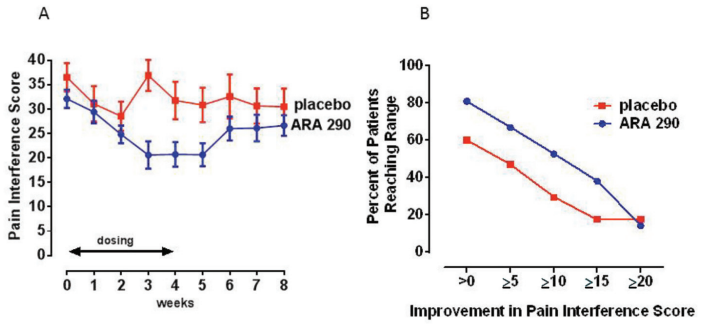

Figure 5. ARA 290 treatment improves the BPI pain interference score. (A) Weekly pain interference scores significantly decline over the 4 wks of daily dosing for the ARA 290 compared with the placebo group. (B) A proportional responder display illustrates that the ARA 290 group responded to a larger extent at all levels of improvement.

age BPI pain intensity score was reduced ~9% from baseline in both treatment groups, with a mean decrease of –3.4 (out of a maximum of 40). This result represented a significant improvement for both the active and placebo arms with respect to baseline ($p = 0.01$; t test), but with no significant difference between the treatment groups. The individual pain intensity scores were notable for a similar reduction in "most pain" (–1.2; $p = 0.003$), "average pain" (–1.1; $p = 0.004$) and "pain now" (–1.0; $p = 0.03$), whereas "least pain" did not change from baseline (–0.2; $p = 0.54$).

Pain interference: In contrast to the mean pain intensity scores that were significantly improved in both groups by wk 5, the mean change in the BPI pain interference score differed significantly between the treatment groups by the third week of dosing ($p < 0.02$; Figure 5A). Specifically, while the baseline values of the two groups were not different, the ARA 290 group dropped from a mean score of 32.1 ± 2.3 at baseline to 20.6 ± 2.7 by the week after dosing (a 36% reduction from baseline). This result compares to a change in the placebo group from a baseline of 36.5 ± 2.5 to 30.8 ± 3.1 (a 16% change from baseline). Proportional responder analysis (Figure 5B) illustrates that the ARA 290 group exhibited about a 20% greater proportion of responders across the response spectrum up to an improvement total of 20 points. At the time of evaluation 9 months after dosing, two patients in the ARA 290 group were lost to follow-up. For the remaining patients, the pain interference score did not significantly differ from the baseline values. Specifically, the score in the ARA 290 group mean was 30.0 ± 2.2 and in the placebo group was 33.9 ± 2.9.

6MWT. The 6MWT is a measure of functional exercise capacity. Both groups had approximately the same baseline 6MWT distance (Table 1), which was significantly less than normal. By using a prediction formula for a normal population with the same approximate age spread (32), the patients in this study at baseline exhibited a mean reduction of 219 meters ($p < 0.0001$; 95% confidence interval of –186 to –253 m) in the actual distance walked in 6 min from a predicted value of 693 m. After 28 d of daily dosing, the 6MWT showed that the ARA 290 group increased the distance walked by a mean of 18.7 m, whereas the performance of the placebo group fell by a mean of –15.1 m (difference between groups: $p = 0.049$; t test). A proportional responder analysis (Figure 6) illustrates that about half of the patients in both treatment groups had improved their 6-min walk distance by up to 12 m. However, for an increase from >25 m, only 12% in the placebo group improved, compared with 52% of the ARA 290 group. Substantial percentages of the ARA 290 group exhibited even larger increases in the 6-min walk distance, whereas none of the placebo patients did.

A 6MWT was repeated at 9 months after dosing (two ARA 290 patients and one placebo patient were lost to follow-up). The mean change from baseline in the ARA 290 group was 8.3 ± 13.3 m and for placebo was –12.9 ± 13.9, neither of which constituted a significant change from baseline (p = ns; t test).

DISCUSSION

Sarcoidosis complicated by small-fiber neuropathy is a chronic disease characterized by the loss of small nerve fibers with associated pain, decreased temperature sensitivity, thermal allodynia and pronounced autonomic dysfunction that severely degrades quality of life. All patients included in this trial had painful neuropathic symptoms consistent with SFNSL that were unresponsive to the standard therapies for chronic sarcoidosis. Many patients continued on immune suppression and symptom-directed therapy throughout the trial.

The principal hypothesis to be tested in this study was whether exposure to ARA 290, a molecule demonstrating tissue-protective, antiinflammatory and reparative activities in numerous preclin-

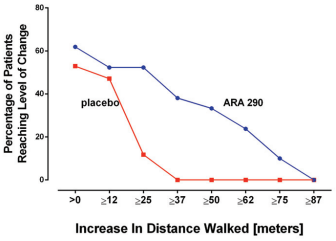

Figure 6. ARA 290 increases the distance patients can walk in 6 min. Similar to the results of symptom questionnaires, patients receiving ARA 290 performed better at all levels of response in the 6MWT.

ical models, would stimulate nerve fiber regrowth with associated improvements in pain and other sensory symptoms, and autonomic function. To accomplish this, the trial was designed to focus on the assessment of objective endpoints such as small nerve fiber quantification by using both skin biopsy and corneal confocal microscopy and to relate these findings to semiobjective sensory testing by using QST, which directly assesses the effects of potential changes in cutaneous innervation. The 6MWT was also included as a simple semiobjective test that requires the integration of complex sensory stimuli of the lower limbs and good exertion by the patient. Finally, patient-reported outcomes were included for subjective assessments of pain and the degree to which pain interfered with activities of daily living, as well as symptoms of autonomic dysfunction that could also be potentially related to changes in nerve fiber density.

Baseline nerve fiber data from this study were analyzed (30), and these data show that corneal nerve quantification (density and length) correlates well with the IENFD of the distal, but not the proximal, lower limb when adjusted for the covariates of sex and age. Further, at baseline, the corneal nerve fiber density (and length) is inversely related to the BPI pain interference score and therefore has relevance for the symptoms that the patients report. Previous work performed in patients with diabetes has also shown a good correspondence between corneal nerve quantification and nerve fiber counts performed in the distal leg (33), thereby confirming the usefulness of corneal nerve assessment in patients with symptoms of small-fiber neuropathy.

The results of nerve fiber assessment after 28 d of dosing show that the corneal nerve fiber density improved significantly in the ARA 290 group when compared with the placebo group at the end of 28 d of dosing. In contrast, no change was observed in the IENFD obtained from the proximal thigh, although a trend was observed for the distal leg biopsy site. Notably, a recent study carried out in a diabetic population has reported positive effects of treatment on corneal nerve fiber density (although over a longer time scale, with no change in the skin biopsy nerve density of the distal extremity) (33). In this study, patients with Type 1 diabetes were followed after curative therapy by pancreas transplantation. Twelve months (but not 6 months) after normalization of blood glucose concentrations, a significant increase in corneal nerve fiber density was documented, whereas no changes were observed in the IENFD of the distal leg or in the results of QST. Additionally, Boyd *et al.* (34) were able to demonstrate a change in skin biopsy nerve densities after drug administration. These investigators studied Type 2 diabetic patients with small-fiber neuropathy after 12 wks of administration of the antiepileptic drug topiramate and documented an increase in cutaneous nerve fiber length at multiple biopsy sites and in nerve fiber density in the proximal leg. It would be of interest to know what assessment of the corneal nerve fibers would have shown.

Prior study (35) of re-innervation after experimental denervation by using capsaicin application to the skin of diabetics with neuropathy or normal individuals has shown that the natural rate of regrowth of sensory nerve fibers is slow in normal individuals and very slow in patients with diabetes. In contrast, regrowth of autonomic fibers is appreciably faster (40–50 d to return to baseline density) than sensory fibers (140–160 d for normalization) (36). Similar experiments have not been performed on corneal nerve fibers, but the results of a preclinical model shows that rapid regeneration (days to weeks) occurs after mechanical injury (37). It is possible that the cornea is an especially useful location to evaluate potential nerve regrowth. Corneal confocal microscopy has the benefit that it is a noninvasive technique that can be repeated many times in the same patient and thus is well suited for longitudinal interventional studies.

As a group, QST showed that the majority of patients in this study had significantly increased cold, warm and vibratory detection thresholds. For patients with sensitivity to cold or heat, this could translate into less pain during activities of daily living. Previous study of patients with diabetic neuropathy has reported similar findings in patients who specifically complained of pain (38). Because thermal sensory function depends on small fiber function, the admission criterion of neuropathic pain may have specifically selected patients that possess a high degree of fiber loss. This possibility was confirmed by the intraepidermal and corneal nerve fiber assessments that showed a marked reduction in the mean number of small fibers innervating cutaneous and corneal sites compared with a normal population.

It is currently unclear what sensory changes may be associated with the axon regeneration that occurs during the short time frame of this clinical trial, since the results of few relevant studies have been reported. Clinical studies performed by using nerve growth factor show that a single injection into normal individuals produces both mechanical and thermal hypersensitivity at the site of injection, which is rapid, reaching a maximum by 21 d and 3 d, respectively (39). Hypersensitivity has been observed at the injection site in longer-term clinical trials, with repeated injections carried out on patients with neuropathy, for example, diabetic polyneuropathy (40). As mentioned above, no injection site pain was noted after ARA 290 administration in the current study; yet changes in sensory thresholds occurred at distant test sites. This observation suggests that ARA 290 has effects within the central nervous system that underlie the altered sensory thresholds.

On the basis of preclinical work, it also appears that changes in responsiveness may occur within the time frame of the present clinical study. Tanelian and Monroe (37) studied a rabbit model in which they produced corneal nerve fiber injury by a small punch biopsy and subsequently used electrophysiological methods to directly determine the behavior of

regenerating small nerve fibers to cold stimuli. Their findings document electrophysiological changes that returned to normal by 30 d after injury. If similar changes occur in patients during the early period of regrowth, we would expect to observe changes in thermal thresholds to the extent that axon sprouting has occurred. However, no assessments were carried out during the period of dosing that can provide relevant information. Additionally, the questionnaires administered do not provide information that is helpful in determining thermal sensory thresholds. It will be important to add these assessments in future trials. However, it is highly likely that any changes that might occur in the sensory system as a result of effects of 28 d of dosing with ARA 290 would not have reached a steady state.

The results of this study show that ARA 290 administration to patients with painful small-fiber neuropathy is associated with a significant improvement in patient-reported symptoms compared with patients receiving placebo, without any evident adverse events attributable to the drug. The changes in level of discomfort as assessed by the SFNSL after 4 mg ARA 290 administered subcutaneously daily was remarkably similar to what was observed in the previous blinded trial, in which 2 mg ARA 290 was administered three times weekly by the IV route (20). In the prior trial, approximately 80% of the patients in the active arm exhibited some improvement and ~40% showed improvement of ~50% over baseline. In contrast, whereas about 45% of the patients in the placebo arm showed some improvement, only ~12% showed a 50% improvement. Daily administration of 4 mg ARA 290 administered subcutaneously was well tolerated without any evident adverse effects. Also similar to the previous blinded trial, a large proportion of the change in SFNSL score was attributable to questions that are relevant to autonomic symptoms. Finally, it is remarkable how sustained the response to ARA 290 appears to be. This result may reflect the growth of small nerve fibers, as the corneal confocal nerve fiber data reveal.

Self-assessment of pain intensity by using the BPI showed that similar to the first blinded trial (20), both groups improved equally, indicating a significant placebo effect on this dimension. In contrast, assessment of to what extent the level of pain interfered with activities of daily living, mood and enjoyment of life showed that patients who received ARA 290 had an immediate reduction in mean score, reaching a nadir that was significantly different from placebo by the end of the dosing period. This result suggests that ARA 290 has a complex activity that extends beyond the sensation of pain to include effects on activities of daily living.

The 6MWT was originally developed to assess functional exercise capacity (that is, a measure of the ability to engage in physically demanding activities) in patients with chronic cardiopulmonary diseases. Since its introduction, the 6MWT has been used to evaluate functional capacity in a wide range of diseases and in healthy normal individuals (32) and has been used as a means to assess the effects of therapeutic interventions. Studies evaluating patients with chronic sarcoidosis have observed that ~50% of these patients have a markedly impaired baseline 6MWT (22,41). In the current study, we found that all of the patients had a reduction in expected walk distance (some very severe). The reason for the higher prevalence in this patient population is not clear, but could arise from the fact that the patients were selected for the presence of neuropathic symptoms that involved the feet, which could contribute to a poor performance on a walk test due to sensory deficits and pain.

At the end of dosing, the ARA 290 group had improved a mean of ~19 m, while the placebo group had declined by ~15 m, about a 4% improvement and 3% decrease of baseline, respectively. Although only about half of the patients improved in both groups (Figure 6), the improvement in distance walked in the 6MWT was limited in the placebo group to <37 m, whereas almost one-quarter of the ARA 290 patients improved the distance walked by up to 75 m. A minimally clinically significant difference has not been established for sarcoidosis patients with painful neuropathy, but for patients with cardiopulmonary disease, the minimally clinically significant difference was determined to be a low as 25 m (42).

The most prevalent form of small-fiber neuropathy occurs in patients with prediabetes or diabetes, and in this group, retinal edema and visual acuity changes are very common. Additionally, another major clinical manifestation of chronic sarcoidosis is ocular inflammation, especially uveitis, which often affects the retina (2). Alternatively, a recent study has shown that patients with neurosarcoidosis frequently have macular edema, even in the absence of ocular symptoms (43). It was of interest, therefore, to evaluate retinal thickness and visual acuity before and after dosing. At baseline, there were no significant abnormalities observed in the optical coherence tomographic evaluation of either retinal or optic nerve head thickness. Similarly, almost all patients had good visual acuity at baseline. Therefore, retinal abnormalities and visual acuity impairment do not appear to be a common feature of sarcoidosis complicated by small-fiber neuropathy.

The patients included in this trial all had longstanding sarcoidosis, with a mean time since diagnosis of 8.4 years. They all had failed existing therapy for neuropathy, including the use of antiinflammatory agents (nonsteroidal antiinflammatory drug [NSAIDs], glucocorticoids and methotrexate principally), as well as antiepileptics and antidepressants. About 30% of the patients were using a variety of these drugs during the conduct of this trial. Because of the small numbers of patient studied, it is not possible to evaluate synergistic effects with any of these agents. It will be interesting to assess for this possibility in future trials with ARA 290.

The principal limitations of this study are that only patients with pain were studied, and these patients did not have

known active sarcoid involvement of any other organ. Circulating markers of inflammation were not significantly elevated and presumptive markers of active sarcoidosis (for example, angiotensin-converting enzyme levels) were only mildly increased in a minority of patients. Small nerve fiber loss is also well known to occur without associated painful symptoms, for example, in the prediabetic state (44). It will be of interest to determine whether corneal nerve fiber density is also abnormal in this patient group.

CONCLUSION

ARA 290 is the first drug that exhibits the ability to induce small nerve fiber regeneration in the cornea without serious side effects, showing a potential of true disease modification, not just symptom improvement. In addition, this trial design using the combination of objective and subjective endpoints offers insight into correlations with patient-reported outcomes and may provide a blueprint for superior trial design for future studies. Most importantly, the results of this study can provide some hope for sarcoidosis patients suffering from small nerve fiber loss and damage, since ARA 290 could substantially improve their quality of life.

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DISCLOSURE

A Dunne, A Cerami and M Brines are employees of Araim Pharmaceuticals and have stock or stock options in the company.

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CHAPTER 7

ARA 290 for treatment of small fiber neuropathy in sarcoidosis.

EXPERT OPINION

ARA 290 for treatment of small fiber neuropathy in sarcoidosis

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Introduction: Painful peripheral neuropathy is a common, difficult-to-treat complication associated with a variety of diseases, including diabetes mellitus and sarcoidosis. It is caused by damage of small and autonomic nerve fibers, resulting in potentially debilitating symptoms of neuropathic pain and autonomic dysfunction. The limited efficacy of current treatment options dictates a rationalized design of novel compounds.
Areas covered: The authors present the recent data from two Phase II clinical trials on ARA290, an erythropoietin derivative with tissue protective and healing properties that does not stimulate erythropoiesis. ARA 290 treatment was consistently associated with a significant improvement of neuropathic pain symptoms in sarcoidosis patients, evidenced by a decrease in pain scores on validated questionnaires. Moreover, ARA 290 treatment resulted in significant increases in corneal nerve fibers, improved sensory pain thresholds, improved quality of life and physical functioning.
Expert opinion: Current treatment modalities of neuropathy are based on a trial-and-error approach, have limited efficacy and come with significant side effects. Given the excellent safety profile while reducing neuropathy symptoms, the prospects of ARA 290 treatment in sarcoid neuropathy seem promising. The long-lasting beneficial effects of ARA 290 on both pain-related and non-pain-related symptoms in sarcoidosis patients prompt additional studies on potential disease-modifying properties of ARA 290.

Keywords: ARA 290, innate repair receptor, neuropathy, pain, Phase II study, sarcoidosis, small fiber neuropathy.

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

Many chronic diseases and syndromes (including diabetes, sarcoidosis, HIV, paraneoplastic syndromes, alcoholism) can result in damage to peripheral nerves [1,2]. The neuropathy is often limited to small and autonomic nerve fibers, that is, small fiber neuropathy (SFN), which leads to the destruction and/or retraction of the small diameter nerve fibers in the skin and dysfunctional autonomic fibers, although in some patients a small and large fiber neuropathy coexists. While some SFN patients remain relatively symptom-free, the majority of patients develop complaints that significantly affect their quality of life (QoL). Most frequently, pain and autonomic symptoms dominate the clinical picture. Unfortunately, SFN remains repeatedly under-diagnosed by the physician specialists involved in treatment of the underlying disease [2]. Pain from SFN has specific characteristics, which include spontaneous (burning, itching, shooting, or electrical) pain, allodynia (touch-evoked pain) and hyperalgesia (increased pain sensitivity), often in combination with fasciculations or paresthesias [3]. Comorbid conditions may be present in some patients, including insomnia, anxiety, depression and catastrophization, tiredness and obesity. These comorbidities further deteriorate the physical and mental

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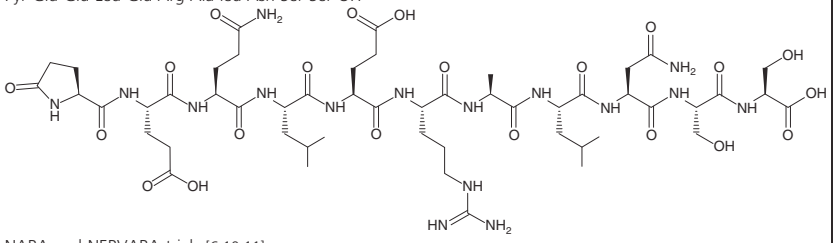
Box 1. Drug summary.

Drug name	ARA 290
Phase	II
Indication	Small fiber neuropathy (SFN) related to sarcoidosis
Mode of action	Activation of the innate repair receptor (IRR)
Route of administration	Intravenous or subcutaneous
Chemical structure	Pyr-Glu-Glu-Leu-Glu-Arg-Ala-leu-Asn-Ser-Ser-OH 
Pivotal trial(s)	NARA and NERVARA trials [6,10,11]

state of the patient, and may affect his or her socioeconomic status often with grave consequences, such as unemployment and social isolation.

Current treatment of SFN pain is symptomatic, and conventional therapy consists of antidepressants, anticonvulsants, prolonged-release opioids and local applications of lidocaine or capsaicin. Irrespective of the treatment or the underlying disease, the efficacy is limited, with just 30 – 40% of patients showing adequate-to-good pain relief [4,5]. In the remaining patient population, the occurrence of severe side effects prevent effective treatment at the required dose or patients have no effect or very limited effect of treatment at all. In some diseases, the initial neuropathic pain treatment is aimed at intervening with the underlying disease process. In the case of sarcoidosis, although conventional immunosuppressive and experimental immune-modulatory and cytotoxic therapies are frequently applied, little relief of neuropathic pain symptoms is seen [3]. We recently initiated a program evaluating the response of patients diagnosed with sarcoidosis and SFN with a new treatment option: ARA 290, an erythropoietin-derivative with tissue protective and healing properties [6]. Sarcoidosis is an orphan disease with ORPHA number ORPHA797 (www.orpha.net) and ARA 290 has received designation as Orphan Drug Product for the treatment of neuropathic pain in sarcoidosis from both the FDA and European Medicines Agency.

A series of experimental studies showed that ARA 290 is effective in the reduction of allodynia in animal models of peripheral nerve injury (which coincided with a reduction in spinal cord inflammation) and inflammatory neuritis [7,8]. Furthermore, administration of related non-erythropoietic tissue protective derivatives have resulted in regrowth of intra-epidermal nerve fibers (IENF) in diabetes mellitus (DM)- and cisplatinum-induced SFN in animals [9]. An initial open-label trial in humans showed that ARA 290 is without side effects, caused relief of neuropathic pain, and improved the QoL of patients with SFN related to diabetes type 2 and sarcoidosis [6]. These positive results prompted the development of the program on ARA 290 in SFN in sarcoidosis patients. Currently, two Phase II trials have been completed and analyzed (the NARA and NERVARA trials [10,11]) and one Phase II trial is ongoing (the DOSARA trial). A first Phase III trial is planned for 2014. We previously reported on the safety, pharmacokinetics and pharmacodynamics of ARA 290 in animals and humans and discussed the open-label trial in DM and sarcoidosis patients [6,11]. Here we will discuss SFN in sarcoidosis and briefly reiterate the mechanism of action and pharmacological properties of ARA 290. Our main focus is the discussion of the two completed and analyzed Phase II trials on the effect of ARA 290 in sarcoidosis patients with painful peripheral neuropathy.

2. Painful small fiber neuropathy in sarcoidosis

Sarcoidosis is a multisystem inflammatory disease of unknown origin, characterized by the development of noncaseating granulomas in various tissues [12,13]. More than 50% of patients have symptoms of pulmonary involvement (dyspnea, coughing), while the majority of patients have nonspecific complaints including fatigue, general feelings of malaise, pain, and symptoms of autonomic dysfunction [14]. The incidence of the disease varies between the sexes and ranges from 1:5300 in females to 1:6300 in males [13,14]. A considerable percentage of patients with persistent sarcoidosis have symptoms consistent with SFN and the diagnosis of SFN is made

Table 1. The small fiber neuropathy screening list.

Part 1: These questions are aimed at finding out how often you experience the following complaints:	
I have painful arms	Never/sometimes/fluctuating/often/always
I suffer from palpitations	Never/sometimes/fluctuating/often/always
I have problems with bowel movements	Never/sometimes/fluctuating/often/always
I have difficulty urinating (either in emptying my bladder or being able to hold my water)	Never/sometimes/fluctuating/often/always
My food does not seem to go down well	Never/sometimes/fluctuating/often/always
I suffer from muscle cramps	Never/sometimes/fluctuating/often/always
My feet and/or hands are colder than I am use to	Never/sometimes/fluctuating/often/always
I have chest pain	Never/sometimes/fluctuating/often/always
Part 2: These questions are aimed at finding out how serious your complaints are:	
I have the feeling that my food gets stuck in my throat	Not at all/slightly/fluctuating/moderately/seriously
At night I throw the bedclothes off my legs	Not at all/slightly/fluctuating/moderately/seriously
I have difficulty urinating (either in emptying my bladder or being able to hold my water)	Not at all/slightly/fluctuating/moderately/seriously
I have dry eyes	Not at all/slightly/fluctuating/moderately/seriously
I have blurred vision	Not at all/slightly/fluctuating/moderately/seriously
I feel dizzy when I get up	Not at all/slightly/fluctuating/moderately/seriously
I have sudden hot flushes	Not at all/slightly/fluctuating/moderately/seriously
My feet and/or hands are colder than I am use to	Not at all/slightly/fluctuating/moderately/seriously
I have painful arms	Not at all/slightly/fluctuating/moderately/seriously
The skin of my leg is over-sensitive	Not at all/slightly/fluctuating/moderately/seriously
I have a tingling sensation in my hands (pins and needles)	Not at all/slightly/fluctuating/moderately/seriously
I have a tingling sensation in my legs (pins and needles)	Not at all/slightly/fluctuating/moderately/seriously
I have chest pain	Not at all/slightly/fluctuating/moderately/seriously

For each question, scores range from 0 (never or not at all) to 4 (always or seriously). The highest score possible is 21 × 4 = 84 (40 points for pain-related symptoms, 44 for autonomic symptoms). The cutoff for SFN is set at 22.
Reproduced from [17] with permission of Elsevier.

in about one-third of sarcoid patients with pain [15]. SFN is characterized by dysfunction, damage and/or destruction of thinly myelinated Aδ and unmyelinated C fibers of the sensory and autonomic nerve system that is not due to granuloma formation [2,3,16]. The symptoms of SFN related to sarcoidosis are similar to SFN from other systemic, inflammatory and autoimmune diseases with complaints of spontaneous pain, allodynia, hyperalgesia and symptoms of autonomic dysfunction (diarrhea or constipation, hypo or hyperhidrosis, urinary incontinence or retention, gastroparesis, dry eyes and mouth, blurry vision, flushes, orthostatic hypotension, erectile dysfunction) [2]. Moreover, sleep-related problems (allodynia-related insomnia from bedsheet intolerance) and restless legs are frequent symptoms of SFN. All symptoms may initially be episodic but gradually become permanent with exacerbations during the nightly hours. In many patients pain is the dominant complaint and varies in presentation between patients (but also within patients) ranging from spontaneous burning and shooting to vibration and electrical shock-like sensations. Finally, the distribution of sensory symptoms can vary between patients, such as a nerve fiber length-dependent stocking/glove presentation or a more patchy, scattered distribution of SFN-related symptoms.

Diagnosis of SFN is primarily based on existing symptoms. The Small-Fiber Neuropathy Screening List (SFNSL) is specifically developed and validated for SFN in sarcoidosis [17]. The SFNSL consists of 21 questions related to neuropathic pain and to autonomic dysfunction (**Table 1**). The latter is important as until recently autonomic complaints were seldom associated with SFN. The specificity of the SFNSL is very high, as a cutoff score has been defined identifying patients with SFN [17]. Additional tests for SFN are also of importance and include quantitative sensory testing (QST), autonomic function testing, and skin biopsies or corneal confocal microscopy, the latter two to assess nerve fiber density.

QST consists of a battery of psychophysical tests where the patient is requested to respond to a specific sensory stimulus to the skin [18]. Tests include cold and warm detection threshold (WDT), cold and warm pain threshold, paradoxical heat sensation, allodynia, and vibration detection threshold. Loss of function (i.e., an increased response threshold) for cold and WDT are indicative of SFN (**Figure 1A**). More objective QST measures include laser-evoked potentials and contact heat-evoked potentials where a short stimulus results in activation of thermo-nociceptive cutaneous nerve fibers. The electroencephalography-potentials (amplitude and delay) give a measure of the functionality of small nerve fibers although more central pathology cannot be excluded. Experimental new tests are being developed to increase specificity and patient compliance. One such example is offset-analgesia, a 30-s test quantitative sensory test in which a disproportionally large

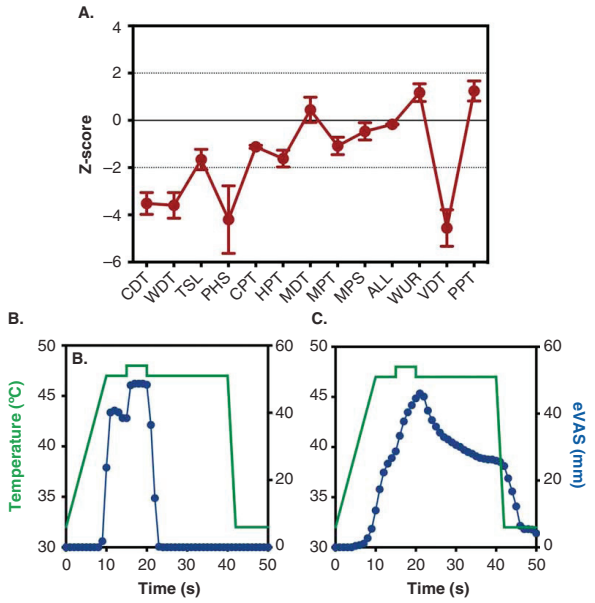


Figure 1. A. Quantitative sensory testing in a mixed population of patients with small fiber neuropathy (n = 10). Values are *z*-scores (mean ± SEM). Values below -2 or above 2 indicate a significant deviation relative to a healthy control population ($p < 0.05$). Abnormal values at the painful extremity were observed for CDT and WDT, PHS and VDT. TSL, CPT, HPT, MDT, MPT, MPS, ALL dynamic mechanical allodynia, WUR, and PPT. **B.** Offset analgesia in a healthy male volunteer 60 years of age. The green line is the temperature change at the skin of the volar surface of the underarm induced by a thermode. Blue dots represent the electronic visual analogue scores. Following the 1°C decrease in temperature a large analgesic response is observed with VAS = zero despite a heat stimulus of 45°C. **C.** An offset analgesia response in a patient with SFN showing no analgesic response following the 1°C decrease in temperature. VAS scores follow closely the noxious temperature stimulus.
A. Data adapted from [45] with permission of Oxford University Press.
B. and **C**. Data adapted from [21] with permission of Wolters Kluwer Health.
ALL: Dynamic mechanical allodynia; CDT: Cold detection thresholds; CPT: cold pain threshold; HPT: Heat pain threshold; MDT: Mechanical detection threshold; MPS: Mechanical pain sensitivity; MPT: Mechanical pain threshold; PHS: Paradoxical heat sensation; PPT: Pressure pain threshold; tsl: Thermal sensory limen; VAS: visual analog scale; VDT: Vibration detection threshold; WDT: Warm detection thresholds; WUR: Windup ratio.

amount of analgesia becomes apparent upon a slight decrease in noxious heat stimulation (**Figure 1B** and **C**) [19-22]. We recently showed that this test has a > 90% sensitivity and specificity to discriminate between healthy volunteers and patients with SFN [21].

Skin biopsies are considered the gold standard for diagnosis of SFN [23,24]. The technique, which involves a 3-mm punch biopsy of the skin (most commonly a site 10 cm above the lateral malleolus chosen), identifies the presences of the small Aδ and C-nerve fibers that penetrate the dermal-epidermal junction (**Figure 2**). Several studies show that SFN in sarcoidosis is associated with a reduction of the intraepidermal nerve fiber density (IENFD) albeit with high specificity and low sensitivity [2]. The punch biopsy is an invasive, time-consuming test, and few medical centers offer this technique. Furthermore, there is a dependence on age and gender and high variability of normal values. Also, diseased patients (up to 60% in sarcoidosis) may have a normal IENFD despite severe SFN pain-like complaints [25]. These numbers indicate that small nerve loss of function precedes actual destruction or retraction (**Figure 2A**). Moreover, sarcoidosis-related SFN can have a non-length-dependent, patchy bodily distribution, so that the detection of SFN could be missed by a single ankle skin biopsy [26,27].

A relatively new technique involves the quantification of C-fibers between the layer of Bowman and basal epithelium of the cornea [28-31]. Using cornea confocal microscopy (CCM), the nerve fibers of the cornea are systematically photographed and quantified with respect to length, density, branching, and tortuosity. Recently we demonstrated that in sarcoidosis patients with SFN, corneal nerve fibers density and length are reduced and, in contrast to intraepidermal fiber

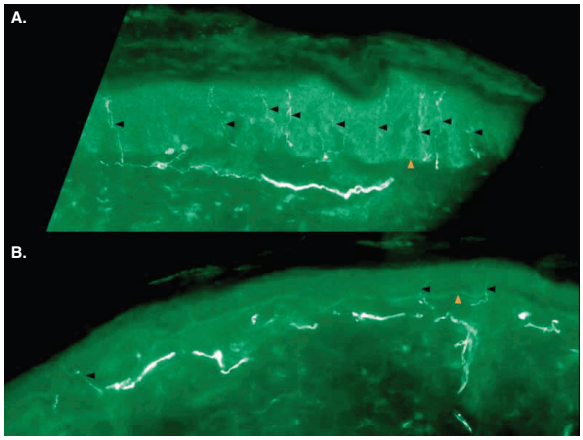


Figure 2. Skin biopsies obtained 10 cm above the lateral malleolus of two patients (A and B) with sarcoidosis and small fiber neuropathy. Both patients had moderate to severe neuropathic pain with pain scores > 5. Note the difference in intraepidermal nerve fiber density. Orange arrowheads point toward the basal membrane between dermis and epidermis, the black arrowheads point toward the small nerve fibers (white). In the dermis the nerve plexus are visible (white).

density, the CCM data correlated with pain-interference score (i.e., the degree at which pain interferes with activities of daily living) derived from the Brief Pain Inventory (BPI) questionnaire [28]. These results, together with the fact that CCM allows rapid and noninvasive assessment of the cornea nerve fibers, this technique may be a further aid in the diagnosis and follow-up of SFN. The corneal nerve plexus is a highly dynamic structure, allowing easy detection of density changes in time [32]. Furthermore, the association between cornea fiber density and pain interference designates this measure as a promising biomarker of SFN pain.

Taken the fact that autonomic dysfunction is an important symptom occurring in SFN, autonomic tests are important for diagnosis as well as assessment of the clinical condition of the patient [2]. Apart from specific questionnaires aimed at diagnosing dysautonomy [17], specific tests are available, including quantitative sudomotor axon reflex testing where the sweat output is measured in response to acetylcholine iontophoresis at the upper and lower extremities [23,24]. Other tests involve cardiac testing, including heart rate variability tests.

3. ARA 290

There is ample evidence that the anti-inflammatory cytokine erythropoietin (EPO) has tissue protective properties [33]. For example, recombinant human EPO (5000 IU/kg) reduces concussive injury in an animal model of blunt head trauma [34]. In humans, EPO (40,000 IU/week) reduces mortality by 50% in trauma patients admitted to the intensive care [35]. In various animal nerve injury models (including SFN due to DM), EPO improved nerve recovery and function [9,36]. The anti-inflammatory property of EPO is exerted through activation of the innate repair receptor (IRR). The IRR is a hetero-complex composed of β-common receptor and erythropoietin receptor subunits. The IRR has a low affinity for EPO, but is expressed on the cell surface in response to local tissue injury and inflammation and is effectively activated by locally produced EPO. IRR activation results in engagement of multiple anti-inflammatory and tissue protective pathways and ultimately results in reduced apoptosis, tissue restoration, and nitric oxide formation by endothelial cells (hence improving tissue perfusion). The EPO-IRR system is programmed to increase the probability of non-hematopoietic cell and tissue survival.

Since a high dose of exogenous EPO, required to activate the IRR, concurrently stimulates erythropoiesis and results in various serious side effects (including pro-thrombotic effects, hypertension, myocardial infarction), novel molecules have been developed that exclusively activate the IRR [37]. One such molecule is ARA 290 (Araim Pharmaceuticals Inc., Ossining, NY), an 11-amino acid linear peptide (molecular weight 1257 Da, amino acid sequence: Pyr-Glu-Glu-Leu-Glu-Arg-Ala-leu-Asn-Ser-Ser-OH [**Box 1**]), which mimics the spatial configuration of EPO that interacts with the IRR. Like EPO, derivative peptides, such as ARA 290 reduce apoptosis, are anti-inflammatory and promote healing in a variety of animal nerve injury models (including peripheral nerve injury and SFN in DM) [9,36,38,39]. In animals (maximum dose given 11,000 μg/kg ARA 290), healthy volunteers (up

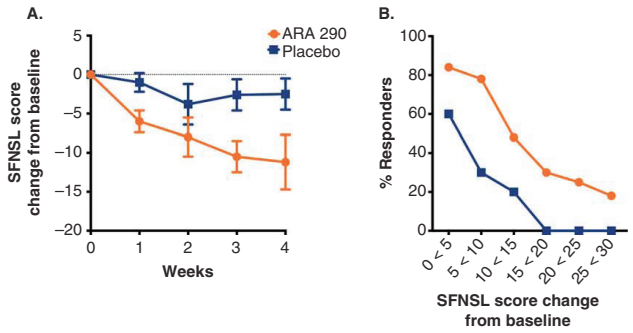


Figure 3. A. Effect of ARA 290 in the first Phase II trial (NARA). ARA 290 produces a time-dependent improvement of the small fiber neuropathy screening list (SFNSL) score, significantly greater than placebo ($p < 0.05$; values are mean ± SEM). **B.** Cumulative percentage responder analysis at six SFNSL score ranges. At each range, the responder percentage of ARA 290-treated patients exceeds that of placebo-treated patients.
Data adapted from [11] with permission of Molecular Medicine.

to 30 µg/kg), and patients (25 – 50 µg/kg) no safety issues were identified (Araim, data on file [6]). In healthy volunteers, ARA 290 has a short intravenous half-life (2 min following intravenous and 20 min following subcutaneous administration). This indicates a rapid passage of ARA 290 eliciting a long-lasting effect. Indeed, the pharmacodynamic analysis of pain relief data of the Phase I trial (data obtained in sarcoidosis and diabetes type 2 patients following a 3-day intravenous treatment with 2 mg ARA 290 on Monday, Wednesday, and Friday) yielded an effect half-life, of 2 – 3 days [6].

4. Clinical efficacy

4.1 The NARA trial protocol [11]

The first exploratory Phase II study was designed in light of the findings from the open-label study. The NARA study was a double-blind, randomized, controlled trial (RCT) performed in sarcoidosis patients with moderate to severe neuropathic pain. Only patients with confirmed sarcoidosis were eligible to participate. Inclusion criteria related to the presence of painful SFN were: i) the presence of at least one of the following symptoms: distal symmetrical dysesthesia or paresthesia; burning feet and; allodynia at the lower extremities (bed sheet intolerance); and ii) spontaneous pain scores of 5 or greater (on an 11-point scale). Confirmation of SFN was further obtained by quantitative sensory testing. Patients could remain on their pain medication (e.g., corticosteroids, antidepressants) but were not allowed to change intake during the study. Patients were randomized to receive ARA 290 (intravenous dose of 2 mg dissolved in 6 ml of normal saline) or placebo (6 ml normal saline) three times weekly (Monday, Wednesday, Friday) for 4 weeks; follow-up period was 12 weeks. The main end points of the study included pain intensity scores from the BPI questionnaire, neuropathic symptoms as determined from the SFNSL questionnaire (**Table 1**), and QoL score from the short form of the RAND36 (SF-36).

4.2 The NARA trial results

A total of 13 patients of either sex were randomized to ARA 290 (12 analyzed, 1 patient did not receive allocated treatment), 13 to placebo (10 analyzed, 2 did not receive the allocated medication, 1 was excluded because of noncompliance). Mean age of the patients was 48.6 years. All patients were treated at home by the trial physician. The SFNSL scores showed a 30% decrease in ARA 290-treated patients (**Figure 3A**) compared to baseline with a maximum difference between treatments at week 4. SFNSL responder rate was greatest in the ARA 290-treated patients: 83 versus 60% of patients had a reduction in score of 1 – 5 points (ARA 290 vs placebo), 42 versus 0% had a reduction in score of at least 15 points (**Figure 3B**). The SFNSL reports on pain-related and autonomic symptoms; ARA 290 caused improvement in both dimensions (autonomic symptoms including dry eyes, blurred vision, orthostatic hypotension: $p < 0.05$ vs placebo; pain symptoms: NS). QoL improved in ARA 290-treated patients ($p < 0.05$ vs placebo). Interestingly, pain scores derived from the BPI reduced in both treatment groups similarly by 2.1 versus 2.3 points. Pain score and physical functioning score derived from the SF-36 improved significantly in ARA 290 treated patients than in placebo-treated patients (difference in score reduction > 50%, $p < 0.01$).

The first Phase II trial confirmed the observations made in the open-label study showing a significant improvement in neuropathic pain symptoms including autonomic symptoms. No safety issues were raised during or in the 3 months following treatment. As expected, ARA 290 had no effect on hemoglobin concentrations.

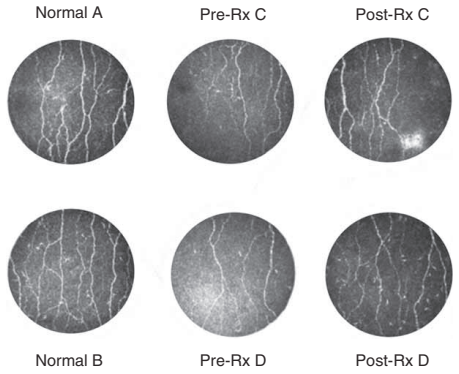


Figure 4. Cornea nerve fibers obtained via corneal confocal microscopy in two normal subjects (Normal A and Normal B) and two sarcoidosis patients with small fiber neuropathy before treatment (Pre-Rx C and Pre-Rx D) and after a daily 28 week 4 mg subcutaneous injection with 4 mg ARA 290 (Post-Rx C and Post Rx D). The paucity in nerve fibers before treatment and the improvement in nerve fiber density after treatment are evident.
Data are from [10] with permission of Molecular Medicine.

4.3 The NERVARA trial protocol [10]

The positive observations from the NARA trial prompted a second, larger Phase II trial, the NERVARA trial. This double-blind, randomized, placebo-controlled RCT was also performed in sarcoidosis patients with painful neuropathy. Inclusion criteria were: i) the confirmed diagnosis of sarcoidosis; ii) pain levels > 5, and SFNSL score > 22 (indicative of SFN-related neuropathic symptoms); and iii) the presence of at least one of the following symptoms: dysesthesias, burning or painful feet worsening at night, and bedsheet intolerance. In contrast to the NARA trial, patients were treated daily with 4 mg subcutaneous ARA 290 injections for 28 days; follow-up was 12 weeks. The primary end points of the study were treatment-induced changes in corneal nerve fiber density, intra-epidermal nerve fiber density, and cutaneous quantitative sensory sensitivity. The secondary end points of the study were treatment-induced changes in the SFNSL score, BPI score, exercise capacity determined by the 6-min walk test (6MWT).

4.4 The NERVARA trial results

Twenty-one patients were randomized to ARA 290 treatment (21 analyzed), 20 patients to placebo treatment (17 analyzed, 2 did not receive allocated medication; 1 patient excluded because of noncompliance). Mean age of the patients was 49.5 years (range 28 – 65 years) with 18 men and 20 women. There were on average 7 (ARA 290) and 10 years (placebo) between the current study and the diagnosis of sarcoidosis. No safety issues became apparent during the study or the follow-up period.

The test results were as follows:

1) *Corneal Nerve Fiber Density (CNFD).* All patients exhibited a reduced CNFD, about 50% of that of healthy age- and sex-matched controls. ARA 290 treatment caused a significant increase in CNFD by 14.5% (compared to a small decrease in placebo treated patients, p = 0.02) (**Figure** 4).
2) *IENFD.* The IENF densities of the proximal (thigh) and distal leg were about 50% reduced compared to healthy age- and sex-matched controls. Treatment had no significant effect on the IENF densities.
3) *QST.* Pretreatment measurements showed loss of function of small fibers of the skin with abnormal cold and WDTs. ARA 290 but not placebo caused an increase in sensory pain thresholds: cold pain threshold increased by about 6°C, warm pain threshold by 3°C. Baseline values of these modalities were within the normal range.
4) *SFNSL.* Baseline scores of the SFNSL were 43 (pain component: 23, autonomic component 21). ARA 290 produced a 30% improvement of the SFNSL score at week 5 of the study versus a 9% improvement for placebo (p < 0.01). The improvement was most apparent for symptom severity than for symptom frequency. The observed changes persisted for at least 3 months post-treatment (**Figure 5A** and **B**). In a large subset of patients (36 patients) a final measurement was obtained at 9 months demonstrating a 6-point difference between treatment groups favoring the ARA 290 patients. Improvement in SFNSL score by ARA 290-treated patients occurred in both pain and autonomic symptom subscores.
5) *BPI.* Mean pain intensity scores were reduced similarly in ARA 290- and placebo-treated patients by a significant 9%. Pain interference scores improved significantly in ARA 290 patients by 36 versus 16% in placebo patients (p < 0.05).
6) *6 MWT.* On average patients were able to walk 470 m during the 6 min of the test. This is over 200 m less than predicted for the study population. After treatment, ARA patients increased the walking distance by almost 19 m versus a reduction of 15 m in placebo patients (p < 0.05). Responder analysis showed that an improvement of > 25 m occurred in 52% of ARA 290 patients versus 12% in placebo patients.

5. Conclusion

The picture that emerges from the first Phase II trials, NARA and NERVARA, is that the effect of the non-hematopoietic EPO analogue ARA 290 is consistent, showing improvement of pain, neuropathic symptoms (including symptoms related

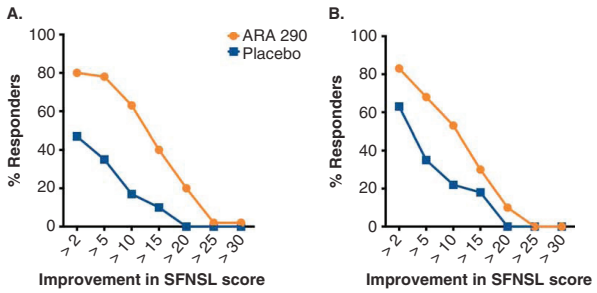


Figure 5. A. Cumulative responder percentage at study week 5 (i.e., the first week following treatment) of the small fiber neuropathy screening list (SFNSL) score. The responder percentage of ARA 290-treated patients exceeds that of placebo-treated patients. **B.** Responder percentage at week 16. The larger responder percentage of ARA 290-treated patients over placebo-treated patients is maintained at 12 weeks after termination of therapy.
Data adapted from [10] with permission of Molecular Medicine.

to autonomic dysfunction), QoL, and exercise capacity during and following the 4-week treatment. In both trials, SFNSL showed robust changes with significant improvement in the two dimensions queried (pain or autonomic function), indicative of an effect of ARA 290 on sensory and autonomic nerve fibers. An important observation in the NERVARA trial is the observation of regrowth of corneal nerve fibers, a clear and visible consequence of the reparative properties of the ARA 290 peptide. The lack of effect on IENFD suggests that longer treatment periods may be needed before an effect on intraepidermal nerves becomes visible. A caveat of these studies is evidently the small patient numbers studied with ARA 290 (33 patients in total). To further complete the ARA 290 dossier and to strengthen the evidence for a beneficial effect of ARA 290, additional studies are ongoing or being planned in SFN resulting from sarcoidosis or other origins (e.g., DM).

6. Expert opinion

Chronic pain and especially chronic neuropathic pain is difficult to treat. Common treatments are effective in just a portion of patients and efficacy is often limited. Furthermore, most (if not all) of these treatments come with side effects that significantly lower the QoL and consequently patient compliance. SFN is a form of neuropathy that is associated with a variety of common diseases and has a high incidence of occurrence in these diseases (e.g., DM, leprosy, HIV, alcoholism, chemotherapy-induced peripheral neurotoxicity, and sarcoidosis). SFN causes a series of debilitating symptoms that, besides neuropathic pain, includes autonomic symptoms ranging from dry mouth to erectile dysfunction, palpitations, and orthostatic hypotension.

ARA 290 is the first treatment option aimed at treatment of SFN in sarcoidosis. The search for compounds that produce relief of neuropathic symptoms (without producing toxic side effects) is important, as there is an unmet need for the treatment of pain and autonomic symptoms in this disease as well as other diseases. Given the first results obtained from Phase I and Phase II clinical trials, the prospects for SFN treatment in sarcoidosis seem promising, although further proof from larger trials is required. Safety data are promising as well but longitudinal data in large patient cohorts should be collected, especially during and following longer treatment periods (> 28 days).

Many pharmacological studies on chronic pain focus on simple quantitative pain-related end points [40]. This is not surprising as most treatments are symptomatic aimed at increasing pain detection thresholds without any modulatory role on the underlying disease process. The most common end point therefore is a pain score, either by numerical rating, visual analogue scale, or a pain score derived from a questionnaire (e.g., the BPI). However, many symptoms (pain- and non-pain-related) deserve investigation and treatment in chronic pain patients. For example, exercise capacity and autonomic symptoms are equally important as they may be affected causing an appreciable reduction in patients' QoL. Although treatment may produce pain relief, the lack of improvement of other symptoms (e.g., physical functioning, orthostatic hypotension) will reduce patient compliance, even more so when treatment causes annoying side effects or worsens present symptoms (e.g., lightheadedness upon standing up causing an increased probability of falling and trauma, especially in the elderly). In the NERVARA trial a systematic and extensive set of end points was chosen to assess pain-related, autonomic, and disease-modulating symptoms. End points were multiple and included neuropathic pain and autonomic scores from several questionnaires (e.g., SFNSL, BPI), nerve fiber density in the epidermis and cornea, sensory thresholds, and exercise capacity (as assessed

by the 6MWT). Moreover, assessments were made prior to and at the end of treatment and during follow-up (at 3 months and an additional assessment was made at 9 months following the start of treatment). ARA 290 had long-lasting beneficial effects on a variety of these end points, suggesting long-lasting anti-inflammatory and tissue restorative effects. In this respect ARA 290 seems unique as other anti-inflammatory drugs that have recently been tested in neuropathic pain syndromes were either ineffective or did produce pain relief but were without improvement in functionality [3,41]. One such treatment is ketamine, which exclusively reduces pain but does not provide improvements in function in chronic pain patients with complex regional pain syndrome [42]. Anecdotal treatment of SFN in sarcoidosis with immune-modulating drugs, such as steroids or anti-tumor necrosis factor agents, suggest some improvement of pain symptoms and support anti-inflammatory mediated beneficial effects [3,43,44]. We argue that ARA 290 should not be considered an analgesic but rather a disease-modifying drug that intervenes in the disease process responsible for SFN-related symptoms. Note that at this point there are no indications that ARA 290 actually interferes with the underlying disease itself (i.e., sarcoidosis). The data presented here suggest that ARA 290 will be equally effective in SFN associated with other syndromes including DM. Indeed, the initial open-label trial showed efficacy in DM SFN similar to sarcoidosis SFN. A more extensive RCT of ARA 290 effect in DM-related SFN has recently been completed and presentation of the results is pending.

Declaration of interest

A Dunne and M Brines are employees of Araim Pharmaceuticals. A Dahan is on the scientific board of Araim pharmaceuticals. A Cerami is the CEO of Araim pharmaceuticals. All other authors declare no conflict of interest.

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CHAPTER 8

Summary and conclusions/ Samenvatting en conclusies

Chapter 8. Summary, General Discussion and Future Perspectives

Small-fiber neuropathy (SFN) selectively affects the thinly-myelinated A-δ and unmyelinated C-fibers. These fibers have their neurons in the dorsal root ganglia (DRG) and are involved in various functions ranging from autonomous functions to the conduction of specific sensations (*e.g.* temperature, itch, pain, touch). Afferent information from the epidermis, where the sensory small-fibers end (*i.e.* as nociceptors), travels via the DRG to spinal cord dorsal horn. SFN, irrespective of its cause, is characterized by specific symptoms such as burning pain (often restricted to the extremities), allodynia (*e.g.* sheet intolerance), hyperalgesia but sometimes also hypoesthesia, often accompanied by signs of autonomous dysfunction (dry mouth, diarrhea, erectile dysfunction, etc.). In this thesis I discuss SFN due to sarcoidosis and propose a treatment with the tissue-healing peptide ARA290 in 6 distinct chapters.

In **Chapter 2** sarcoidosis and pain caused by small-fiber neuropathy is discussed. SFN is one of the disabling and often chronic manifestations of the disease. SFN presents with peripheral pain and symptoms of autonomic dysfunction. The character of the pain can be burning or shooting. Besides, allodynia and hyperesthesia can exist. Diagnosis is usually made on the basis of clinical features, in combination with abnormal specialized tests. The aim of treatment is often to reduce pain; however, total pain relieve is seldom achieved. The role of TNF-α in the pathogenesis of SFN in sarcoidosis appears interesting to explore. TNF- α is an proinflammatory cytokine that may be crucial in the pathogenesis of SFN in sarcoidosis. Novel therapeutic agents such as ARA290, a non-hematopoietic erythropoietin analogue with potent anti-inflammatory and tissue protective properties, are interesting novel treatment options of SFN in sarcoidosis. This agent binds, similar to hypoglycosylated erythropoietin, to the innate repair receptor and as such activates pathways leading to healing and tissue repair.

In **Chapter 3** a case series is presented of patients with sarcoidosis and neuropathic pain due to the presence of small-fiber neuropathy. Various testing modalities have been designed to characterize nerve fiber dysfunction affected patients. In the current study, we characterized the sensory phenotype of sarcoidosis patients with neuropathy using quantitative sensory testing (QST), skin biopsy, and cornea confocal microscopy (CCM). On average, patients displayed sensory abnormalities and decreased nerve fiber densities. However, some cases are discussed that show distinct disease characteristics. We identified an abnormal albeit heterogeneous sensory phenotype in sarcoidosis patients with neuropathic pain. Discrepancies between used methods, such as the assessment of nerve fiber density using skin biopsies *vs.* CCM, indicate that combinatory techniques should be used to link disease features with symptom expression. The sensitivity and specificity of cornea microscopy should be determined and correlated to skin-biopsy-obtained intra-epidermal nerve fiber densities in a larger cohort of neuropathic pain patients. The construction of sensory profiles or subgroups aids in the identification of commonalities in neuropathy phenotypes and will guide personalized, more successful therapy. Current pain treatment is symptomatic with limited efficacy.

The occurrence of severe side effects often prevents effective treatment at the required dose and decreases patient compliance. To improve outcome, future therapeutic trials should take into account both the heterogeneity of neuropathy symptoms, as well as the sensory profile in which patients could be stratified.

Chapter 4 describes the pharmacokinetics of ARA290, a novel treatment option in sarcoidosis-induced SFN. ARA290 is an 11-amino acid peptide with tissue protective properties. It is effective in the treatment of neuropathic pain in patients with sarcoidosis and diabetes mellitus type 2. Since ARA290 requires frequent parenteral administrations, various routes of administration are being examined that allow treatment outside the hospital setting with high patient compliance. In this study we determined the safety and pharmacokinetics of subcutaneous (SC) compared to intravenous (IV) ARA290 administration. 10 healthy volunteers received on one occasion 2 mg IV ARA290, and on another occasion 2 mg SC ARA290. On a third occasion, 5 subjects received 4 mg SC and the five others 6 mg SC ARA290. Serial plasma samples were obtained to determine the time-dependent plasma concentrations of ARA290. Safety parameters were obtained; the pharmacokinetics were assessed by non-compartmental analysis. No safety issues were identified. The mean peak plasma concentration after IV dosing was estimated at 111 ng/mL. After SC administration peak concentrations were observed between 12 and 15 min and were greater than 1.25 ng/mL for all SC doses, the minimum concentration believed to be necessary to trigger the receptor mediating ARA290 biological effects. The terminal half-life of the IV and SC doses were 1.1 min and 17-26 min, respectively. Based on the area-under the plasma-concentration curve the bioavailability of SC ARA290 ranged from 11 to 25%. ARA290 is safe and well tolerated up to 6 mg SC. All three SC doses exhibited peak concentrations greater than the assumed minimum effective concentration. Despite the short plasma residence time of ARA290 in this study, long-term pharmacodynamics effects have been observed in animals and in humans. These observations suggest that ARA290 initiates a cascade of events involving several steps. For neuropathic pain, the site of action of ARA290 is within the central nervous system, and is likely mediated via the innate repair receptor, triggering anti-inflammatory, neuroprotective and healing effects in the damaged tissue.

Chapter 5 describes a first phase II trial on the efficacy and safety of ARA290 in sarcoidosis-induced SFN. ARA290 (a peptide designed to activate the innate repair receptor that arrests injury and initiates cytoprotection, antiinflammation and healing) reduces allodynia in preclinical neuropathy models. We studied the safety and efficacy of ARA290 to reduce symptoms of small fiber neuropathy (SFN) in patients with sarcoidosis. A total of 22 patients diagnosed with sarcoidosis and symptoms of SFN were enrolled in a double-blind, placebo-controlled exploratory trial consisting of three times weekly intravenous dosing of ARA290 (2 mg; n = 12) or placebo (n = 10) for 4 wks. Inclusion criteria were a diagnosis of neuropathy and a spontaneous pain score of ≥5 (Brief Pain Inventory [BPI]). Endpoints assessed were changes in pain intensity and the small fiber neuropathy screening list (SFNSL) score, quality of life (SF-36), depressive

symptoms (Inventory of Depressive Symptomatology) and fatigue (Fatigue Assessment Scale [FAS]). No safety concerns were raised by clinical or laboratory assessments. The ARA290 group showed significant ($p < 0.05$) improvement at week 4 in SFNSL score compared with placebo (Δ -11.5 ± 3.04 versus Δ -2.9 ± 3.34 [standard error of the mean]). Additionally, the ARA290 group showed a significant change from baseline in the pain and physical functioning dimensions of the SF-36 (Δ -23.4 ± 5.5 and Δ -14.6 ± 3.9, respectively). The mean BPI and FAS scores improved significantly but equivalently in both patient groups. No change was observed in the depressive symptoms. ARA290 appears to be safe in patients with sarcoidosis and can reduce neuropathic symptoms, , as assessed by questionnaires focused on pain and quality of life. The most robust effect of ARA290 was on the SFNSL score, indicating an effect on symptom severity rather than frequency. Notably, in the current study an improvement of autonomous symptomatology was observed. Autonomous nerve fibers show a faster regeneration pattern than sensory nerve fibers. The less robust effects of ARA290 treatment on pain suggests that prolonged or intensive dosing regimens are required.

Chapter 6 describes a next phase II trial studying SFN symptoms and cornea nerve fiber density in patients with painful sarcoidosis. Small nerve fiber loss and damage (SNFLD) is a frequent complication of sarcoidosis that is associated with autonomic dysfunction and sensory abnormalities, including pain syndromes that severely degrade the quality of life. SNFLD is hypothesized to arise from the effects of immune dysregulation, an essential feature of sarcoidosis, on the peripheral and central nervous systems. Current therapy of sarcoidosis-associated SNFLD consists primarily of immune suppression and symptomatic treatment; however, this treatment is typically unsatisfactory. ARA290 is a small peptide engineered to activate the innate repair receptor that antagonizes inflammatory processes and stimulates tissue repair. Here we show in a blinded, placebo-controlled trial that 28 days of daily subcutaneous administration of ARA290 in a group of patients with documented SNFLD significantly improves neuropathic symptoms. In addition to improved patient-reported symptom-based outcomes, ARA290 administration was also associated with a significant increase in corneal small nerve fiber density, changes in cutaneous temperature sensitivity, and an increased exercise capacity as assessed by the 6-minute walk test. On the basis of these results and of prior studies, ARA290 is a potential disease-modifying agent for treatment of sarcoidosis-associated SNFLD. The cornea appears to be an especially useful location to evaluate potential nerve regrowth, and assessment of cornea nerve fiber density is fast, non-invasive and can be used in longitudinal, interventional studies. It is currently unclear what sensory changes may be associated with the nerve regeneration that occurs during the short time frame of the trial, but it is likely that any changes have not reached a steady state. Moreover, most patients had longstanding sarcoidosis with failing existing therapy. Possible synergistic effects of concomitant medication should be evaluated in the future.

Finally, in **Chapter 7**, the results of the various ARA290 trials in painful sarcoidosis are discussed. Painful peripheral neuropathy is a common, difficult-to-treat complication

associated with a variety of diseases, including diabetes mellitus and sarcoidosis. It is caused by damage of small and autonomic nerve fibers, resulting in potentially debilitating symptoms of neuropathic pain and autonomic dysfunction. The limited efficacy of current treatment options dictates a rationalized design of novel compounds. The authors present the recent data from two Phase II clinical trials on ARA290, an erythropoietin derivative with tissue protective and healing properties that does not stimulate erythropoiesis. ARA290 treatment was consistently associated with a significant improvement of neuropathic pain symptoms in sarcoidosis patients, evidenced by a decrease in pain scores on validated questionnaires. Moreover, ARA290 treatment resulted in significant increases in corneal nerve fibers, improved sensory pain thresholds, improved quality of life and physical functioning. Current treatment modalities of neuropathy are based on a trial-and-error approach, have limited efficacy and come with significant side effects. Given the excellent safety profile while reducing neuropathy symptoms, the prospects of ARA290 treatment in sarcoidosis-related neuropathy seem promising. The long-lasting beneficial effects of ARA290 on both pain-related and non-pain-related symptoms in sarcoidosis patients prompt additional studies on potential disease-modifying properties of ARA290. We argue that ARA290 should not be considered an analgesic but rather a disease-modifying drug that intervenes in the disease process responsible for SFN-related symptoms. There are no indications that ARA290 actually interferes with the underlying disease itself (*i.e.* sarcoidosis). The data collected so far suggest that ARA290 will be equally effective in SFN associated with other syndromes, including diabetes mellitus.

General Discussion of the Data

Heterogeneity of patient population

Despite the fact that all patients treated with ARA290 in Chapters 5 and 6 had neuropathy related to sarcoidosis, therapy efficacy was limited both in magnitude and responder rate. For example, just 50% of patients in Chapter 5 and 40% of patients in Chapter 6 showed a reduction in SFNSL score of 50% or greater at the end of the ARA290 treatment period. From Chapter 3 it becomes clear that different patients present with different phenotypes with respect to cornea fiber and skin fiber abnormalities, responses to the different questions in the neuropathic-pain related questionnaires, and outcome of sensory tests. Possibly multiple well defined specific subgroups of patients exist that may respond differently to therapy. For example, Martini et al.[1] showed previously that pain ratings in the days prior to therapy predicts the response to topical capsaicin or placebo in patients with post-herpetic neuralgia. A large placebo response was predicted by a high variability in the pain ratings in the days prior to treatment. Another example is the observation that patients with diabetic polyneuropathy respond best to treatment with tapentadol when they have defects in their endogenous pain modulatory system.[2] Tapentadol is an analgesic with a dual mode of action, it activates the mu-opioid receptor system and inhibits re-uptake of neuronal noradrenaline at spinal and supraspinal sites. Consequently, postsynaptic α_2-adrenergic

receptors are activated causing inhibition of nociceptive trafficking. Especially patients with defects in their endogenous pain modulatory system (as measured by conditioned pain modulation) are sensitive to the noradrenergic enhancement of opioid analgesia. These data suggest that indeed individualized pain management based on specific biomarkers is possible. Hence, identification of biomarkers may be a step forward towards understanding the pathophysiology of chronic pain and individualized pain medicine.

In this thesis sarcoidosis patients were all profiled prior to treatment with ARA290. Specific tests were performed to assess whether these patients present with abnormalities in intra-epidermal nerve fibers and cornea nerve fibers (as measured by cornea confocal microscopy, CCM). Additionally, quantitative sensory tests were performed to get an indication of the presence of gain- or loss-of-function of specific sensory modalities. And finally the small-fiber-neuropathy screening list (SFNSL) questionnaire was completed by all patients. Figure 1 is a summarizing box plot of the data. Each column represents a single patient and a colored square indicates a value for that biomarker that deviates from a sex- and age-matched “normal” reference population without pain (see the legend of Figure 1 for further explanation). This population clearly had abnormal functioning of their peripheral small nerve fibers as more than 90% of patients had an abnormal cold, warm or mechanical detection threshold (CDT, WDT and MDT, in Fig 1). Interestingly, quantification of the intra-epidermal nerve fibers (IENF) showed a normal density in 72% of the population and more than half (56%) had normal cornea C-fibers. Forty percent of patients had allodynia (ALL) or an increased wind-up ratio (WUR), indicative of central sensitization. Phenotyping patients based on CCM and the presence/absence of central sensitization results in 4 groups: (1) Abnormalities in CCM with central sensitization (n = 6/26); (2) Abnormalities in CCM without central sensitization (n = 20/26); (3) Normal CCM with central sensitization (n = 18/32); and (4) Normal CCM without central sensitization (n = 14/32). The absence of abnormalities in the cornea confocal nerve morphology data does not indicate that small nerves in the skin are unaffected. The abnormalities in CDT, WDT and MDT suggest otherwise and 9/36 of patients had abnormal skin fibers while the CCM indicated no abnormalities. Several conclusions may be drawn from the data presented in Figure 1: (i) Sensory testing based on CDT, WDT and MDT suggests abnormalities of small fibers in most tested patients; (ii) IENF abnormalities overlap with CCM abnormalities in just 50% of patients with IENF (7 of 16); (iii) neither IENF nor CCM quantification may correspond with clinical (or preclinical) small fiber abnormalities; (iv) the SFNL showed signs of small fiber neuropathy in 10/26 patients with CCM abnormalities and 6/32 patients without CCM abnormalities; (v) central sensitization is observed in about half of the population. I am aware that this is a first approach to profile the rather complex sarcoidosis patient with chronic pain. Various other factors have not been accounted for such as sex, age, duration of disease, medication, progression of the underlying disease and genotype. Further studies in a much larger population are required to allow a more complete profile of this patient population.

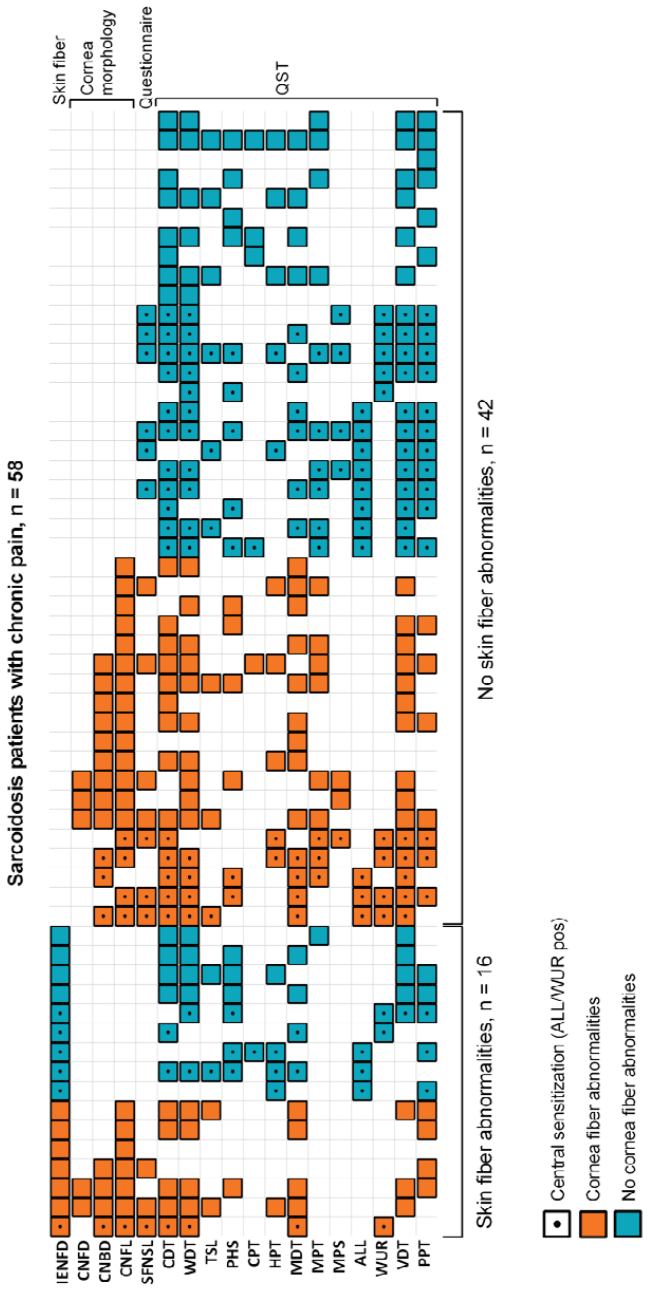

Figure 1. Fire plot of 58 sarcoidosis patients with chronic pain. Each column represents 1 patient. On the y-axis the following biomarkers are presented: IENFD = intra-epidermal nerve fiber density; CNFD cornea nerve fiber density; CNBD = cornea nerve branching density; CNFL=cornea nerve fiber length; SFNSL = small fiber neuropathy screening list; CDT= cold detection threshold; WDT= warm detection threshold; TSL= thermal sensory limen; PHS= paradoxical heat sensation; CPT= cold pain threshold; HPT= heat pain threshold; MDT= mechanical detection threshold; MPT= mechanical pain threshold; MPS= mechanical pain sensitivity; ALL= dynamic mechanical allodynia; WUR= wind-up ratio; VDT= vibration detection threshold; PPT= pressure pain threshold. Colored squares indicate abnormalities in the tests: for cornea testing values outside the 95% interval of normal reference data are considered abnormal; for quantitative sensory tests (QST) values less than –2 (loss of function) or greater than 2 (gain of function) x the SD of normal reference data are considered abnormal, for the questionnaires values > 37 (indicative of neuropathic pain) are colored. In blue lines the CCM data and the data indicative of central sensitization (ALL and WUR).

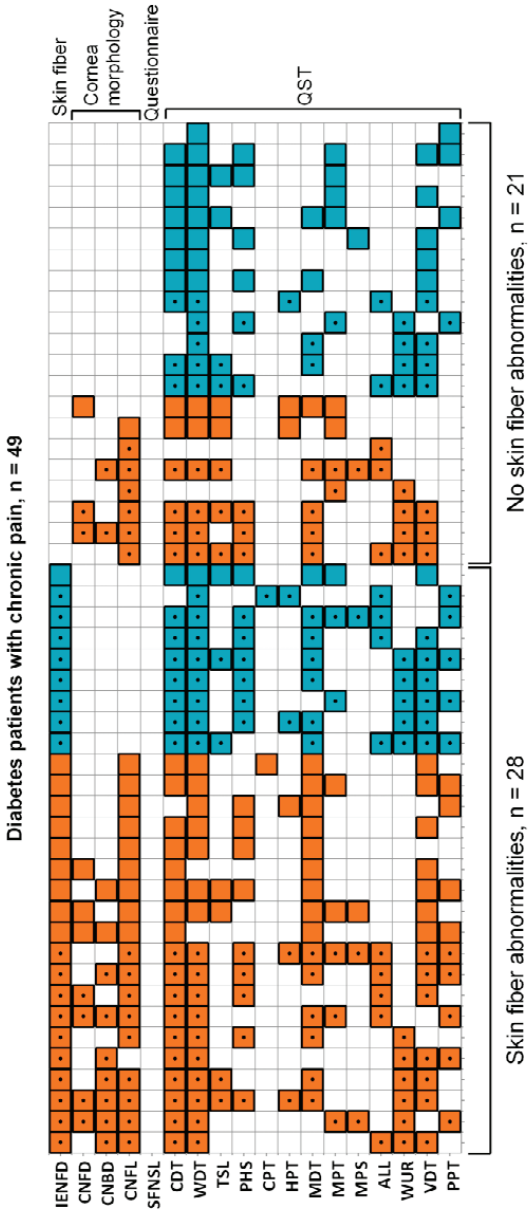

Figure 2. Fire plot of 49 diabetes mellitus type 2 patients with chronic pain. See legend of Figure 1 for explanation.

It is of interest to compare the sarcoidosis data set to small-fiber neuropathies of other origins. In Figure 2, I present a summarizing box plot of data obtained from 49 patients with diabetes mellitus type 2 and chronic neuropathic pain that were tested in our laboratory at LUMC. The plot is comparable to the data obtained in sarcoidosis patients. Again CDT, WDT and MDT abnormalities were present in the complete population with just 1 exception. Twenty-nine of 49 patients had signs of central sensitization. Again the data are well described by 4 phenotypes (absence/presence of CCM abnormalities with and without central sensitization) in close agreement to the sarcoidosis data. A similar observation was made in patients with fibromyalgia (data not shown).

These data collectively indicate that chronic pain is a complex entity with similar phenotypes determined from a small subset of biomarkers, regardless of the underlying disease (and consequently the initial cause of the chronic pain). The 4 phenotypes that I present here may indicate that the progression of disease or involvement of specific systems differs between patients. In cases with central sensitization prolonged afferent input to central sites may have caused upregulation of excitatory receptors at the spinal levels and/or may have caused a profound spinal inflammatory response. In cases without ALL and WUR the disease appears to be restricted to the periphery, without (as yet) any central involvement. Perhaps more importantly, these 4 phenotypes may require different treatment approaches. For example patients with just signs of central sensitization may need a centrally acting drug such as pregabalin; patients with just signs of small fiber abnormalities may benefit from a drug that causes tissue repair such as ARA290; patients with combinations of the two may require a combination therapy targeting peripheral and central pathology. An interesting group is the population without abnormalities of peripheral nerve fibers (CCM) and absence of signs of central sensitization (most of them also have a normal IENF density, Fig. 1). Since most of these patients have an abnormal CDT, WDT or MDT the peripheral nerves respond abnormal to cold, warm or pressure stimulation. Possibly the morphology of the relevant nerves is still intact, which may be due to a less aggressive underlying disease progression, or the disease has just recently manifested. Irrespective, these results indicate the importance of sensory testing in diagnosing neuropathic pain from small-fiber pathology.

Treatment effect of ARA290

Virtually all pharmacotherapy for chronic pain causes 30 to 50% pain relief in just 30 to 50% of patients.[3] One way of improving medication efficacy is by phenotyping patients and treating patients on a mechanistic basis (see above) or by determining responder groups and linking specific biomarkers or covariates to these responder groups. Martini et al.[1] identified 5 responder groups to treatment with topical capsaicin in patients with post-herpetic neuralgia; the responder groups ranged from non- to super-responders. Specific covariates such as age, baseline pain and duration of disease predicted the super-response to capsaicin treatment. The data presented in Chapters 5 and 6 point towards the same principle, a limited effect of treatment in a limited portion of the population. However, the ARA290 data are more complex than data from “regular” analgesic trials as ARA290 is not an analgesic in the sense that morphine is an analgesic

but it is rather a disease modifier. Morphine blocks nociceptive trafficking from the periphery to spinal and supra-spinal areas involved in pain processing. ARA290 through its actions at the innate repair receptor (Chapter 7) causes tissue healing and repair of affected small nerve fibers. Animal data further indicate profound anti-inflammatory effects at the level of the spinal cord.[4] ARA290's effects on pain are therefore more indirect and pain relief may not be the first disease symptom to be alleviated. The reduction of autonomic symptoms and increase in activity level that was observed in Chapters 5 and 6 point towards a more general (healing) effect of ARA290 on the diseased system. Moreover, pain relief from pharmacotherapy may be a rather insufficient and possibly even illogical end-point in chronic pain trials. Pain perception is a multifactorial process that is influenced by various often-interacting complex bio-psychosocial factors such as the pathophysiology of the underlying disease, activity level, energy level, inflammation, nociceptive damage, presence of a neuropathic component, spinal inflammation, central sensitization, ability to activate descending inhibition, presence of psychiatric symptoms (depression and/or anxiety), physical and mental resilience, previous painful diseases/symptoms, medication, sex, socioeconomic and marital status, etc. While some drugs may affect one factor (*e.g.* nociceptive afferent input to the brain) many of the other factors remain undertreated. Hence, a composite end-point in trials of chronic pain is needed.

Future perspectives

The results from the presented trials indicate that ARA290 is beneficial to some extent but much more work is needed before we can definitively conclude that ARA290 is effective in alleviating sarcoidosis-induced neuropathic pain.

Future ARA290 trials should focus on:

(1) More prolonged exposure to ARA290. Current trials that have been performed on ARA290 (in sarcoidosis and diabetes mellitus) were relatively short in duration. The studies presented in this thesis (Chapters 5, 6 and 7) and one additional trial in diabetes mellitus patients (Ref. 5) were performed in patients treated for 28 days. One later trial was performed in sarcoidosis patients treated for 3 months (unpublished observation). It is difficult to imagine that such a short treatment period is beneficial with respect to the many factors involved in pain perception in a complex disease such as sarcoidosis. Assuming that ARA290 affects the disease process I suggest a much longer treatment duration, such as 6-12 months and only then re-assess the pain as well as other related factors (activity as assessed by the 6-min walk, depression, autonomic function, etc.).

(2) Dose finding studies. Just one small multicenter dose-finding study has been performed so far suggestive that once daily ARA290 4 mg sc. is the optimal dose (unpublished observation). However, taken the short half-life of ARA290 in the body (Chapter 4), possibly multiple exposures during the day are even more beneficial. ARA290 is given by subcutaneous injections very similar to insulin injections. Studies on

repeated injections with multiple doses in large populations are required focusing on efficacy and toxicity. The current data shows little toxicity but the data set is small and the exposure doses and durations limited.

(3) Treatments based on body weight. ARA290 dosing is currently based on a fixed dose (2-4 mg) irrespective of the patient's body weight. This approach is based on the fact that ARA290 is rapidly transported to its main target, the innate repair receptor, and that it initiates a cascade of events that do not require prolonged and intense stimulation of the receptor. In fact, ARA290 likely loses its necessity once the cascade is activated (Chapter 7). Still I predict that fixed doses of ARA290 may be less efficacious in morbidly obese patients. Hence dosing based on total body weight seems a logical step in improving the efficacy of ARA290.

(4) Multicenter studies in large patient populations. The number of patients so far dosed with ARA290 is limited (< 100 patients). Hence, a true indication of ARA290's efficacy is lacking. Large multicenter trials are needed to determine a true treatment effect. However, as suggested above, first the study's end-points have to be defined. It may be clear that the end-point pain perception will require large patient numbers and that, irrespective of study size, the effect-sizes will be relatively limited. This is especially true taken the relatively large placebo effect that is currently observed in pain trials.[6] Also in the ARA290 trials the effect of placebo treatment was substantial. A composite end-point, combining pain symptoms with improvement in physical function, autonomic function and mental state may be the more optimal end-point.

Additional issues that require future discussion include:

(5) Are there alternatives for ARA290 with possibly an improved efficacy? ARA290 mimics the effect of erythropoietin (EPO) and as such is an agent that counteracts the effects of tumor necrosis factor α (TNF-α; Chapter 7). The literature indicates that administration of both EPO and TNF-α to chronic patients is an exception (see Chapters 2 and 7) due to the side-effects these drugs produce. Still both drugs are useful for other indications and it may well be worthwhile to compare ARA290 with either drug to parallel efficacy and toxicity.

(6) Is it preferable to combine ARA290 with an analgesic agent? In modern pain medicine patients are treated according to a multimodal approach. Pharmacotherapy is an often-important component of such an approach. Combining analgesic drugs with different mechanisms of action will cause enhanced efficacy with a reduced side effect profile. Since the overall treatment effect of ARA290 seems limited, combining ARA290 with an analgesic may result in a better outcome for the patient. The question then remains, which analgesic is the best choice? Given the prolonged treatment duration, the use of opioids seems less of an option due to its known negative or harmful effects such as the possibility of overdose, abuse and addiction, and tolerance development with reduced efficacy over time. The choice of co-medication should depend on the patient's phenotype. Two drug classes may be considered, GABAergic medication

(gabapentin or pregabalin) in case of central sensitization and drugs that (re-)activate descending inhibition (*eg.* drugs that increase serotonergic or noradrenergic neurotransmission in the spinal cord such as duloxetine, tapentadol and other serotonin-noradrenaline reuptake inhibitors). The phenotyping approach that was discussed above, however, does not assess descending inhibition. Additional tests for descending inhibition should therefore be added to the test battery. One such test is Conditioned Pain Modulation (CPM), which provides a surrogate marker for the presence or absence of descending inhibition. In CPM testing two painful stimuli are applied to the skin at remote areas. An active CPM response is when pain inhibits pain, *i.e.* the first painful stimulus decreases in intensity due to application of the second stimulus.[2] An alternative test is offset analgesia (OA; see also Chapter 7). Both CPM and OA tests are relatively simple to execute and will give the clinician an indication of the vitality of the endogenous pain modulatory system. In case of CPM or OA abnormalities, duloxetine or tapentadol may be added to the patient's pharmacotherapy. In cases in which CPM and OA responses are normal and also no central sensitization is detected, ARA290 may be combined with opioids for no longer than three months. The opioids will subdue the initial pain while ARA290 initiates a healing process that in the end will effectively reduce the neuropathic pain component on multiple levels. The confirmation of this hypothesis, however, requires future studies.

Conclusions

- SFN is one of the more disabling and often chronic manifestations of sarcoidosis.
- Using specific diagnostic tests such as quantitative sensory testing, skin biopsy, and cornea confocal microscopy allows determination of specific sensory phenotypes of sarcoidosis patients with SFN.
- Cornea confocal microscopy examines the densely innervated cornea as a surrogate for the small nerve fiber state, and can serve as a quantitative and qualitative measure of small fiber pathology in a reproducible, non-invasive manner.
- ARA290 is a small (11 amino acid) peptide engineered to activate the innate repair receptor that antagonizes inflammatory processes and stimulates tissue repair.
- Despite that fact that patients with sarcoidosis and SFN have less pain during treatment with ARA290, this peptide cannot be considered a classical analgesic. ARA290 is rather a disease-modifying molecule.
- ARA290 is a potential disease-modifying agent for treatment of sarcoidosis-

associated SFN. Whether it modulates the underlying disease process is currently unknown and merits further study.

- Using multiple biomarkers of chronic pain leads to identification of specific phenotypes that may require different treatments and as such may form the basis of individualized pain medicine.

- Pain perception is a multifactorial process that is influenced by various often-interacting factors. A composite end-point, combining pain symptoms with improvement in physical function, autonomic function and mental state may be the more optimal end-point in clinical trials.

- ARA290 may be used in combination with true analgesics to enhance treatment efficacy. The choice of co-medication depends on the patient's phenotype.

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