

Factors that Impact Symptomatic Diabetic Peripheral Neuropathy in Placebo-treated Patients from Two 1-year Clinical Trials

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## **ABSTRACT**

**Objective:** To evaluate the change in neuropathy symptoms and disease progression in placebo-treated patients from two 1-year studies that tested the impact of ruboxistaurin (RBX) in mild diabetic peripheral neuropathy (DPN).

**Research Design and Methods:** Data from 262 placebo-treated patients from two identical phase 3, randomized, double-blind trials were combined and analyzed.

**Results:** After 1 year, change in Neuropathy Impairment Score of Lower Limbs (NIS[LL]; -0.63 points;  $p = 0.005$ ), vibration detection threshold (VDT, -0.42 JND Units;  $p = 0.003$ ) and Neuropathy Total Symptom Score-6 (NTSS-6, -3.73 points;  $p < 0.001$ ) improved while some electrophysiology measures and Heart Rate Deep Breathing (HRDB - 0.78 beats;  $p = 0.003$ ) worsened when compared with baseline values. There was a small but significant worsening of A1C (0.28%;  $p < 0.001$ ) and a greater percentage of patients were using analgesics at the end of the trials (33.6%;  $p = 0.003$ ). At 1 year, the change in NTSS-6 directly correlated with changes in NIS(LL) and VDT, and inversely correlated with the peroneal nerve conduction velocity. On logistic regression analyses, a  $\geq 50\%$  reduction in NTSS-6 score was less likely in patients that used anti-hypertensive or chronic symptom medication at baseline.

**Conclusions:** In placebo-treated patients with mild symptomatic DPN: 1) there was a progressive improvement in symptoms over 12 months whilst nerve conduction studies and HRDB declined; 2) clinically significant worsening of DPN would require greater than 1 year of observation.

In the Rochester Diabetic Neuropathy Study cohort, the prevalence of diabetic peripheral neuropathy (DPN) was 54% in patients with type 1 diabetes mellitus and 45% in patients with type 2 diabetes mellitus (1). The two main clinical consequences of DPN, painful neuropathy and foot ulceration (sometimes leading to amputation), are associated with much patient morbidity and mortality (2). It is well established that lack of glycemic control and a longer duration of diabetes are major risk factors for the development of DPN (3,4). In addition, a major European prospective study has recently shown that potentially modifiable, traditional markers of macrovascular disease such as hypertension, hyperlipidemia and smoking are also independent risk factors for DPN (5).

The most consistent early abnormality in DPN is an abnormality in nerve electrophysiology. Clinical signs resulting from nerve dysfunction may include loss of light touch and pressure sensation, a decrease in vibration detection threshold (VDT), decreased motor strength, and areflexia. Symptoms may or may not develop with the onset of functional abnormalities or mild clinical impairments and are therefore not essential for the diagnosis of DPN. However, it is well recognized that pain is the most distressing symptom of DPN and the main factor that prompts the patient to seek medical advice (6). There are few studies that have examined the prevalence and progression of painful DPN and they report a prevalence rate ranging from 7-26% (7-8). The variation in prevalence reporting reflects the heterogeneity of the population studied, the criteria used

to define symptomatic neuropathy, and the changes in the standard of care or alternatively the degree of DPN symptoms or use of concomitant therapy in that patient population.

Virtually all clinical trials involving pain relieving drugs have been short term and solely evaluated changes in the symptoms of DPN without carefully assessing neuropathy parameters. Thus, information regarding symptomatic improvement in relation to underlying disease state progression over an extended period of time is lacking. We present data from the placebo-treated patients from two large, randomized, double-blind, identical, 12-month clinical trials in order to investigate which factors may impact not only the disease-state progression but also the change in symptoms in patients with mild but clinically symptomatic DPN.

## RESEARCH DESIGN AND METHODS

Two identical, phase 3, parallel, randomized, double-blind, placebo-controlled trials (MBBP and MBCW) were performed at 64 centers (see appendix for complete list of investigators), investigating the effects of 32 mg/day of the protein kinase C  $\beta$  inhibitor, ruboxistaurin (RBX) mesylate compared to placebo in patients with diabetes mellitus and symptomatic DPN. The studies were conducted according to the principles expressed in the Declaration of Helsinki.

Patients studied were 18 years or older with type 1 or type 2 diabetes mellitus who had clinically diagnosed sensory symptoms due to distal symmetrical polyneuropathy. Patients needed to have mild DPN, which included a VDT  $\geq$  95<sup>th</sup> percentile, a sural sensory nerve action potential (SNAP)  $\geq$  1  $\mu$ V, and a baseline

neuropathy total symptom score-6 (NTSS-6) > 6 points. Patients who had a VDT > 23 just noticeable difference (JND) units, a hemoglobin A1c (A1C) value of > 12.0%, or neuropathy due to diseases other than diabetes mellitus, were excluded. Assessments included the NTSS-6, VDT, Neuropathy Impairment Score of the Lower Limbs (NIS[LL]), heart rate variation during deep breathing (HRDB), and electrophysiology measured by nerve conduction studies.

### **Measurements**

To evaluate symptoms of DPN, the NTSS-6 was utilized to measure frequency and intensity of neuropathic sensory symptoms (numbness and/or insensitivity, prickling sensation, aching pain, burning pain, lancinating pain, allodynia and/or hyperalgesia) (9-10). Surface stimulation and recordings of nerve conduction were obtained from the sural, peroneal and tibial nerves of the lower extremity. Conduction velocities were calculated from these measurements using standard methods (11). In addition, the study limb was tested for vibratory perceptions over a 30-minute period. A noninvasive detector was placed at predetermined skin locations and '4-2-1 stepping' algorithm was followed and the reading center conducted quality control assessments prior to data capture (12).

### **Concomitant Medication Use**

All concomitant medication use was recorded on the case report form. Analgesics were permitted and medications taken for DPN symptoms were separately noted. Chronic symptom medications were defined as medications that are typically prescribed for the treatment of DPN symptoms on

an ongoing basis (>1 month). These drugs include anticonvulsants and antidepressants; some examples include gabapentin, topiramate, amitriptyline, duloxetine, and nortriptyline. Patients who required medication to relieve DPN symptoms were prescribed analgesic medications according to the following algorithm: Week 1: aspirin, acetaminophen, paracetamol, or aspirin-like compounds; Weeks 2 through 4 (if needed and indicated): nonsteroidal anti-inflammatory medication; Weeks 5 through 8 (if needed and indicated): Class 4 controlled substances such as propoxyphene or propoxyphene combined with another analgesic such as aspirin or acetaminophen; Week 9 and beyond (if needed and indicated): codeine or codeine combined with another analgesic such as aspirin or acetaminophen. If Class 2 controlled substances were required (with the exception of codeine), then the patient was discontinued from the study medication.

Patients were required to have stable glucose control prior to entering the study. Patients with an A1C between 9% and 12% at screening were required to lower their A1C before entering the study by use of insulin or other measures (diet and exercise with or without oral antihyperglycemic agents). Patients with an A1C > 12% were excluded from the study. The patient's antihyperglycemia therapy could have been altered at any time during the trial in accordance with good clinical practice and the local standards of diabetes care.

### **Analyses and Statistical Methods**

Analyses were conducted using the intent-to-treat population, which includes all randomized patients. For patients missing post-baseline measurements, the last observation carried forward (LOCF) approach was applied by imputing the last

non-missing post-baseline value. Pearson's correlation coefficient was used to evaluate the disease progression within the placebo-treated patients by correlating the change in sensory symptoms (as measured by the NTSS-6) with the change in NIS[LL], VDT, or electrophysiological measures. Change from baseline to endpoint in medication use at baseline as compared to post-baseline were also investigated.

Stepwise logistic regression was then conducted with the following patient characteristics included in the model: age, A1C, gender, origin (Caucasian vs non-Caucasian), diabetes type, alcohol use, tobacco use, body mass index, blood pressure assessments, insulin use, and baseline measures of neuropathy. In addition, the use of the following medications was included: statins, antihypertensives, ACE/ARB, and chronic symptom medications. In all stepwise logistic regression models, the probability level to enter the model was set to 0.3 and the probability to remain in the model was set to 0.1. The first stepwise logistic regression included the above factors; in addition protocol (MBBP vs MBCW) was forced into the model as a factor. The second analysis was conducted in the same manner, but also forced age and baseline A1C into the model, as these are known predictors of diabetic neuropathy disease state progression (3,4). The goal of these analyses was to assess the likelihood of a clinically significant symptom improvement while adjusting for all characteristics together.

## RESULTS

Of the 519 patients randomized at 64 centers, 262 received placebo and 211 of the placebo-treated patients completed the 1-year study (see

appendix for patient disposition diagram). Baseline characteristics of the placebo group patients are presented in Table 1. Significant symptom improvement within each treatment group was demonstrated as early as 1 month and this was observed throughout the course of one year ( $p < 0.001$  in the placebo and  $p < 0.001$  in the RBX groups). The combined data for the primary endpoint from these two clinical trials indicated that there was no significant difference between RBX- and placebo-treated groups for the NTSS-6 change at any point during the 1-year trials.

At baseline, placebo-treatment patients had a mean NTSS-6 total score of  $9.76 \pm 3.3$  points (mean  $\pm$  standard deviation); NIS [LL] score of  $6.95 \pm 5.0$  points; and VDT results of  $20.43 \pm 2.1$  JND units. The change from baseline to endpoint exhibited a statistically significant mean improvement for each of the following parameters (Table 2): the NTSS-6 total score ( $3.73 \pm 3.8$ ;  $p < 0.001$ ), the NIS [LL] ( $0.63 \pm 3.4$  points;  $p = 0.005$ ), and the VDT ( $0.42 \pm 2.1$  JND units;  $p = 0.003$ ). In contrast, the HRDB difference (inspiration – expiration at baseline =  $11.9 \pm 6.7$  beats/minute) had a statistically significant mean worsening ( $0.78 \pm 3.9$  beats/minute;  $p = 0.003$ ) from baseline to the end of the 1-year study evaluation (Table 2).

Most electrophysiology attributes numerically worsened over the 1-year study period. A statistically significant worsening was observed for peroneal motor nerve conduction velocity (NCV), tibial motor nerve F-wave latency, sural sensory nerve amplitude and sural sensory peak latency (Table 2).

Although the change was small, a significant mean increase in baseline to endpoint A1C was observed ( $0.28\% \pm 1.2$ ,

$p < 0.001$ ). The percentage of patients using insulin at baseline and at the end of the study was comparable (60.7% versus 62.2%,  $p = 0.720$ ), while the use of statins slightly increased from 26.3% at baseline to 31.7% at the end of the study ( $p = 0.178$ ). However, the use of analgesic medications did significantly increase in the placebo-treated patients from 21.8% at baseline to 33.6% by the end of the study ( $p = 0.003$ ). Regardless of analgesic medication use, whether never taken, taken at baseline or initiated during the trial, there was a similar degree of improvement in the mean change from baseline in the NTSS-6 score for placebo-treated patients.

A change in sensory symptoms as measured by the NTSS-6 significantly correlated with change in VDT ( $r = 0.169$ ,  $p = 0.010$ ); NIS (LL) ( $r = 0.166$ ,  $p = 0.010$ ); and peroneal NCV ( $r = -0.213$ ,  $p = 0.001$ ), although the correlations were mild. No consistent correlation was observed between change in symptoms and change in other electrophysiological measures in placebo-treated patients. In addition, no statistically significant correlation between change from baseline in each of the individual NTSS-6 symptoms and change from baseline in measures of neuropathy was observed.

As shown in Table 3, when the patient characteristics were assessed in a univariate fashion, a clinically significant ( $\geq 50\%$ ) reduction in NTSS-6 score was less likely in patients that used anti-hypertensive (65.2% vs 52.8%;  $p = 0.0464$ ) or chronic symptom medication (18.7% vs 8.5%;  $p = 0.0248$ ) at baseline. A similar trend was observed with the use of statins at baseline (30.3% vs 19.8%;  $p = 0.0591$ ). Patients that had clinically significant

improvement in neuropathy symptoms at 1 year had lower mean baseline score for NTSS-6, milder neuropathy (eg, lower VDT, lower NIS[LL], higher sural sensory amplitude, peroneal NCV, tibial F-wave latency), lower BMI, type 1 diabetes, lower systolic blood pressure and were younger. Additionally, there was a significant difference in the change in peroneal NCV between patients that had clinically significant improvement in neuropathy symptoms compared to those patients that did not (Table 3).

The results from the stepwise logistic regression analysis to assess the impact of patient characteristics on change in symptoms at 1 year are presented. Patients who used anti-hypertensive ( $p = 0.025$ ) and chronic symptom medications ( $p = 0.01$ ) at baseline and had a higher VDT ( $p = 0.013$ ) at baseline were less likely to improve in symptoms. When the stepwise logistic regression analysis was performed with A1C and age in the model (results not shown), patients who used anti-hypertensive medications and chronic symptom medications at baseline were less likely to have symptom improvement. In addition, those with a higher body mass index and, as anticipated since age and A1C were forced into the model, older patients and those with a higher A1C at baseline were less likely to improve.

## CONCLUSIONS

Accompanying the change in the standard of care, there has been a decrease in the incidence, prevalence and progression of diabetic microvascular complications (10,13-15). However, the impact of improved care on neuropathy symptoms is unclear (10,13-15). It has also been conventionally assumed that the placebo effect on pain relief would be short-lived, lasting only 3-6 months. This

has not been confirmed by long-term, randomized, controlled trials. Therefore longer, randomized controlled trials are clearly important as virtually all previous symptom-based trials have lasted less than 16 weeks and information is lacking on the continued efficacy of drugs currently in use for painful DPN. This analysis addresses the evolution of neuropathy symptoms in placebo-treated patients with mild DPN over 1 year. The findings of this study may be relevant for designing future longer-term studies. In addition, the natural history and progression of the symptoms of DPN in relation to the underlying neuropathy is poorly understood (13), an issue that is also addressed by this study.

In the patients that we studied, described as having mild DPN, we demonstrated variable progression of signs and symptoms. During the 1-year time course, there was statistically and clinically significant improvement in symptoms, signs (on neurological examination) and sensory testing of vibration, while HRDB, a marker of autonomic neuropathy and small nerve fiber function, actually worsened. It is commonly believed that autonomic and sensory neuropathies are progressive complications of diabetes. We observed worsening autonomic function, (HRDB), while sensory function (VDT), improved. This may be due to a differential effect on large fiber sensation versus small fiber function. Additionally, electrophysiology was uniformly and numerically worse after 1 year but peroneal NCV, tibial F-wave latency, sural peak latency and sural sensory amplitude were the only attributes to demonstrate a statistically significant worsening.

In contrast to positive results observed in the phase 2 trial investigating the effect of RBX in patients with DPN (16), the change in the NTSS-6 score was not statistically significant when comparing RBX and placebo groups in the two phase 3 studies (17). However, the change in symptoms from baseline to endpoint after this one year period was statistically significant in this patient population with mild DPN, regardless of treatment group. What could have impacted symptom improvement in this study? Possibilities including the psychological effects of frequent study required visits (18), a placebo effect (19), change in the diabetic and neuropathic disease states, glucose control, and use of symptom medication were considered. The improvement in symptoms as well as in the neurological examination score and VDT was unexpected since the patients were on a stable regimen (both diabetic medications and symptom medications) prior to the study and symptoms had been present for at least 6 months and as long as 5 years prior to enrollment. Although there was a slight increase in A1C at 1 year, this change could not be expected to greatly affect the clinical course. Thus, the most plausible explanation for the significant improvement in symptoms in the placebo-treated patients is likely to be the “placebo effect”. The “placebo effect” would include the psychological effect of taking the medication as well as more frequent interactions with a team of researchers interested in their well-being, thereby increasing the expectation of improvement. The “placebo-effect” is unlikely to be explained by the increase in analgesic medication use from baseline to endpoint as placebo-treated patients that never received medications for symptoms of DPN during the trial had also had a similar degree of

improvement in the mean change from baseline in the NTSS-6 score. It is interesting that not only was the improvement in symptoms significant and progressive, but it appeared to be increasing over the 1-year time period.

Alternatively, other factors may have influenced change or improvement of symptoms in the patient population. We chose a  $\geq 50\%$  improvement as a clinically meaningful change (20). We used logistic regression analysis which identified the patients with antihypertensive and chronic symptom medication use at baseline and worse VDT were less likely to have symptom improvement. Additionally, milder symptoms and milder disease state at baseline, as defined by a composite score of nerve function, VDT, peroneal NCV change from baseline or baseline sural sensory amplitude was identified as important. Finally, younger age, lower BMI and lower blood pressure were associated with symptom improvement.

Similar to our previous study, and distinct from the literature in this patient population, symptoms appeared to correlate positively with the neurological examination and vibration, and inversely with the worsening of electrophysiological measures, such as peroneal nerve conduction velocity. Although this may support previous assertions that symptoms are unreliable in assessing neuropathy disease state progression, it also brings into question the value of other measures of neuropathic change, such as the neurological examination.

The neurological examination, which may or may not include a quantitative evaluation of sensation, has long been considered the gold standard

by neurologists in making the diagnosis of DPN. The examination has subjective components which are not always amenable to describe the presence of neuropathy or disease state progression in a strictly quantifiable manner. Moreover, it is well known that muscle strength (a prominent part of the neurological examination) cannot be fully evaluated and quantified when pain is present. Thus, the use of quantitative evaluation for the neurological examination may not accurately predict the presence or degree of neuropathy when painful symptoms are present. The recent consensus report advocating the use of the clinical examination as an important endpoint in defining the presence of neuropathy for clinical research purposes may be questionable (21).

Finally, in contrast to symptoms and signs, electrophysiological parameters consistently demonstrated worsening during the course of a 1-year period. Peroneal nerve conduction velocity, tibial F-wave latency, sural peak latency and sural sensory amplitude were the only tests of nerve function to demonstrate a statistically significant worsening although the remaining attributes were numerically worse. These measures are relatively objective with less variability and would thus be suitable endpoints for disease state progression in clinical trials (22). Clearly, several years of follow up would be required for any clinically meaningful change even in electrophysiological measures.

This study has some limitations. These clinical trials were only 1 year studies, which may not have been long enough to observe changes in the disease state progression. Additionally, the trials screened approximately 8500 patients for evidence of mild DPN with loss of

vibration sensation. Enrollment into the study was limited to patients with mild DPN. Hence patients with severe symptoms and more advanced DPN were not enrolled into the trials. For this reason, only 1 of 7 patients identified as having any severity of DPN qualified for the study. Therefore, these patients may not be representative of all symptomatic DPN patients. Finally, the closer contact in a clinical trial setting may allow for a better patient care and glucose control resulting in a reduction of disease state progression.

In conclusion, in patients with mild symptomatic DPN followed closely in a clinical trial, there was a significant and progressive improvement in symptoms over a 12-month period attributable to the “placebo effect”. Intervention with RBX during this one year period did not significantly alter

symptom or disease state progression. The NIS(LL) and VDT improved from baseline to endpoint while the more objective measures, including most electrophysiology attributes and the autonomic nerve function (HRDB), worsened over the course of 1 year. Finally, clinically significant worsening of DPN in placebo-treated patients in a clinical study would require greater than 1 year of observation.

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**REFERENCES**

1. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, Wilson DM, O'Brien PC, Melton LJ: The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology* 43: 817-824, 1993
2. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM: Diabetic somatic neuropathies. *Diabetes Care* 27: 1458-1486, 2004
3. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329: 977-986, 1993
4. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352: 837-853, 1998
5. Tesfaye S, Chaturvedi N, Eaton SEM, Witte D, Ward JD, Fuller J: Vascular risk factors and diabetic neuropathy. *New Engl J Med* 352: 341-350, 2005
6. Quattrini C, Tesfaye S: Understanding the impact of painful diabetic neuropathy. *Diabetes Metab Res Rev* 19: S2-S8, 2003
7. Harris M, Eastman R, Cowie C: Symptoms of sensory neuropathy in adults with NIDDM in the U.S. population. *Diabetes Care* 16: 1446-1452, 1993
8. Davies M, Brophy S, Williams R, Taylor A: The prevalence, severity, and impact of painful diabetic peripheral neuropathy in Type 2 diabetes. *Diabetes Care* 29: 1518-1522, 2006
9. Bastyr EJ, Price KL, Bril V for the MBBQ Study Group: Development and validity testing of the neuropathy total symptom score-6: questionnaire for the study of sensory symptoms of diabetic peripheral neuropathy. *Clin Ther* 27(8):1278-1294, 2005
10. Dyck PJ: Detection, characterization, and staging of polyneuropathy: assessed in diabetics. *Muscle Nerve* 11(1):21-32, 1988
11. Olney RK: Clinical trials for polyneuropathy: the role of nerve conduction studies, quantitative sensory testing, and autonomic function testing. *J Clin Neurophysiol* 15(2):129-137, 1998
12. Dyck PJ, O'Brien PC, Kosanke JL, Gillen DA, Karnes JL: A 4, 2, and 1 stepping algorithm for quick and accurate estimation of cutaneous sensation threshold. *Neurology* 43(8):1508-1512, 1993
13. Muraleedharan V, Shanmugarajah PD, Dodd T, Caddick L, Hardisty C, Scott A, Tesfaye S. The diabetes NSF: are targets being met 5 years on? *Diabetic Medicine* 2006; 23 (suppl 2): P293
14. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ: The 30-year natural history of type 1 diabetes complications: The Pittsburgh Epidemiology of Diabetes Complications Study Experience. *Diabetes* 55: 1463-1469, 2006
15. Martin CL, Albers J, Herman WH, Cleary P, Waberski B, Greene DA, Stevens MJ, Feldman EL: Neuropathy Among the Diabetes Control and Complications Trial Cohort 8 Years After Trial Completion. *Diabetes Care* 29(2): 340-344, 2006
16. Vinik AI, Bril V, Kempler P, Litchy WJ, Tesfaye S, Price KL, Bastyr EJ for the MBBQ Study Group: Treatment of symptomatic diabetic peripheral neuropathy with the protein kinase C beta-inhibitor ruboxistaurin mesylate during a 1-year, randomized, placebo-controlled, double-blind clinical trial. *Clin Ther* 27: 1164-1180, 2005

17. Tandan R, Skljarevski V, Price KL, Kles KA, Bastyr EJ, for the Ruboxistaurin Treatment of DPN Study Group: Neuropathy progression in patients with symptomatic diabetic peripheral neuropathy: Experience from phase 3 ruboxistaurin clinical trials. *Neurology* 66(5), Suppl 2: A191, 2006
18. Low PA, Opfer-Gehrking TL, Dyck PJ, Litchy WJ, O'Brien PC: Double-blind, placebo-controlled study of the application of capsaicin cream in chronic distal painful polyneuropathy. *Pain* 62(2):163-168, 1995
19. Hrobjartsson A, Gotzsche PC: Is the placebo powerless? Update of a systematic review with 52 new randomized trials comparing placebo with no treatment. *J Intern Med* 256(2): 91-100, 2004
20. Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL: Defining the clinically important difference in pain outcome measures. *Pain* 88: 287-294, 2000
21. England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ: Distal symmetric polyneuropathy: A definition for clinical research: Report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology* 64(2): 199-207, 2005
22. Dyck PJ, O'Brien PC, Litchy WJ, Harper CM, Klein CJ, Dyck PJB: Monotonicity of nerve tests in diabetes: Subclinical nerve dysfunction precedes diagnosis of polyneuropathy. *Diabetes Care* 28(9): 2192-2200, 2005

## **APPENDIX**

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**Table 1. Patient Baseline Characteristics**

Characteristic	Placebo (n = 262)
Female, n (%)	147 (56.1)
Type 1 Diabetes, n (%)	68 (26.0)
Age, (years)*	48.1 ± 9.4
Caucasian, n (%)	207 (79.0)
Body Mass Index, (kg/m <sup>2</sup> )*	30.0 ± 6.5
Hemoglobin A <sub>1c</sub> , (%)*	7.6 ± 1.4
Used Insulin, n (%)	159 (60.7)
Duration of Diabetes, (yrs)*	11.4 ± 9.2
Duration of neuropathy, (yrs)*	2.7 ± 2.8
Statin Medication Use, n (%)	68 (26.0)
Chronic Symptom Medication Use, n (%)	38 (14.5)
Antihypertensive Medication Use	157 (59.9)
ACE Inhibitor or ARB Use, n (%)	131 (50.0)

\*Mean ± Standard deviation

Abbreviations: ACE=angiotensin-converting enzyme; ARB=angiotensin II receptor blocker

**Table 2. Baseline to Endpoint Change at 1 Year in Placebo-Treated Patients**

<b>Characteristic</b>	<b>Baseline</b>	<b>Baseline to Endpoint Improvement</b>	<b>P value*</b>
NTSS-6 Total Score (points)	9.76 ± 3.3	3.73 ± 3.8	<i>P</i> < 0.001
NIS (LL) (points)	6.95 ± 5.0	0.63 ± 3.4	<i>P</i> = 0.005
Quantitative Sensory Testing (JND units)	20.43 ± 2.1	0.42 ± 2.1	<i>P</i> = 0.003
<b>Characteristic</b>	<b>Baseline</b>	<b>Baseline to Endpoint Worsening</b>	<b>P value*</b>
Heart Rate Deep Breathing Difference – (beats/minute) (Inspiration – Expiration)	11.92 ± 6.7	0.78 ± 3.9	<i>P</i> = 0.003
Peroneal NCV (m/sec)	43.05 ± 4.9	0.38 ± 2.2	<i>P</i> = 0.012
Tibial F-wave Latency (msec)	54.93 ± 6.1	0.33 ± 2.4	<i>P</i> = 0.045
Sural Amplitude (μV)	9.10 ± 5.3	1.12 ± 3.7	<i>P</i> < 0.001
Sural Peak Latency (msec)	3.95 ± 0.49	0.058 ± 0.37	<i>P</i> = 0.021
Hemoglobin A <sub>1c</sub> (%)	7.58 ± 1.4	0.28 ± 1.2	<i>P</i> < 0.001

P values assess within placebo treatment baseline to endpoint change

Data Mean ± Standard Deviation

In order to assess disease progression within the placebo-treated patients, changes from baseline to endpoint were assessed using a t-test for the following neuropathy measures: NTSS-6 total score, NIS[LL] score, VDT, the HRDB (heart rate difference between inspiration and expiration), and attributes of electrophysiology (sural, peroneal and tibial nerves). Change from baseline to endpoint in A1C was also investigated.

Abbreviations: m/sec = meters per second; msec = milliseconds, NCV=nerve conduction velocity; NTSS-6=neuropathy total symptom score -6; NIS(LL)=neuropathy impairment score of the lower limbs; JND=just noticeable difference; μV=microvolts

**Table 3. Patient Characteristics that Impact Clinically Significant Improvement in Neuropathic Symptoms\***

Characteristic	Symptom Improvement $\geq 50\%$	No Symptom Improvement $< 50\%$	P value <sup>†</sup>
Baseline NTSS-6 Total Score (points)	9.17 $\pm$ 2.87	10.19 $\pm$ 3.58	0.0168
Baseline NIS(LL) (points)	6.45 $\pm$ 4.25	7.31 $\pm$ 5.41	0.1714
NIS(LL) Changes from Baseline (points)	-1.21 $\pm$ 3.37	-0.21 $\pm$ 3.41	0.0277
Baseline NIS(LL)+7 (points)	13.28 $\pm$ 5.99	15.26 $\pm$ 7.17	0.0219
NIS(LL)+7 Change from Baseline (points)	0.027 $\pm$ 7.7	2.51 $\pm$ 12.7	0.0969
Baseline Vibration Detection Threshold (VDT; JND Units)	20.00 $\pm$ 2.06	20.71 $\pm$ 2.07	0.0087
VDT Change from Baseline (JND Units)	-0.582 $\pm$ 2.39	-0.304 $\pm$ 1.87	0.3228
Baseline Peroneal NCV (m/sec)	43.34 $\pm$ 4.96	42.85 $\pm$ 4.90	0.4273
Peroneal NCV Change from Baseline (m/sec)	0.015 $\pm$ 2.32	-0.674 $\pm$ 2.15	0.0260
Baseline Tibial F-wave Latency (msec)	54.54 $\pm$ 6.19	55.20 $\pm$ 6.06	0.3939
Tibial F-wave Latency Change from Baseline (msec)	0.285 $\pm$ 2.66	0.362 $\pm$ 2.21	0.8165
Baseline Sural Amplitude ( $\mu$ V)	10.19 $\pm$ 5.44	8.34 $\pm$ 5.13	0.0076
Sural Amplitude Change from Baseline ( $\mu$ V)	-1.23 $\pm$ 3.55	-1.04 $\pm$ 3.76	0.6985
Age (years)	46.30 $\pm$ 9.15	49.28 $\pm$ 9.36	0.0128
Baseline BMI (mg/kg <sup>2</sup> )	29.07 $\pm$ 7.14	30.67 $\pm$ 5.95	0.0528
Baseline SBP (mmHg)	124.22 $\pm$ 14.21	128.26 $\pm$ 15.68	0.0361
Type 1 Diabetes	33 (31.1)	34 (21.9)	0.0962
Baseline Chronic Symptom Medication Use	9 (8.5)	29 (18.7)	0.0248
Baseline Antihypertensive Medication Use	56 (52.8)	101 (65.2)	0.0464
Baseline Statin Use	21 (19.8)	47 (30.3)	0.0591

\*A patient was considered to have a clinically significant symptom improvement if the patient had at least a 50% reduction from baseline in the NTSS-6 total symptom score.

<sup>†</sup> P-value calculated using a logistic regression analysis, with the categorical outcome (symptom improvement versus no symptom improvement) as the dependent variable and the characteristic (eg, age) as the independent variable.

The patient characteristics that may predict clinically significant symptom improvement were also investigated. For these analyses, a patient was considered to have a clinically significant symptom improvement if at least a 50% reduction in the NTSS-6 total symptom score was observed from baseline to end of study. Each patient characteristic was initially evaluated using a univariate logistic regression model, with clinically significant improvement status as the dependent variable and the patient characteristic as the independent variable.

Abbreviations: ACE=angiotensin-converting enzyme; ARB=angiotensin receptor blocker; BMI=body mass index; DBP=diastolic blood pressure; HbA1c=hemoglobin A1c; JND=just noticeable difference; m/sec=meters per second; msec = milliseconds; NCV= nerve conduction velocity; NIS(LL)=neuropathy impairment score (lower limbs); NTSS-6=Neuropathy total symptom score-6; SBP=systolic blood pressure;  $\mu$ V=microvolts