3,4-Diaminopyridine Base Effectively Treats the Weakness of Lambert-Eaton Myasthenia[1]

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Abstract word count: 149

Manuscript word count: 3,911 exclusive of references and tables

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Reprint requests: not available

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The remaining authors have no conflicts of interest.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ABSTRACT

Introduction: 3,4-diaminopyridine has been used to treat Lambert Eaton myasthenia (LEM) for thirty years despite the lack of conclusive evidence of efficacy.

Methods: We conducted a randomized double-blind placebo-controlled withdrawal study in LEM patients who had been on stable regimens of 3,4-diaminopyridine base (3,4-DAP) for ≥ 3 months. The primary efficacy endpoint was >30% deterioration in Triple Timed Up-and-Go (3TUG) times during tapered drug withdrawal. The secondary endpoint was self-assessment of LEM–related weakness (W-SAS).

Results: 32 participants were randomized to continuous 3,4-DAP or placebo. None of the 14 receiving continuous 3,4-DAP had >30% deterioration in 3TUG time vs 72% of the 18 who tapered to placebo (p<0.0001). W-SAS similarly demonstrated an advantage for continuous treatment over placebo (p<0.0001). Need for rescue and adverse events were more common in the placebo group.

Discussion: This trial provides significant evidence of efficacy of 3,4-DAP in the maintenance of strength in LEM.

INTRODUCTION

LEM is a rare autoimmune disorder often associated with a malignancy, usually small cell lung cancer.[1]   Epidemiologic studies suggest that there may now be approximately 800 patients with LEM in the USA and up to 170 new cases annually.[2,3]

The symptoms that characterize LEM result from reduced release of acetylcholine (ACh) from the presynaptic terminals of the neuromuscular junctions.[1]   Autoantibodies that target presynaptic voltage-gated calcium channels (VGCCs) impair entry of calcium into nerve terminals, thereby decreasing ACh release.[4] By blocking presynaptic potassium channels, 3,4-diaminopyridine, also known as amifampridine, prolongs depolarization from impulses arriving at the nerve ending, allowing VGCCs to remain open longer, thus increasing entry of calcium. Since the quantal release of ACh depends on the intracellular concentration of calcium, 3,4-diaminopyridine increases the release of ACh.

The first use of 3,4-diaminopyridine formulated as the free base (3,4-DAP) in the treatment of LEM was described in 1983[5] and subsequent uncontrolled studies reported benefits in small numbers of patients.[6] In a double-blind, placebo-controlled crossover study of 3,4-DAP in 12 LEM patients in 1989,[7] oral doses up to 100 mg/day were effective in treating both the motor and autonomic deficits, and the amplitude of compound muscle action potentials (CMAPs) nearly doubled. A randomized, placebo-controlled study of 26 LEM patients in 2000 demonstrated that those who received oral 3,4-DAP had a greater improvement in Quantitative MG (QMG) score and in the summated amplitude of CMAPs in 3 sentinel muscles,[8] although the magnitude of change in QMG score in this study was not clinically significant. A randomized, placebo-controlled crossover study demonstrated efficacy of intravenous 3,4-DAP using

dynamometry and CMAP in 9 LEM patients.[9] None of these studies was used to file for

regulatory approval in the USA or in Europe. Other clinical studies failed to provide definitive

objective evidence of efficacy of 3,4-diaminopyridine.[10, 11]

In 1990 the US Food and Drug Administration granted orphan designation to 3,4-

diaminopyridine, and compassionate use of the free base formulation, (3,4-DAP), became

available through physician-held INDs for named patients in the USA and through special

governmental approvals in other countries.

To provide definitive evidence of efficacy, we designed the 3,4-DAP Product Efficacy

Research (DAPPER) trial, a prospective, placebo-controlled withdrawal study of 3,4-DAP in

LEM.

METHODS

Outcome Measures:

Because physiologic endpoints of dynamometry and CMAP do not capture function, and QMG performance in LEM had proven questionable in previous clinical trials, we used the 3TUG walking test, a modification of the validated Timed Up and Go test,[12] as the quantitative measure of proximal muscle function.[13] Based on minimal detectable change data reported in Parkinson disease, the primary efficacy outcome was defined as a >30% deterioration in the 3TUG time.[14,12]

Study Participants:

Given the paucity of eligible subjects, we targeted LEM patients being actively treated with 3,4-DAP, for whom responsiveness could be objectively documented before randomization.[15] To be eligible, participants had to indicate a need to wait briefly after the first morning dose of 3,4-DAP for improvement in LEM-related dysfunction, and they also needed to display quantifiable functional benefit after the first morning dose.[15] This approach avoided entering participants whose LEM may have improved or remitted over time.[16]

Fifty-two LEM patients, all participants in the Sponsor's 3,4-DAP compassionate use program, were screened for eligibility. Fifty of them were receiving treatment under 21 different physician-held INDs in the USA. One participant each from Canada and Argentina had special access through appropriate governmental agencies and local neuromuscular specialists. The diagnosis of LEM was confirmed by independent neurologist (DBS) review of clinical records, including VGCC antibody and electromyography test results. The dose of 3,4-DAP was at least 10 mg 3 times a day and up to a total daily dose of 100 mg, and permitted participants to walk,

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with or without an assistive device. All LEM-related treatments were stable for at least 3

months, and other concomitant medications for at least 1 month before study entry.

Patients were excluded if they had previous respiratory failure while on 3,4-DAP or an

insufficient 3TUG response to 3,4-DAP during the baseline observation period. (See eTables 1 &

2 for detailed inclusion and exclusion criteria.)

Study Oversight:

Written informed consent was obtained from all study participants. The study was conducted in

accordance with International Conference on Harmonization guidelines and principles of the

Declaration of Helsinki. The protocol was approved by the institutional review board at each

study site and registered with clinicaltrials.gov (NCT01511978).

Criteria for rescue requiring reinstitution of baseline dosing were: new dysphagia, a drop

in pulse oximetry of 5% from baseline, or a decrease in oxygen saturation to < 90% with

accompanying shortness of breath. Inability to get out of bed or inability to arise from a chair,

even with assistance, after 2 efforts about 1 hour apart, also merited reinstitution of baseline 3,4-

DAP.

Continuous safety monitoring was built into the study design, precluding the need for a

formal safety monitoring committee.

The definition for eligibility was concealed during the study. Individual 3TUG results

were shared with the Sponsor, who determined if the eligibility criterion had been met. The

initial eligibility criterion for randomization required ≥ 30% improvement in 3TUG after the first

3,4-DAP dose on 2 consecutive mornings. This criterion threshold was lowered to ≥27% after

approximately 20% of participants had been randomized due to the constrained number of

potential participants and concern that participants who were responsive to 3,4-DAP were being excluded. The eligibility criterion was further modified after approximately 30 to 40% of participants had been randomized to accommodate a potential stacking effect of 3,4-DAP (i.e., the additive effect of multiple doses of 3,4-DAP throughout the day) and allowing consideration of 3TUGs performed in the afternoon as well as in the morning to determine if subsequent participants displayed a sufficiently large response to 3,4-DAP during the baseline observation period. A later modification also permitted consideration of the evening post-dose 3TUG. The four thresholds used for each participant are detailed in eTables 3 & 4. Each modification made it easier for subjects to qualify for participation and less likely that they would weaken enough during the drug taper to reach the study endpoint.

Vital signs including pulse oximetry were measured at least 6 times daily in conjunction with 3TUG testing, with continuous pulse oximetry monitoring overnight. Post-dose W-SAS was obtained three times daily as was an inventory of LEM-related signs or symptoms. Electrocardiograms (ECGs) and CMAPs were obtained before and after the first doses of the morning and afternoon throughout the study. A Lower Extremity Function Scale (LEFS) score was obtained at baseline and at the end of the study.[17] Blood for plasma drug and metabolite levels was drawn at regular intervals surrounding doses during the baseline observation period and after randomization.

Participants were classified into one of four baseline treatment categories according to their baseline LEM treatment regimen: 3,4-DAP plus pyridostigmine, 3,4-DAP alone, 3,4-DAP plus pyridostigmine plus immunomodulators/immunosuppressants, and 3,4-DAP plus immunomodulators/ immunosuppressants. To assure that the two study arms were balanced for

possible disease severity, participants within each category were randomized centrally in a 1:1 ratio.

Study Design (Figure 1)

A screening visit was scheduled up to 4 weeks before admission to the inpatient clinical research unit. After determination of initial eligibility, participants were hospitalized and observed for 2.5 days on their usual dose and schedule of 3,4-DAP. Responsiveness to 3,4-DAP, the final determinant of eligibility, was assessed by 3TUG testing before and after the first doses of the morning, afternoon, and evening, regardless of a participant's total number of daily doses. The 2 hour post-dose period was selected as the estimated time for peak pharmacodynamic effect.[9] Because LEM patients usually do not take 3,4-DAP during the night and the half-life following oral doses is approximately 3.5 to 4 hours, patients effectively experience a withdrawal from drug every night and frequently have mobility issues before their first dose of the morning. This suggested that morning would be the ideal time to detect drug responsiveness.

Subjects with a sufficient 3TUG response were randomly assigned to the taper to placebo group or the continuous 3,4-DAP group on the afternoon of the second day.

Tapered withdrawal began with the last dose of the second full hospital day, which was decreased to 90% of the participant's usual dose. Each consecutive dose was then decreased so that it was 50% of the usual dose by the end of the third full day, 20% at the end of the fourth day and 0% for the third dose of the fifth full day (eFigure 1). Thereafter, there was an additional up to 16 hours with no 3,4-DAP before the participant's usual dose was restored. Participants were then observed for 0.5 days or until deemed clinically stable, and then discharged. Standardized weekly follow-ups continued for one month after discharge.

Enrollment was planned to cease when approximately 15 participants were enrolled in each of the 2 study groups.

Treatments:

The Sponsor prepared tablets of 0.5, 2, 3, 4, 5, and 10 mg 3,4-DAP and placebo, all identical in appearance. Combinations of 4 tablets were pre-packaged into a series of blisters to permit a smooth taper (eFigure 2) based on each participant's individualized pre-randomization dosing regimen.

Efficacy Endpoints:

Primary Endpoint: The primary efficacy measure was the percent change in the last completed post-dose 3TUG during the treatment period compared with the average of the 2 time-matched 3TUGs obtained during the baseline. The 3TUG involves 3 repetitions without rest (laps), of rising from an 18" straight-backed armchair, walking to a line 10 feet from the chair, and returning to sit in the chair.[15] Participants are instructed to walk at their normal pace and only prompted with the word "Go" for the first lap and "Go again" after they come to a full stop in the chair for the subsequent laps. Each lap is timed and the score is the average of the 3 lap times. In addition to timing the 3TUGs "live" using a traceable stop watch, the tests were videotaped and the lap times were later measured in triplicate by a remote assessor blinded to the treatment and to the date, time, and sequence of the 3TUGs. The blinded assessor's times were used to determine the primary endpoint. The calculation of percent change in 3TUG for the primary endpoint utilized the last completed post-dose 3TUG during the withdrawal period:

Percent change = 100 x [1 - (final post-dose 3TUG / time-matched baseline 3TUGs)].

Rescue was an anticipated event and rescued participants were considered to have completed the trial.

Secondary Endpoint: The secondary efficacy measure was the W-SAS, a 7 level categorical scale developed by the Sponsor to demonstrate participant-perceived change in overall strength from study entry; responses ranged from "Much Much Weaker (-3)" to "Much Much Stronger (+3)". The W-SAS was obtained 3 times daily corresponding to the post-dose 3TUGs. The last completed post-dose W-SAS during the randomized treatment period was the endpoint.

Additional Efficacy Endpoints: Change in CMAP was measured in the nerve-muscle pair determined to be most responsive to 3,4-DAP during acclimation (ulnar nerve - abductor digiti minimi, median nerve — abductor pollicis brevis, or peroneal nerve – extensor digitorum brevis). A reviewer blinded to the treatment and to the date, time, and sequence of the CMAPs used pre-determined criteria to assess CMAP test quality. Tests approved by this reviewer were used to determine change in CMAP amplitude at the end of the blinded treatment period compared with baseline.

The LEFS at the end of the blinded treatment period was compared with baseline. This questionnaire consists of 20 items, each scored from 0 (extreme difficulty) to 4 (no difficulty); a change of 9 points has been determined to represent a clinically-meaningful functional change in subjects with a lower extremity musculoskeletal condition.[17]

Need for rescue was considered both as an efficacy and a safety endpoint. Blinded physician assessment of treatment effect was performed once at the end of the study.

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Safety Endpoints: Safety assessments included need for rescue during blinded treatment and/or the emergence of LEM-related signs and symptoms or pre-dosage LEM-associated weakness, 3TUG, W-SAS, and treatment-emergent adverse events. Changes in ECG parameters, vital signs and pulse oximetry were also examined.

Plasma Drug Levels: Pharmacokinetic analyses of plasma drug and metabolite levels were used to confirm the taper.

Statistical Analysis

Setting α at 0.05 and the power at 80%, 10 participants were required in each study group. To allow for departures in the assumptions, 30 participants were planned, 15 for each treatment arm. Thirty-two participants were actually enrolled. No interim analysis was performed. Statistical analyses followed the intention-to-treat principle. Fisher's exact test was used to compare primary efficacy outcomes between treatment groups (i.e., >30% prolongation in 3TUG times) and t-test was used to compare W-SAS assessments. ECGs were analyzed for changes in cardiac intervals and a concentration response analysis exploring changes in QTcF and plasma drug concentrations was performed. Statistical analysis of the other outcome measures was not performed.

RESULTS

Subjects: Fifty-two subjects were screened at 7 study sites and agreed to participate

(Figure 2). Eighteen were ineligible due to insufficient improvement in 3TUG (eTable 4).

Two subjects were excluded due to positive toxicology testing. Thirty-two participants were

eligible and were randomized, 14 to continuous 3,4-DAP and 18 to taper to placebo. There

were no important differences between the two treatment groups in demographics (Table 1),

LEM-related history or LEM treatments (eTables 5 & 6). All randomized participants

completed the study.

Efficacy:

Primary Outcome Measure: >30% deterioration in final 3TUG time during tapered

withdrawal of study drug.

The 3TUG primary efficacy endpoint demonstrated a highly significant difference

between treatment groups in favor of continuous 3,4-DAP (Table 2, Figure 3). There was very

strong agreement and a high correlation between the blinded 3TUGs and the on-site 3TUGs

(correlation coefficient=0.9192, eFigure 2).

A significantly greater proportion of subjects tapered to placebo had >30% deterioration

in the final post-dose 3TUG test compared to subjects in the continuous 3,4-DAP group. Results

were consistent for efficacy, intent-to-treat, and per protocol populations.

Secondary Outcome Measure: W-SAS.

The W-SAS secondary efficacy endpoint (Table 2) also demonstrated a highly significant

difference in favor of the continuous 3,4-DAP group, with most participants who continued

3,4-DAP being "about the same" and most of those who tapered to placebo being "Much

Weaker" or "Much Much Weaker" at the last W-SAS assessment . Results were consistent for the efficacy, intent-to treat, and per-protocol populations.

Additional Outcome Measures

The final post-dose CMAP measurements approved by the blinded reviewer favored the continuous 3,4-DAP group, that had a median CMAP of 3.4 mV (all nerve-muscle pairs) and a median percentage change of -9.5 %, while the taper to placebo group, for whom the median final CMAP was 2.3 mV, had a median percent change from baseline of -42.1%.

The continuous 3,4-DAP group had a median change in LEFS score of -1.5 points compared with -27 points for the taper to placebo group. In a repeat LEFS assessment one week post-discharge in the taper to placebo group, the median score increased from 10 to 42, returning to baseline.

Two participants in the continuous treatment group (14.3%) and 5 in the taper to placebo group (44.4%) were rescued for new dysphagia during the withdrawal phase, without reaching the primary study endpoint (eTable 3). In addition, one participant in the tapered group was rescued due to a 5% drop in oxygen saturation and another required rescue due to inability to rise from a chair. Two of the seven rescued participants in the tapered group did not meet the primary endpoint prior to rescue (eTable 3a). Another participant in the tapered group had baseline medication reinstituted upon request due to anxiety and a sense of impending doom, preceded by intermittent nocturnal hypoxemia.

Blinded physician assessment of treatment effect at the end of the study favored the continuous 3,4-DAP group, with physicians indicating that 12 of 14 (86%) subjects had no change, while 1 each was somewhat worse or much worse than during baseline. In contrast, 14

of the 18 (78%) participants in the taper to placebo group were considered much worse, 3 (17%) were somewhat worse, and only one had no change from baseline.

Recovery of 3TUG After Reinstitution of 3,4-DAP:

The morning post-dose 3TUG returned to baseline after reinstitution of baseline 3,4-DAP doses in all participants who tapered to placebo and in those who were rescued or advanced early (Figure 3).

Recovery of W-SAS After Reinstitution of 3,4-DAP:

Participants in the taper to placebo group reported return to their baseline strength after the first dose of their baseline 3,4-DAP dosage. A similar recovery in the W-SAS assessment was observed in rescued participants.

Unblinding:

There were no identified instances of unblinding during the study. At the end of the study, participants and investigators were independently asked to which treatment arm they believed they had been randomized. Participants and physicians agreed in all cases; both guessed treatment assignment incorrectly in 3 cases.

Pharmacokinetic Results:

Pharmacokinetic sampling was assayed post hoc and confirmed the randomization. First morning pre-dose plasma drug levels confirmed that most participants, regardless of their treatment arm, had effectively withdrawn from drug overnight.

Safety:

Treatment-Emergent Adverse Events (TEAEs): TEAEs were reported with a higher frequency in the taper to placebo group, with 23 AEs in 12 of the 18 participants (67%) vs 9

in 5 of 14 participants (36%) in the continuous 3,4-DAP group. The most common non-LEM-related signs and symptoms TEAEs were abdominal discomfort and respiratory tract infection (2 in the taper to placebo group). One in each treatment group had back pain, headache, nasopharyngitis, or oropharyngeal pain. One serious AE of pneumonia occurred in a participant in the taper to placebo group more than 3 weeks after completing the inpatient trial. There were no deaths in this study.

Re-emergence of LEM-related signs and symptoms

The most common LEM-related signs and symptoms to emerge during drug withdrawal were decreased oxygen saturation in 3 participants in the taper to placebo group, muscle spasms and nausea each in 2 participants in the taper to placebo group, and arthralgia, in 1 participant in each group. One participant in the taper to placebo group had a severe episode of anxiety with a sense of impending doom.

Based on the morning pre-dose 3TUGs, participants who were not rescued were no weaker at the end of the 3.5-day taper than they were every morning on their usual steady regimen of 3,4-DAP.

Clinical Laboratory Tests:

With the exception of minor blood glucose increases, there were no clinically meaningful laboratory value changes. End of Study measurements of blood glucose were increased compared to Screening in both treatment groups, mean blood glucose being increased by

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21.3 and 15.9 mg/dL in the taper to placebo group and continuous 3,4-DAP group, respectively.

Vital Signs:

Small increases in average pulse rate of 4 to 6 bpm were observed at some time points post-dose in both treatment groups.

Low oxygen saturation occurred in 5 participants in the taper to placebo group and was reported as a LEM-related sign/symptom AE in 3 participants who had pre-existing pulmonary disease and/or sleep apnea and who received supplemental oxygen. Low oxygen saturation resolved spontaneously or with reinstitution of 3,4-DAP treatment. Oxygen saturation did not worsen significantly in any participant who received continuous 3,4-DAP.

ECGs:

None of the participants had clinically significant abnormal ECG findings. All ECG parameters (RR, QRS, PR, QT and QTcF) as well as the interpretative statements by the investigators showed little or no differences between baseline and study days or between the treatment groups.

An increased heart rate up to 5 bpm was observed in the taper to placebo group. Short-term withdrawal of 3,4-DAP had no apparent effect on standard ECG parameters. Linear regression analysis indicated that there was no relationship between changes in QTcF interval and the respective plasma drug concentrations, regardless of treatment group.

Tolerability:

3,4-DAP in doses from 30 to 100 mg daily was well-tolerated by participants in the continuous 3,4-DAP group.

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Discussion:

This trial provides highly significant evidence of efficacy and demonstrates that 3,4-DAP is essential for the maintenance of strength in patients with LEM. Lowering 3,4-DAP resulted in significant deterioration of strength when doses reached approximately 50% of the usual individual dosage (Figure 3). Rescue was required in 44% of participants who tapered 3,4-DAP and resulted in prompt resolution of weakness.

DAPPER contained 2 embedded efficacy studies in addition to the overall randomized withdrawal trial: (1) a series of N of 1 trials of each participant's daily responses to 3,4-DAP and (2) the responses to reinstitution of 3,4-DAP at the end of the study. The recovery of strength and function with reinstitution of 3,4-DAP supports the lack of deconditioning, rebound weakness or sustained negative effects from short-term withdrawal of 3,4-DAP.

Key to the potential success of the withdrawal design was the identification of 3,4-DAP-responsive LEM patients, which ensured an enriched population for the study.

While the TUG has been validated and used to study a variety of conditions, the use of 3 repetitions of the TUG for the DAPPER study was developed to accommodate the neuromuscular fatigue or facilitation that may affect LEM patients to different degrees. Initial pilot testing indicated the ability of the 3TUG to detect drug effect 1 to 2 hours post-dose. A subsequent study demonstrated the reliability of the 3TUG in controls, in non-LEM patients with neuromuscular disease and in LEM patients.[13] Pharmacokinetic/pharmacodynamic analysis of plasma levels of 3,4-DAP from the DAPPER study demonstrated a concentration-response

relationship with 3TUG times, supporting the use of the 3TUG as a measure of disease-related weakness in patients with LEM.[18]

The 3TUG data used in the analysis of the DAPPER study were timed from videos made on site. In order to eliminate a potential source of bias in outcome assessment, the video reader was remote from any of the study sites and blinded to the sequence of the tests and treatment assignments There was very strong agreement and a high correlation between the blinded 3TUGs and the on-site 3TUGs.

The study can be criticized for changing the eligibility criterion after the study had started. However, the study endpoint was not changed and decreasing the required level of responsiveness for eligibility to <30% should have made it less likely that participants would have >30% deterioration when tapered to placebo. To avoid potential biasing of the 3TUG results used to determine eligibility, site personnel were blinded to the eligibility threshold. The Sponsor determined eligibility from data reported by the sites using pre-set criteria that were not subject to bias.

Although only one study participant had paraneoplastic LEM (eTable 3a, Screen No. 50.0), previous reports have demonstrated clinical responsiveness to 3,4-DAP in paraneoplastic LEM and that there is no difference in responsiveness in paraneoplastic and non-paraneoplastic LEM.[7,19]

That 2 participants in the continuous 3,4-DAP treatment group were rescued for dysphagia is evidence for the effectiveness of the blind. Both participants and study personnel may have been overly attuned to swallowing issues, knowing that participants would be rescued under the protocol to avoid aspiration. The onset of dysphagia in these 2 participants

demonstrates that even with usual treatment, LEM patients may experience unexpected weakness that can be addressed by an extra dose of 3,4-DAP.

The highly significant findings for both the primary and secondary outcome measures are strengthened by the rapid recovery of function upon resumption of the participants' usual regimens and the finding that the majority of subjects in the continuous 3,4-DAP group had daily demonstrable benefit with >30% improvement in 3TUG times compared to the morning pre-dose 3TUG.

Conclusion

3,4-Diaminopyridine base (3,4-DAP) is safe and effective treatment for LEM-related weakness.

Table 1. Demographics and Baseline Characteristics

	Taper to Placebo	Continuous 3,4-DAP base
N	18	14
Age, years, Mean (SD),	59.3 (14.99)	50.7 (15.97)
Range	28 - 78	23 - 83
Gender: Male, N (%)	7 (38.9%)	4 (30.8%)
Female, N (%)	11 (61.1%)	10 (71.4%)
Race: White, N (%)	18 (100%)	11 (78.6%)
Black, N (%)	0 (0.0%)	3 (21.4%)
Ethnicity: Hispanic or Latino, N (%)	1 (5.6%)	0 (0.0%)
Not Hispanic or Latino, N (%)	17 (94.4%)	14 (100%)
BMI, kg/m^2, Mean (SD)	27.7 (5.14)	27.3 (5.92)
Range	18.9 - 35.4	20.3 - 39.0
Positive P/Q VGCC-Ab at screening, N (%)	17 (94.4%)	12 (85.7%)
CMAP facilitation >100% at Screening, N (%)	10 (55.6%)	7 (50.0%)
Paraneoplastic LEM, N (%)	1 (5.6%)	0 (0.0%)
Duration of LEM prior to randomization (years)	6.7 (6.08)	6.7 (5.70)
Mean (SD) / Range	0.3 - 22.3	1.1 - 19.8
Duration of 3,4-DAP treatment at entry (years)	5.5 (4.92)	6.2 (5.30)
Mean (SD) / Range	0.3 - 18.3	0.7 - 18.9
Total Daily Dose of 3,4-DAP at entry (mg)	74.7 (22.26)	76.4 (19.46)
Mean (SD) / Range	30 - 100	35 - 100
LEM treatment before and during study		
3,4-DAP + PB, N (%)	11 (61.1%)	9 (64.3%)
3,4-DAP, N (%)	0 (0.0%)	1 (7.1%)
3,4-DAP + PB + IM, N (%)	4 (22.2%)	2 (14.2%)
3,4-DAP + IM, N (%)	3 (16.7%)	2 (14.2%)

VGCC Ab, voltage-gated calcium channel antibodies

PB, pyridostigmine bromide; IM, immunomodulators/immunosuppressants

Table 2. Primary and Secondary Efficacy Endpoints: Change in the final 3TUG Times and W-SAS upon withdrawal of study drug

Study Group		Taper to Placebo N=18	Continuous 3,4-DAP base N=14
3TUG Change	No Change or Faster	5 (27.8%)	14 (100%)
N (%)	>30% Slower	13 (72.2%)	0 (0%)
		P<0.0001*	
Final W-SAS	Much Much Weaker (-3)	10 (55.6%)	1 (7.1%)
N (%)	Much Weaker (-2)	6 (33.3%)	1 (7.1%)
	Somewhat Weaker (-1)	1 (5.6%)	1 (7.1%)
	About the Same (0)	1 (5.6%)	9 (64.3%)
	Somewhat Stronger (+1)	0 (0.0%)	1 (7.1%)
	Much Stronger (+2)	0 (0.0%)	1 (7.1%)
	Much Much Stronger (+3)	0 (0.0%)	0 (0.0%)
		P<0.0001†	

*, P value based on Fisher's Exact Test

†, P value based on CMH test for categorical data

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Abbreviations

3,4-DAP	3,4-diaminopyridine base
3TUG	triple timed up and go test
Ab	Antibodies
ACh	Acetylcholine
AE	adverse event
ANCOVA	analysis of covariance
CMAP	compound muscle action potential
CMH	Cochrane-Mantel-Haenszel statistic
CONSORT	Consolidated Standards of Reporting Trials
DAPPER	Diaminopyridine (DAP) Prospective Efficacy Research
ECG	electrocardiogram
IM	immunomodulators/immunosuppressants
LEFS	Lower extremity function scale
LEM	Lambert-Eaton Myasthenia
PB	pyridostigmine bromide
QMG	Quantitative Myasthenia Gravis score
QTcF	electrocardiographic QT interval with Fridericia's correction
TDD	total daily dose
TEAE	treatment-emergent adverse event
VGCC	voltage-gated calcium channels
W-SAS	LEM-related weakness self-assessment scale

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Figures

Figure 1. Study design schematic. See text for details.

Figure 2. CONSORT diagram

Figure 3. Percent change from baseline in 3TUG at 2 hours after dosing vs time, by treatment

group.

M: Morning; A: Afternoon; E: Evening.

* P-value <0.05; ** P-value <0.01; *** P-value <0.001. P-value is based on the one-way
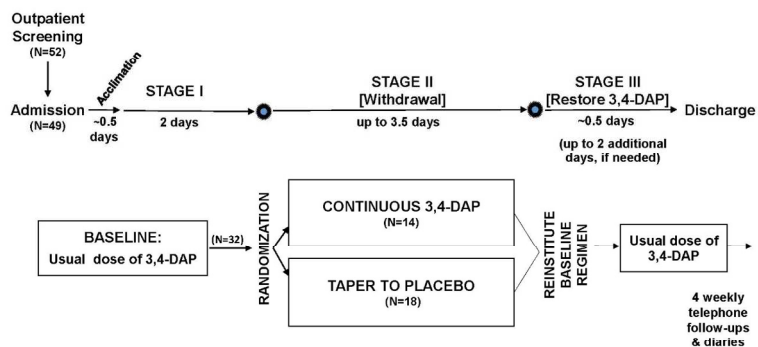ANCOVA model, with the baseline 3TUGs as the covariate.

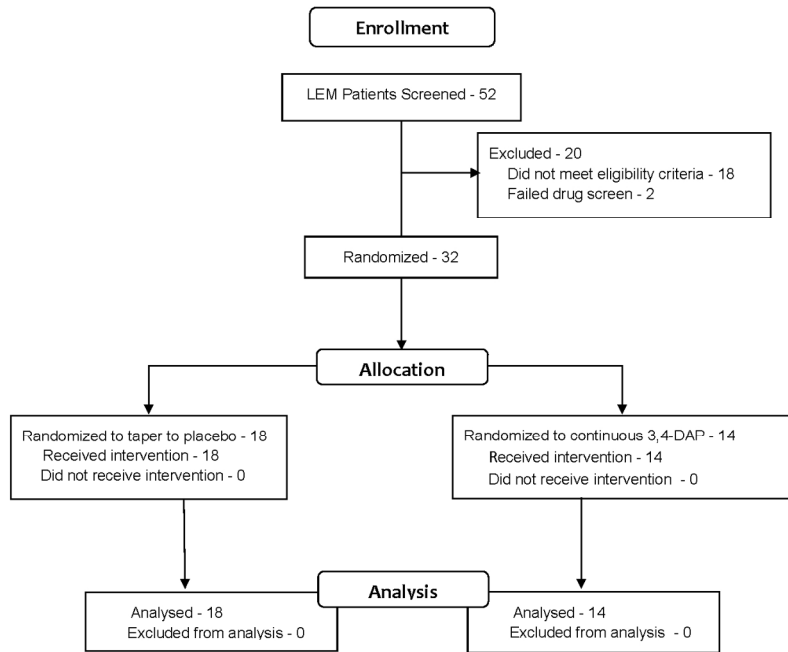Figure 1. Study design schematic. See text for details.

198x111mm (220 x 220 DPI)
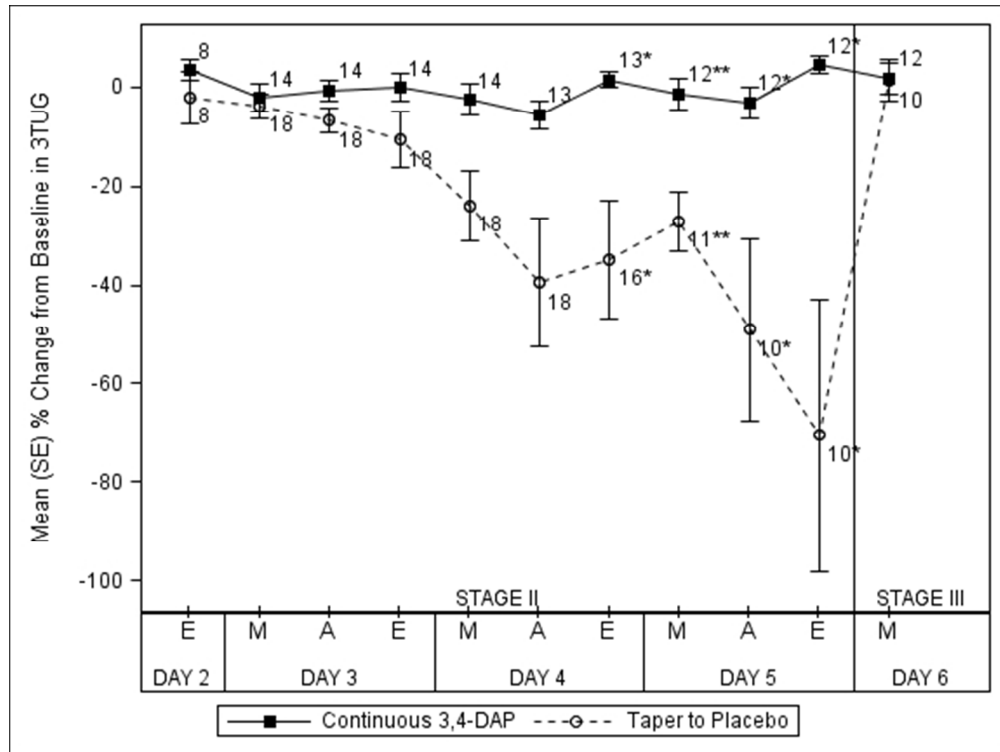
Figure 2. CONSORT diagram

215x279mm (200 x 200 DPI)

Figure 3. Percent change from baseline in 3TUG at 2 hours after dosing vs time, by treatment group.
M: Morning; A: Afternoon; E: Evening.
* P-value <0.05; ** P-value <0.01; *** P-value <0.001. P-value is based on the one-way ANCOVA model,
with the baseline 3TUGs as the covariate.

152x114mm (96 x 96 DPI)