Amifampridine phosphate (Firdapse®) is Effective and Safe in A Phase 3 Clinical Trial in LEMS

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Running title: Amifampridine phosphate in LEMS
Abstract

Objective: We evaluated the efficacy and safety of amifampridine phosphate (Firdapse®) for symptomatic treatment in Lambert-Eaton myasthenic syndrome (LEMS).

Methods: Phase 3, randomized, double-blind, study. Patients were treated initially with amifampridine phosphate for 7–91 days, followed by randomization to continue amifampridine phosphate for 14 days or placebo (7-day taper, 7-day placebo). The primary efficacy endpoints were changes from baseline at day 14 in Quantitative Myasthenia Gravis and Subject Global Impression scores.

Results: The coprimary efficacy end points and 1 of the secondary efficacy end points were met, showing a significant benefit of amifampridine phosphate over placebo at Day 14. All 5 primary, secondary, and tertiary endpoints achieved statistical significance at Day 8. Amifampridine phosphate was well tolerated; the most common adverse events were oral and digital paresthesias, nausea, and headache.

Conclusions: This study provides Class I evidence of efficacy of amifampridine phosphate as a symptomatic treatment for LEMS.

Key Words
1. Amifampridine phosphate; 2. 3,4-diaminopyridine; 3. 3,4-DAP; 4. Lambert-Eaton myasthenic syndrome; 5. LEMS; 6. Potassium channel blockers; 7. Small cell lung cancer (SCLC)
Introduction

Lambert-Eaton myasthenic syndrome (LEMS) is a rare, frequently disabling autoimmune neuromuscular disorder that produces serious muscle weakness and symptoms of autonomic dysfunction. Sanders estimated the prevalence of LEMS in the United States (US) to be 1/100,000 on the basis of prevalence of small cell lung cancer.

In LEMS, voltage-gated calcium channel (VGCC) auto-antibodies block influx of calcium ions (Ca\(^{2+}\)) into the nerve terminal, inducing insufficient release of acetylcholine (ACh) that produces muscle weakness. About 50% of patients with LEMS, particularly male smokers, present with an underlying malignancy, usually small cell lung cancer.

For symptomatic treatment, pyridostigmine, guanidine, 4-aminopyridine, and 3,4-diaminopyridine (3,4-DAP) have been tried. Among these, 3,4-DAP (amifampridine) in its base form has been found to be most effective and safe.

3,4-DAP base has not received Food and Drug Administration (FDA) approval for the symptomatic management of LEMS in the United States (US), and at present has limited availability through Treatment Investigational New Drugs (IND) studies, expanded-access programs, clinical trials, and compounding pharmacies. The potential exists for batch-to-batch variability and issues of lack of reliability in drug quality, potency, and risk of under and overdose due to compounding errors. A review and analysis of compounded 3,4-DAP products concluded that they are subject to substantial variability (22.2% - 125.2%) in active drug substance content and are noncompliant with the good manufacturing practice (GMP) standard of 95% to 105% range limit of declared label content. To overcome the limitations currently associated
with the use of 3,4-DAP base, an effort was made to develop a formulation that meets FDA regulatory requirements. Amifampridine phosphate, the salt form of 3,4-DAP, has been shown to have superior stability compared with the base, and an oral formulation containing amifampridine phosphate, equivalent to 10 mg base, has been developed under current GMPs.

Amifampridine phosphate has been recommended as a first-line symptomatic treatment for LEMS by the European Federation of Neurologic Societies and was approved for use in this indication by the European Medicines Agency in December 2009. Amifampridine phosphate has received orphan drug designation by the FDA and has also been granted Breakthrough Therapy designation. The multicenter, randomized, double-blind, placebo-controlled withdrawal study we report was designed to evaluate the efficacy and long-term safety of the phosphate salt form of amifampridine in patients with LEMS.

Materials and Methods

This multicenter, randomized, phase 3 “withdrawal trial” was conducted at 18 sites in the US, European Union (EU), and Russian Federation. This study was approved by the local ethic committees or institutional review boards for compliance with the international and US regulations and good practice guidelines for pharmaceuticals for human use. All patients gave written informed consent. This study was registered as NCT01377922 at www.ClinicalTrials.gov. This study was designed and sponsored by Biomarin Pharmaceutical Inc and Catalyst Pharmaceuticals Inc.

Patients
Patients enrolled in the study were at least age 18 years and had a confirmed LEMS diagnosis, either previously treated with 3,4-DAP base (treatment experienced) or naïve (treatment naïve) with documented acquired (typical) proximal muscle weakness and at least 1 of the following: compound muscle action potential that increased ≥ 2-fold after maximum voluntary contraction of the tested muscle or a positive anti-P/Q-type VGCC antibody test. For treatment-naïve patients, the Quantitative Myasthenia Gravis (QMG) score was required to be ≥5. A forced vital capacity (FVC) of ≥80% of predicted and ≥60% of predicted was required among patients who were and were not receiving 3,4-DAP base at screening, respectively. Patients were required to have normal swallowing function, as defined by the ability to swallow 4 ounces of water without coughing or throat clearing, and to have completed anti-cancer treatment ≥3 months prior to the screening visit. If the patient was receiving a peripherally acting cholinesterase inhibitor, a stable dose was required for >7 days prior to screening. If the patient had received permitted oral immunosuppressants, a stable dose was required for >90 days prior to screening. Patients with known seizure(s), brain metastasis, or serious electrocardiogram (ECG) abnormalities were excluded.

Study Design

The study was conducted in 4 parts (Figure 1). Subjects who successfully met all inclusion criteria at screening were entered into the open-label run-in (Part 1). During Part 1, amifampridine phosphate was titrated for each individual patient to a dose that produced optimal neuromuscular benefit and tolerability in the opinion of the investigator. Treatment-naïve patients were required to have an improvement of at least
3 points in the QMG score from the initial evaluation. An improvement in the QMG score was not required at the time of study entry for treatment experienced patients. Those who continued to the double-blind treatment discontinuation part were required to have received ≥91 consecutive days of treatment (base or phosphate), including at least 7 consecutive days of open-label amifampridine phosphate, and to be on a stable background therapy regimen.

In Part 2 (double-blind treatment discontinuation), patients were randomly allocated to either continuation of treatment of amifampridine phosphate at the dose established during the open-label run-in, or to downward titration of the dose to zero and discontinuation of treatment. This was accomplished by substituting an increasing proportion of matching placebo tablets for amifampridine phosphate tablets starting on Day 2 and ending on Day 7, at which point all tablets were placebo.

Blood samples were collected for pharmacokinetic analysis on Day 1 of Part 2 prior to dosing.

Patients in Part 3 (double-blind treatment), who were randomized to amifampridine phosphate in part 2 continued to receive the same dose regimen for 7 additional days. Patients for whom the dose was titrated downward to placebo in Part 2 remained on placebo for 7 days. Efficacy and safety assessments were performed on Days 8 and 14. If required, rescue treatment was permitted during the treatment discontinuation and double-blind parts of the study.

In Part 4 (open-label, safety assessment), patients were offered the option to participate in a long-term safety assessment for up to 2 years (not reported here).
**Drug Regimen, Placebo, and treatment**

Active treatment was administered as tablets of amifampridine phosphate (Firdapse®, Catalyst Pharmaceuticals) equivalent to 10 mg of amifampridine (3,4-DAP) base. During Part 1 (open-label), patients received a total daily dose of amifampridine phosphate of 15 to 80 mg/day, given in 3 to 4 divided doses, with a maximum single dose of 20 mg. Treatment experienced patients were converted to amifampridine phosphate at either an equivalent or lower dose of 3,4-DAP base. Amifampridine phosphate was initiated in treatment naïve patients and escalated in 5- to 10-mg increments every 4 or 5 days to a maximum of 80 mg/day, based on optimal benefit. Patients had to receive at least 30 mg/day to enter part 2 of the study. Patients were required to be on stable background therapy during the study. Placebo tablets were indistinguishable from amifampridine phosphate tablets and were administered consistent with the dose and dose regimen of amifampridine phosphate.

**Efficacy Assessments**

Efficacy endpoints were identified based upon discussions with the US FDA. The coprimary efficacy endpoints were changes from baseline to Day 14 (the end of Part 3) in QMG score and in Subject Global Impression (SGI) score. Baseline was defined as the measurements obtained at the beginning (Day 1) of Part 2 Double-blind Treatment. Efficacy assessments were also performed at Day 8 in Part 2.

The QMG is a physician-rated evaluation consisting of 13 assessments mainly designed for clinical trials in patients with myasthenia gravis. Each assessment is rated 0 to 3, where 0 indicates “no weakness” and 3 indicates “severe weakness.” The 13 individual assessment scores were totaled to obtain a QMG score that provided a
quantitative assessment of muscle strength. For QMG assessment, lower scores reflected better muscle strength. \(^{12,14}\)

The SGI is a 7-point scale on which patients rate their global impression of the effects of a study treatment (1 = terrible to 7 = delighted) on their LEMS symptoms. For SGI assessment, higher ratings reflected a higher level of patient satisfaction. \(^{13}\)

Secondary endpoints included the changes from baseline in Clinical Global Impression of Improvement (CGI-I) and Timed 25-foot Walk test (T25FW) speed at day 14. \(^{15,16}\)

The CGI-I is a 7-point scale that captures the investigator’s global impression of improvement or worsening (1 = very much improved; 7 = very much worse) baseline status. An investigator scored the CGI-I scale based on changes in symptoms, behavior, and functional abilities. \(^{15}\) On this assessment, lower ratings reflected a higher level of improvement as perceived by the investigator. The T25FW is a quantitative mobility and leg function performance test based on a timed 25-foot walk. Following a rest period of at least 5 minutes, the T25FW was repeated. \(^{16}\) The measurement for the T25FW was the average speed, expressed in feet/minute, of the 2 completed walks.

The compound muscle action potential (CMAP), a tertiary endpoint, was obtained in the abductor digiti minimi muscle at rest with a single maximal stimulation of the ulnar nerve at the wrist. \(^{10}\) Because amifampridine phosphate acts rapidly (\(T_{\text{max}} = 0.5\) hour) and has a short half-life (\(T_{1/2} = 2.5 \pm 0.73\) hours), efficacy assessments were required to be performed 45 minutes after taking a dose of medication. \(^{17}\)

**Safety Assessments**

Safety assessments included monitoring for adverse events (AEs) using Medical Dictionary for Regulatory Activities (MeDRA) codes, clinical laboratory assessments,
vital-signs assessments, physical examinations, and ECGs. Pregnancy testing was also required for women of childbearing potential.

Statistical Analyses

A sample size of at least 36 patients was required to detect a 2.44-unit difference in the mean change of the QMG scores between the 2 treatment groups, assuming a type I error of 0.05 and a common standard deviation of 2.5. $^{18,19,20,21}$

Two prespecified populations were analyzed: 1) the full analysis set (intent to treat) consisted of all randomized patients who received at least 1 dose of amifampridine phosphate or placebo in Part 2 and who had at least 1 post-baseline efficacy assessment, and 2) the safety population included all enrolled patients who received at least 1 dose of study drug and had any post-treatment safety information collected. $^{18}$ Efficacy endpoints were summarized using descriptive statistics at each measurement time point. Treatment differences were assessed at a significance level of 0.05 for all endpoints. A mixed-effect model repeated measures (MMRM) model was used to analyze each of the coprimary endpoints. For each coprimary endpoint, the model included treatment, time (Day 8, Day 14), treatment-by-time interaction, and double-blind baseline scores as fixed effects and patient as a random effect. The P-values corresponding to the treatment comparisons were determined using a permutation test. P-values determined by permutation remove all assumptions of normality of distribution. Both the results for the QMG and SGI were required to be statistically significant in order to deem the study positive. A ‘per-protocol’ analysis was also conducted and included patients in whom assessments were conducted in accordance to the protocol. All patients who received at least 1 dose of amifampridine
phosphate and had any post-treatment safety information collected were included in the safety analysis. A final safety analysis will be performed after completion of the Part 4 open-label extension and will be reported separately.

**Results**

*Patients Demographics.*

Seventy-four patients were screened for eligibility, and 54 entered Part 1 of the study (Figure 2); 20 patients did not meet all eligibility criteria. The safety population included all 53 patients who received ≥1 dose of amifampridine phosphate and who had any post-treatment safety information collected (1 patient was excluded from the safety population, because no post-baseline data were collected). Among 54 patients in Part 1, 14 patients did not complete the Part 1 open-label study for various reasons, and 2 patients were advanced to Part 4 of the study.

Thirty-eight patients (placebo, n = 22; amifampridine phosphate, n = 16) completed the 3-month, open-label run-in period (Part 1) and were enrolled in the subsequent 2-week, double-blind, randomization period. Thus, the full analysis set included all 38 patients who received ≥1 dose of amifampridine phosphate or placebo in Part 2 and who had ≥1 post-baseline efficacy assessment.

At baseline, the majority of patients (73.7%) were treatment-naïve, and there were more women (66%) enrolled than men (Table 1). Prior median exposure to treatment was 630 days in the placebo group and 365 days in the amifampridine phosphate group. There were no remarkable differences between groups in other parameters. During Part 2 of the study, 1 placebo patient was rescued because of an increase in the QMG score >5 and was advanced to Part 4. Thus, 37 patients...
completed Part 2 of the study. During Part 3 of the study, 1 placebo patient was rescued because of increase in the QMG score >5 and was advanced to Part 4. Thus, 36 patients completed Part 3 of the study.

Forty patients have entered the 2-year open-label safety extension part (38 patients from the randomized part and 2 from the run-in part). The result of Part 4 will be reported separately.

Primary Efficacy Endpoints at Day 14 in Part 3.

The coprimary efficacy end points of the change in the QMG and SGI scores were met and showed a significant benefit of amifampridine phosphate over placebo at Day 14 in the full analysis. (Table 2) The coprimary efficacy end points for this study were the change in QMG score and change in SGI score from baseline to Day 14. Over this time frame, the mean increase in QMG score was 0.3 among patients randomized to amifampridine phosphate and 2.2 among those randomized to placebo [mean difference, -1.7 in favor of amifampridine phosphate; 95% confidence interval (CI), -3.4, -0.0]. This difference was statistically significant (P=0.0452).

Among the 13 domains in the QMG, the arm domain showed the most change (95% CI, -1.8,-0.6; P < 0.0001). Considering that the arms and legs domains of the QMG represent a measure of proximal muscle weakness characteristic of LEMS, an analysis of the arms plus legs domains was performed. At Day 14 the results were statistically significant in favor of amifampridine phosphate (P=0.0195).

A 'responder analysis' evaluation of whether patients experienced clinically significant disease progression (defined as an increase of at least 3 points) in their QMG scores from baseline to Day 8 and Day 14 showed that amifampridine phosphate
was superior to placebo. At Day 8, 59.1% of placebo patients had clinically significant disease progression versus 6.3% on amifampridine phosphate; and at Day 14, 38.1% versus 12.5% had progression on placebo compared to amifampridine phosphate, respectively.

The mean change in SGI at Day 14 was -0.7 and -2.7 in the amifampridine phosphate and placebo groups, respectively [mean difference, 1.7 in favor of amifampridine phosphate (95% CI, 0.7, 3.0; \( P = 0.0028 \)]. The comparison at Day 8 was also significant in favor of amifampridine phosphate. (Table 2).

An evaluation of patient satisfaction with the treatment (SGI score of 4 to 7, partially satisfied to delighted) at Day 8 and Day 14 showed amifampridine phosphate to be superior to placebo. At Day 8, 45.5% of placebo patients had scores of 4 to 7 versus 93.8% on amifampridine phosphate, and at Day 14, 42.9% versus 75% had scores of 4 to 7 on placebo compared to amifampridine phosphate, respectively.

There was also a strong correlation between the change from baseline in QMG score and the change from baseline in SGI score (the R2 statistic = 0.56), using the full analysis set.

Secondary and Tertiary Efficacy Endpoints at Day 14 in Part 3

At day 14, the CGI-I was significantly improved among patients who received amifampridine phosphate as compared with those who received placebo in the full analysis set. Mean scores were 3.6 and 4.7 for amifampridine phosphate and placebo, respectively, with a statistically significant (\( P = 0.0267 \)) mean difference of -1.1 (95% CI: -2.1, -0.1) in favor of amifampridine phosphate. There was no statistically significant difference in changes in scores in T25FW and CMAP amplitude at Day 14.
Aamifampridine phosphate efficacy at Day 8 in Part 2

All 5 primary, secondary, and tertiary endpoints achieved statistical significance at Day 8 (Table 2), suggesting that placebo patients experienced a clinically and statistically significant worsening of symptoms during the amifampridine phosphate withdrawal period and indicating that amifampridine phosphate was effective. The difference between amifampridine phosphate and placebo in QMG score at day 8 was 3.4 ($P < 0.0001$), satisfying standard criteria for “clinical significance”, as defined as a QMG score change of ≥2.6 points. The efficacy of amifampridine phosphate is further supported by objective CMAP results at Day 8.

Per-protocol analysis at Day 14

To more thoroughly evaluate the effects of treatment and the observed differences between Day 8 and Day 14, a per-protocol analysis was performed in 26 patients (Table 3). This analysis was performed because the assessment protocol at Day 14 was not followed in 10 patients; more specifically, amifampridine phosphate was taken 1 night prior to assessment instead of 45 minutes prior to the assessment. In this analysis, there was a 0.7-point decrease in QMG score among patients who received amifampridine phosphate, as compared with a 2.3-point increase among patients who received placebo. This difference was 3.0, which was statistically significant ($P=0.0048$) in favor of amifampridine phosphate. Thus, the statistical value of significance was increased from 0.0453 in the full analysis to 0.0048 in the per-protocol analysis. Further, the 3.0-point difference between study drug and placebo satisfies the criterion of “clinical significance”, as defined by a difference in QMG score of 3.0. A similar pattern of increased difference among the full analysis set and per-protocol...
population was seen for the SGI and CGI-I scores. No statistical significance was observed for either the T25FW or CMAP in this analysis.

**Safety**

Amifampridine phosphate was well tolerated, and there were no unexpected or notable adverse events. No serious adverse events attributable to amifampridine phosphate were reported during the study. In Part 1 (N = 53), serious adverse events (none attributed to drug treatment) were reported in 3 subjects, only 1 of whom did not proceed into Part 2. Overall, the most common adverse events were oral (40%) and digital (34%) paresthesia, nausea, and headache (Table 4).

During the double-blind treatment discontinuation (Part 2), 37.5% of patients randomized to amifampridine phosphate reported a total of 11 TEAEs (headache, erythema, subcutaneous abscess, upper respiratory infection, dizziness, diarrhea, chest pain, seasonal allergy, oropharyngeal pain) compared to 13.6% of patients randomized to placebo who reported 3 TEAEs (muscle weakness, myalgia, chronic sinusitis). None of these TEAEs were serious or resulted in study drug discontinuation. Only 1 event was deemed drug related by the investigator, and this was in the placebo group.

In Part 3, double-blind treatment, 18.8% of patients randomized to amifampridine phosphate reported 3 TEAEs (nasopharyngitis, otitis externa, urinary tract infection) compared to 27.3% of patients randomized to placebo who reported 11 TEAEs [asthenia, sensation of heaviness, periodontitis, dental pulpitis, fatigue, muscle weakness, blood creatine kinase (CK) increase, depression, dyspnea]. None of these TEAEs were serious or resulted in study drug discontinuation. Two events in the
placebo group were deemed drug related by the investigator. In 1 patient, a liver panel was abnormal; mildly elevated CK was noted in 1 placebo patient. No seizures, serious ECG abnormalities (including prolonged QT interval), or serious laboratory abnormalities were reported in the clinical trial. Side effects were generally mild and waned over time with continued use of amifampridine phosphate. Fewer side effects were reported in treatment-experienced patients compared to treatment-naïve patients.

**Discussion**

Amifampridine is the non-proprietary active ingredient name for 3,4-DAP. In 2009, the European Commission granted marketing approval to amifampridine phosphate as an orphan drug for symptomatic treatment of LEMS. Amifampridine phosphate has been shown to have superior stability compared with the 3,4-DAP base and can be stored without refrigeration; in the EU, amifampridine phosphate has been shown to have a 3-year shelf life. \(^8,22,\)

Amifampridine is a voltage-gated potassium channel blocker. \(^23\) The blocking action prolongs the depolarization of nerve action potentials. This increases the open time of VGCCs, thereby increasing presynaptic calcium levels. The increased calcium influx enhances ACh release, which then binds to muscle receptors, resulting in improved muscle function. Thus, amifampridine is ideally suited for symptomatic treatment for LEMS.

Since the first use of amifampridine in LEMS in 1983, its efficacy and safety have been documented consistently in multiple case reports and 1 open trial in more than 70 patients. \(^24,25\) In these reports, moderate-to-marked functional improvement was seen in patients who received amifampridine at doses ranging from 24 to 80 mg per day. The
maximum daily dose should not exceed 80 mg, because seizure had been reported in 3 patients on a daily regimen of ≥100-mg.\textsuperscript{20, 26}

Three randomized, placebo-controlled trials with oral 3,4-DAP base and 1 with intravenous 3,4-DAP base have demonstrated its efficacy in patients with LEMS.\textsuperscript{19,20,23, 27} These 4 trials showed a significant improvement in muscle strength or QMG score and CMAP amplitude in patients with LEMS.\textsuperscript{19,20,26,27} In a crossover study, patients treated with 3,4-DAP base showed significantly better CMAP, LEMS classification, Medical Research Council muscle strength, QMG, and subjective symptom scores ($P = 0.0017–0.0246$).\textsuperscript{19} Based on analyses of the results of these 4 randomized, controlled studies in a total of 54 patients with LEMS, a Cochrane review in 2011 concluded that there was “limited but moderate to high-quality evidence showing that over days 3,4-DAP improved muscle strength and CMAP in LEMS.\textsuperscript{21}

This is the phase 3, double-blind, randomized study with the largest number of patients that has lasted longer than 8 days. We randomized 38 patients at multiple academic centers and evaluated the efficacy of amifampiridine phosphate at Day 8 (Part 2) and Day 14 (Part 3). All previous randomized trials were conducted at a single academic center, involved 7-26 patients, and analyzed the results at days 3 to 6 in part 2.\textsuperscript{19,20,26} In contrast, our study demonstrated significant efficacy of amifampiridine phosphate over placebo in all 5 endpoints at Day 8 in Part 2 and a significant steady efficacy of amfampiridine phosphate compared with placebo in both primary endpoints and 1 of the 2 secondary endpoints at Day 14 in Part 3 in the full analysis.

If the primary endpoints had been set at Day 8 in Part 2 as was done in the previous studies, this study would have shown that amifampridine phosphate was
significantly more efficacious than placebo based on all primary, secondary, and tertiary endpoints.\textsuperscript{19,20,26}

In 2 previous phase 2 studies, 3,4-DAP base showed a significant improvement in QMG over placebo: a 2.76 score difference in a prior study by Oh et al. and 2.44 in a study by Sanders et al.\textsuperscript{19,20} In these 2 studies, the evaluation was performed at days 3 to 6. In the present study, at Day 8 in Part 2, the highest differential of 3.1 occurred, indicating clinically significant improvement associated with amifampridine phosphate over placebo. This study showed the lowest differential of 1.8 at Day 14 in Part 3, that was, however, statistically significant.

Thus, there was an unexpected decrement in response to treatment in the QMG between days 8 and 14. Amifampridine phosphate has a rapid absorption ($T_{\text{max}}=0.5$ hours), and relatively short half-life (2.5 hours). For this reason, assessments were specified to be performed 45 minutes after taking a dose of medication. A per-protocol analysis of those patients in whom protocol instructions were followed demonstrated that those assigned to amifampridine phosphate had sustained improvement in QMG scores through the end of the double-blind treatment period compared with patients who received placebo ($P=0.0048$). The difference is explained by the finding that 10 patients took their last dose of study drug the evening prior to final assessments instead of the morning of. Based upon the pharmacokinetics of amifampridine phosphate, the medication dosing time would have implications on efficacy evaluations and bias the results against the treatment group. This was confirmed by the per-protocol analysis. Nevertheless, the QMG score was still statistically significant in favor of the treatment group for the full analysis data set. One hypothesis is that some pharmacodynamic
effect may extend beyond those predicted solely on the pharmacokinetics of the drug; however, additional research is required to evaluate this hypothesis. Notably, the SGI score appeared less affected by the medication dosing time. This is due to the fact that SGI is based upon the patient’s impression of the effects of the study medication during the previous week.

According to Barohn et al. the QMG score change should be > 2.6 to be “of clinical significance” based on 5 MG patients and 4 controls. If this criterion is applied, both the Oh et al. study and the present study at Day 8 in Part 2 and at Day 14 in the per-protocol analysis showed a “clinically significant improvement”. Considering that the QMG score was primarily designed for assessing clinical trials in myasthenia gravis, this finding is remarkable. This was also supported by the improvement in the QMG score in the combined arms and legs QMG domains. This indicates that the QMG score is still valid for clinical evaluation in LEMS.

This study also used the subjective evaluation by the patient as measured using SGI-score) as a coprimary endpoint. There was a significant differential of 1.7 in favor of amifampridine phosphate over placebo for this end point. A subjective symptom score was first used in LEMS in the Oh et al. study; consistent with the findings of this study, there was a statistically significant improvement of 3,4-DAP base treated group.

The CGI-I score and T25FW test were secondary endpoints. The T25FW test was used in the 4-aminopiridine study in multiple sclerosis. The CGI-I score showed a significant improvement with amifampridine phosphate over placebo. Thus, one can conclude that subjective evaluation by patients as well as by clinicians confirmed the objective findings of the QMG score. Our study also showed a significant improvement
in CGI-I score with amifampridine phosphate at Day 8. A significant improvement with 3,4-DAP base over placebo has been reported in abstract form in a phase 2 study using the endpoint of 3 TUG study at 3.5 days.28 Our study also showed significant improvement at Day 8 in the CMAP as previously reported.19,20,26

Amifampridine phosphate therapy was well tolerated with an excellent safety profile and without any notable adverse events. The most common side effects reported by patients were tongue and finger paresthesia, mainly in drug-naïve patients. These are drug effects of amifampridine phosphate rather than adverse events, as noted in previous studies. No serious cardiac abnormalities were detected in this study, confirming the previous BioMarin study of amifampridine phosphate in 59 healthy volunteers.29 There has been 1 case report of prolonged QTc interval with 60-mg 3,4-DAP base in a LEMS patient.30

A limitation of our trial was that the protocol was not followed in 10 patients as discussed above.

From these findings, we conclude that this study of amifampridine phosphate (Firdapse) provides Class I evidence of efficacy as a symptomatic treatment for LEMS with an acceptable tolerability and safety profile.
Abbreviations

3,4-DAP, 3,4-diaminopyridine

ACh, acetylcholine

AEs, adverse events

Ca^{2+}, calcium ions

CGI-I, Clinical Global Impression of Improvement

CMAP, compound muscle action potential

CK, creatine kinase

ECG, electrocardiogram

EU, European Union

FDA, Food and Drug Administration

FVC, forced vital capacity

GMP, Good manufacture practice

IND, Investigational new drug

LEMS, Lambert-Eaton myasthenic syndrome

MeDRA, Medical Dictionary for Regulatory Activities

mg, milligram

MMRM: mixed-effect model repeated measure;

NS, not significant
QMG, Quantitative Myasthenia Gravis score

SGI, Subject Global Impression of improvement

T25FW, Timed 25-foot Walk test

US, United States

VGCC, voltage-gated calcium channel
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17. Catalyst Pharmaceutical Partners. A Phase 3, Multicenter, Double-blind, Placebo-controlled Randomized Discontinuation Study Followed by an Open-label Extension Period to Evaluate the Efficacy and Safety of Amifampridine phosphate (3,4-Diaminopyridine Phosphate) in


Figure legends

Figure 1. Study Design

Figure 2. Disposition of study patients
Table 1: Demographic Data

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<th>Parts 2 and 3</th>
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<td>22</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>48 (90.6)</td>
<td>13 (81.3)</td>
<td>21 (95.5)</td>
<td>34 (89.5)</td>
<td></td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>35 (66.0)</td>
<td>9 (56.3)</td>
<td>14 (63.6)</td>
<td>23 (60.5)</td>
<td></td>
</tr>
<tr>
<td>Mean age (range), years</td>
<td>52.1 (20–88)</td>
<td>51.6 (25–67)</td>
<td>51.5 (21–88)</td>
<td>51.5 (21–88)</td>
<td></td>
</tr>
<tr>
<td>Treatment-experienced, n (%)</td>
<td>11 (20.8)</td>
<td>3 (18.8)</td>
<td>7 (31.8)</td>
<td>10 (26.3)</td>
<td></td>
</tr>
<tr>
<td>PEF: ≥100%</td>
<td>32</td>
<td>9</td>
<td>12</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>VGCC antibody-positive, %</td>
<td>48</td>
<td>15</td>
<td>20</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>History of cancer, %</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

PEF=post-exercise facilitation; VGCC=voltage-gated calcium channel.
<p>| Table 2: Full Analysis Scores on Days 8 and 14 in the Primary, Secondary, and Tertiary Endpoints |
|-------------------------------------------------|----------------|----------------|----------------|
| <strong>Primary endpoints</strong>                           | Baseline (N=38) | Day 8* (N=37)  | Day 14† (N=36) |
| QMG Amifampridine phosphate                      | 6.4 ± 3.22      | 6.4 ± 3.08     | 6.7 ± 4.09     |
| Placebo                                         | 5.6 ± 3.99      | 9.5 ± 3.58     | 7.9 ± 2.85     |
| <em>P value</em>                                        | &lt;0.0001         | 0.0452         | 0.0028         |
| 95% CI                                          | -4.9, -1.9      | -3.4, 0.0      |               |
| SGI Amifampridine phosphate                      | 5.6 ± 1.26      | 5.4 ± 1.20     | 4.9 ± 1.57     |
| Placebo                                         | 5.9 ± 1.22      | 3.5 ± 1.99     | 3.2 ± 1.70     |
| <em>P value</em>                                        | 0.0010          | 0.0028         |               |
| 95% CI                                          | 0.9, 3.1        | 0.7, 3.0       |               |
| <strong>Secondary endpoints</strong>                         | Amifampridine phosphate | 2.6 ± 0.63 | 3.5 ± 0.97 | 3.6 ±1.50 |
| CGI-I Amifampridine phosphate                    | 2.5 ± 0.98      | 4.6 ± 1.53     | 4.8 ±1.45     |
| Placebo                                         | 2.5 ± 0.98      | 4.6 ± 1.53     | 4.8 ±1.45     |
| <em>P value</em>                                        | 0.0170          | 0.0252         |               |
| 95% CI                                          | -2.0, -0.2      | -2.1, -0.1     |               |
| TFW25 (ft/min)                                   | Amifampridine phosphate | 254 ± 126 | 263 ± 141 | 253 ± 126 |
| Placebo                                         | 255 ± 111       | 205 ± 109      | 244 ± 116     |
| <em>P value</em>                                        | 0.0302          | NS             |               |
| 95% CI                                          | 4.8, 89.9       | -26.8, 43.8    |               |</p>
<table>
<thead>
<tr>
<th>Tertiary endpoint</th>
<th>CMAP (mV)</th>
<th>Amifampridine phosphate</th>
<th>Placebo</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.7 ± 3.72</td>
<td>5.7 ± 4.12</td>
<td>0.0065</td>
<td>0.5, 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.7 ± 3.72</td>
<td></td>
<td>-1.0, 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.5 ± 3.26</td>
<td>4.9 ± 3.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.0 ± 3.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QMG: Quantitative Myasthenia Gravis score; SGI: Subject Global Impression of improvement; CGI-I: Clinical Global Impression of Improvement; TFW25: Timed 25-foot Walk test; NS: not significant, CMAP: compound muscle action potential.

*1 placebo patient was rescued. † 1 placebo patient was rescued.
Table 3: Per-protocol Analysis Scores on Day 14 in the Primary and Secondary Endpoints

<table>
<thead>
<tr>
<th></th>
<th>Baseline (N=26)</th>
<th>Day 14 (N=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary endpoints</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QMG</td>
<td>Amifampridine phosphate</td>
<td>6.5 ± 3.34</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>5.9 ± 4.12</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>0.0048</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>-4.7, -0.9</td>
</tr>
<tr>
<td>SGI</td>
<td>Amifampridine phosphate</td>
<td>4.9 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>5.6 ± 1.26</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>0.0019</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>0.9, 3.4</td>
</tr>
<tr>
<td><strong>Secondary endpoints</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGICI</td>
<td>Amifampridine phosphate</td>
<td>2.7 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.6 ± 1.03</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>0.0024</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>-3.0, -0.7</td>
</tr>
</tbody>
</table>

QMG: Quantitative Myasthenia Gravis score; SGI: Subject Global Impression of improvement;
CGI-I: Clinical Global Impression of Improvement.
Table 4: Treatment-emergent Adverse Events during Open-label Run-in (part 1)

<table>
<thead>
<tr>
<th>Symptoms*</th>
<th>Treatment-naïve (N=42)</th>
<th>Previously Treated (N=11)</th>
<th>Overall (N=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral paresthesia</td>
<td>20 (47.6)†</td>
<td>1 (9.1)</td>
<td>21 (39.6)</td>
</tr>
<tr>
<td>Digital paresthesia</td>
<td>18 (42.9)</td>
<td>0</td>
<td>18 (34.0)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (11.9)</td>
<td>0</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (11.9)</td>
<td>0</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (9.5)</td>
<td>1 (9.1)</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>Constipation</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>Limb pain</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>Fall</td>
<td>3 (7.1)</td>
<td>0</td>
<td>3 (5.7)</td>
</tr>
</tbody>
</table>

Each symptom listed here was reported in at least 3 patients.

† N (%)
**Figure 1. Study Design**

<table>
<thead>
<tr>
<th>Screening</th>
<th>Open-label Run-in</th>
<th>Double-blind Treatment</th>
<th>Open-label Long-term Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Part 1</td>
<td>Part 2</td>
<td>Part 4</td>
</tr>
<tr>
<td>amfampridine phosphate</td>
<td>amfampridine phosphate</td>
<td>amfampridine phosphate</td>
<td>amfampridine phosphate</td>
</tr>
<tr>
<td>Days 1–4</td>
<td>Days 7–91</td>
<td>Days 1–7</td>
<td>Up to 2 years</td>
</tr>
<tr>
<td>Efficacy assessments screening</td>
<td>Efficacy/Eligibility assessments and dose adjustments</td>
<td>Baseline measurement taken on Day 1</td>
<td>Safety assessments and dosage adjustments</td>
</tr>
<tr>
<td></td>
<td>Dose Taper</td>
<td>Placibo</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 1*

171x89mm (300 x 300 DPI)
Figure 2. Disposition of study patients

- Screening, N=74
  - N=20 did not meet all eligibility criteria

  Part 1: Open-label run-in phase, N=54
  - N=14 did not complete this phase:
    - N=5 withdrawn due to AE
    - N=3 withdrawn due to withdrawal by subject
    - N=2 withdrawn due to lack of efficacy
    - N=1 withdrawn due to physician decision
    - N=3 withdrawn due to other

  Part 2: Double-blind Treatment Discontinuation, N=38
  - N=2 advanced to Part 4 (enrollment target met)

  Part 3: Double-blind Treatment Phase, N=37
  - N=1 did not advance to Phase 3:
    - N=1 due to rescue treatment required (increase in QMG score by >5 points) after completing Part 2. After rescue visit, advanced to Part 4

  Part 4: Open-label Extension, N=40
  - Consisted of the following:
    - N=16 completed Part 3
    - N=2 completed Part 2 (but not Part 3)
    - N=2 went straight from Part 1 to Part 4

Figure 2
154x128mm (300 x 300 DPI)