Symptomatic treatment of myasthenia gravis and its role in the era of emerging immunomodulatory treatments

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CHAPTER

5

Efficacy and safety of amifampridine

Efficacy and safety of amifampridine in myasthenia gravis:
a randomized, double-blind, placebo-controlled crossover trial
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Abstract

Background and objectives

Amifampridine, a short-acting potassium-channel blocker, is the first-line treatment for Lambert-Eaton myasthenic syndrome. Its benefit as add-on to pyridostigmine in acetylcholine receptor (AChR) positive myasthenia gravis (MG) has been proposed, but no randomized controlled trials have been conducted. We aimed to evaluate the efficacy and safety of amifampridine in AChR-MG with insufficient symptom control on pyridostigmine.

Methods

A randomized, double-blind, placebo-controlled crossover trial was conducted at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands, as part two of the IMPACT-MG trial. Eligible patients had either completed part one (pyridostigmine vs. placebo) or failed the initial two-day pyridostigmine washout, and had an MGII score >10. Participants were randomized (1:1:1) to one of three treatment sequences. Each treatment period lasted 5 days, with a 2-day washout between treatments, during which participants received either 30 mg or 60 mg of amifampridine modified release, or placebo. The primary outcome was the difference in MGII score between the two amifampridine dosages and placebo. Pharmacokinetics, safety and tolerability were assessed throughout the trial.

Results

Between March 2023 and April 2024, twenty patients were enrolled (median age 60 years, 55% female). For the MGII score, the estimated mean differences were -1.0 (95% CI -4.1-2.0, p=0.681) for amifampridine 30 mg versus placebo and -1.7 (95% CI -4.7-1.4, p=0.379) for amifampridine 60 mg versus placebo. No statistically significant association was observed between pharmacokinetic parameters and the MGII. No serious adverse events were reported. Adverse events were more common with amifampridine 30 mg (12 patients [60%]), and 60 mg (15 patients [75%]), compared to placebo (6 patients [30%]), with paresthesia, fatigue, numbness, dizziness, and sleep disturbances most frequently reported. Three patients discontinued amifampridine early due to adverse events.

Discussion

The addition of amifampridine to pyridostigmine was not superior to treatment with pyridostigmine alone and was associated with a higher incidence of adverse events.

Introduction

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder characterized by fluctuating muscle weakness due to impaired signal transmission at the neuromuscular junction.

Most commonly, autoantibodies target the acetylcholine receptor (AChR), while in rarer cases, antibodies against other neuromuscular junction structures are found leading to different clinical features and in some cases, a different response to treatment.

Clinical management includes either symptomatic drugs, which directly improve muscle strength, or immunomodulating drugs, which modify immune system activity. Symptomatic treatment is universally considered to be the first step in the treatment of MG. Patients who do not have a satisfactory functional result with symptomatic treatment alone, generally start using immunosuppressive treatment consisting of corticosteroids often in combination with nonsteroidal immunosuppressive treatment.[167]

Symptomatic drugs offer several advantages over immunomodulatory therapies. A key advantage is their ability to improve muscle strength within minutes, whereas immunomodulatory agents typically require weeks to months to demonstrate effects. Additionally, immunomodulatory treatments and in particular corticosteroids, are associated with significant long-term side effects that can severely impact health-related quality of life.[168] Although significant progress has been made in developing novel immunomodulatory agents, their widespread clinical application is limited by high costs and insufficient long-term safety data. These limitations underscore the continued importance of symptomatic treatment in the management of MG.

Pyridostigmine has emerged as the preferred symptomatic drug in MG treatment [169]. However, for the majority of patients, pyridostigmine monotherapy is insufficient; currently, 69% of MG patients require immunosuppressant medication.[86] Other symptomatic treatment options are limited and there remains an unmet need for more effective symptomatic treatments for MG that can delay or reduce the need for immunosuppressive treatment. In patients with a chronic, lifelong autoimmune disease, even a relatively modest clinical benefit would have a substantial impact on long term side-effects and consequently, on quality of life.

Amifampridine, also known as 3,4-diaminopyridine, is a well-known symptomatic treatment for other diseases of the neuromuscular junction such as Lambert Eaton myasthenic syndrome (LEMS),[170] and certain subtypes of congenital myasthenic syndromes.[171] It is a short-acting potassium channel blocker, which blocks potassium efflux presynaptically. This results in a prolonged action potential of the presynaptic nerve terminal, which enhances the release of acetylcholine into the synaptic cleft by an increased calcium influx into the nerve terminal.[172,173] In the Netherlands, amifampridine base modified release (amifampridine

MR) has been used clinically since 1989 and it is the formulation most frequently used in Dutch LEMS patients. Higher peak concentrations are associated with adverse effects and it is hypothesized that amifampridine MR leads to lower peak concentrations than the immediate release amifampridine phosphate salt authorized for the treatment of LEMS. The use of amifampridine as an add-on to pyridostigmine in MG has been primarily described in patients with antibodies against muscle-specific kinase (MuSK).[32,33] In AChR MG its use has been reported only in a limited number of observational studies.[34,99,174] To our knowledge, no randomized controlled trials have systematically evaluated the therapeutic effect of add-on amifampridine treatment in patients with AChR MG already receiving pyridostigmine. In this crossover randomized trial, we therefore aimed to assess the efficacy and safety of add-on treatment with amifampridine MR for patients with AChR MG whose symptoms are insufficiently controlled with pyridostigmine alone.

Methods

Study design and participants

IMPACT-MG was a two-part, investigator-initiated, prospective, randomized, double-blind, placebo-controlled crossover trial that evaluated the efficacy and cost-effectiveness of pyridostigmine in patients with MG (part one), followed by a second randomization to assess the efficacy and safety of amifampridine in combination with pyridostigmine (part two). Part one focused on pyridostigmine optimization and is reported separately. The current manuscript specifically presents part two of the study, designed to evaluate the efficacy and safety of amifampridine MR added to stable pyridostigmine treatment. The study was performed at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands.

The results of part one will be published separately. The independent Medical Ethics Review Committee Leiden The Hague Delft reviewed and approved the study protocol. The protocol was revised once, four months after the first inclusion, to extend the study with an additional observational visit after three months and after six months, to evaluate the long-term effectiveness of amifampridine.

Written informed consent was obtained from each patient before study participation. Subjects gave separate consent for participating in the pharmacokinetic/pharmacodynamic (PK/PD) substudy to provide additional blood samples.

Participants

Eligible patients were aged 18 years or older and had a documented diagnosis of ocular or generalized MG with evidence of elevated anti-acetylcholine receptor antibodies. Patients

had a Myasthenia Gravis Foundation of America (MGFA) clinical classification of class I to IV at screening, were currently using pyridostigmine and had a stable dose of other MG treatments. Patients were excluded if they had a history of thymectomy in the 6 months before screening, or thymectomy (expected) to take place during the trial, use of intravenous immunoglobulin or plasma exchange <4 weeks or planned during the trial or current use of other AChE inhibitors than pyridostigmine.
To be eligible for part two, patients were required to have either completed part one or failed to complete the two-day washout period prior to randomization in part one. In addition, patients were required to have a score of >10 points on the myasthenia gravis impairment index (MGII) questionnaire at inclusion. Patients were excluded from part two if they had contra-indications for treatment with amifampridine MR: a history of epilepsy, uncontrolled asthma, inherited QT syndrome or a prolonged QT interval (as indicated by ECG), or use of any drug known to cause QTc-prolongation or any drug known to lower the epileptic threshold. A full list of the eligibility criteria is provided in the appendix.

Randomization and masking

Eligible participants were randomized in a 1:1:1 ratio and allocated to either one of three treatment sequences as per a balanced Latin square crossover design: amifampridine MR 30 mg, amifampridine MR 60 mg, and placebo (30-60-P); amifampridine MR 60 mg, placebo, and amifampridine MR 30 mg (60-P-30); or placebo, amifampridine MR 30 mg, and amifampridine MR 60 mg (P-30-60). The Latin square design ensured that each treatment appeared once in each study period, thereby balancing potential period effects. As carry-over effects were expected to be minimal given the washout period between treatment periods and the short half-live of amifampridine, and because of the small sample size which would have complicated block randomization, we chose for this specific design.
Randomization was done with the Castor Electronic Data Capturing System (Castor EDC; Amsterdam, the Netherlands) using variable block sizes (3, 6 and 9 patients). The amifampridine MR 30 mg, amifampridine MR 60 mg and placebo tablets appeared identical and were dispensed by the hospital pharmacy and distributed in identical bottles. The study medication allocation sequence was known only to the study pharmacist, while all other study personnel and participants remained blinded until database lock. The success of blinding was evaluated by assessing both the investigator's and the patient's perception of which treatment the patient had received during the past week.

Study procedures

After obtaining informed consent, screening assessments were conducted, including the MGII and an ECG. As part of the study protocol for part one, patients were instructed to discontinue their regular use of pyridostigmine. Patients who were unable to do so were

not eligible for part one, but could directly proceed to part two if they met the in- and exclusion criteria. All other patients first participated in part one for two weeks before proceeding to part two. Upon completion of part one, patients who met the inclusion and exclusion criteria were randomized for part two on the final day of assessments in part one. Participants were randomly assigned to a sequence of three treatment periods for five days separated by a 2-day washout. Patients received two times daily 15 mg (amifampridine MR 30 mg), two times daily 30 mg (amifampridine MR 60 mg) or matching placebo, in addition to their unchanged pre-study dose of pyridostigmine. The amifampridine doses were selected based on the standard dosing used in the Netherlands in patients with LEMS, with 30 mg corresponding to a commonly used low dose and 60 mg to a commonly used high dose. Throughout the trial, patients were required to maintain a stable dose and treatment schedule of their prior pyridostigmine, oral corticosteroids, or other immunosuppressive treatments. Patients had the opportunity to consult an independent neurologist specializing in neuromuscular disorders regarding symptoms and adverse effects. To maintain blinding, this neurologist was unaware of the study medication, and researchers were not informed of any patient consultations.

For evaluation of efficacy, we assessed the following validated outcome measures: the MGII,[148] the Myasthenia Gravis-Activities of Daily Living (MG-ADL) scale,[149] the Quantitative Myasthenia Gravis (QMG) score,[150] and the revised 15-item Myasthenia Gravis Quality of Life (MG-QOL15r) questionnaire.[151] Treatment satisfaction was evaluated using the 9-item Treatment Satisfaction Questionnaire for Medication (TSQM-9).[152] Health-related quality of life was measured using the 5-level EQ-5D (EQ-5D-5L).[153] The MGII was assessed at screening, baseline (without pyridostigmine treatment), and during part two on days 5, 12 and 19. MG-ADL, QMG, MG-QOL15r, and EQ-5D-5L were scheduled at baseline and on days 5, 12 and 19. TSQM-9 was assessed at day 5, 12 and 19.

Serum concentrations of amifampridine MR and pyridostigmine were measured at each visit. In addition, an optional PK/PD substudy was conducted among a subset of patients. Blood samples of pyridostigmine and amifampridine MR were collected before amifampridine MR ingestion, at 30 and 60 minutes post-ingestion, and then hourly up to 8 hours post-ingestion.

Safety evaluations included the collection of vital signs at each visit, and an electrocardiogram (ECG) to monitor corrected QT (QTc) intervals at screening, the last day of part one (or at baseline for patients only participating in part two of the study), days 5, 12 and 19. Patients were actively queried for adverse events during each visit and their severity was graded. Additionally, side effects were evaluated using a questionnaire that was previously developed to assess the side effects of pyridostigmine in a cross-sectional study.[79] For amifampridine MR, the questionnaire was adapted by compiling a list of 21 potential side effects based on the patient information leaflet of amifampridine MR and the European Public Assessment

Report (EPAR) for amifampridine immediate release phosphate. A full list of the side effects queried are in the appendix. Laboratory testing at baseline and day 19 included a blood cell count, assessment of electrolytes, renal function, liver enzymes, creatine kinase and AChR antibody titers.

After the final study visit, patients were given the option to continue using amifampridine MR in an unblinded manner at a dose of their choosing. For those patients, further assessments were conducted at three and six months after the last visit to evaluate long-term efficacy and potential side effects. An overview of the study design, including the first part of the trial, is provided in **Figure 5.1**.

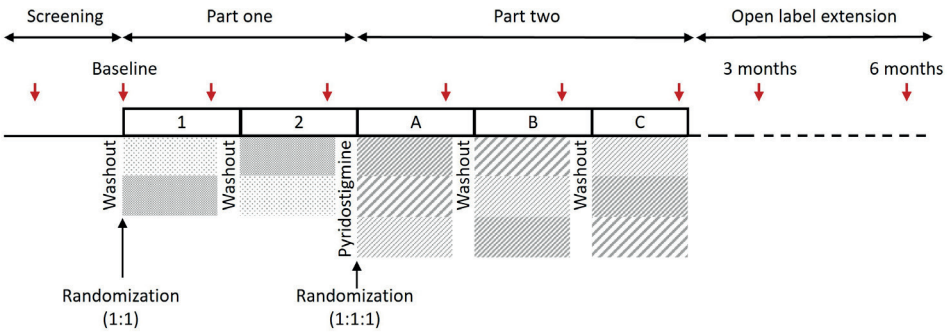

Figure 5.1 Outline of the study design
The study consisted of a screening phase, part one (pyridostigmine vs. placebo, published separately), part two, and an open-label extension. Patients unable to complete the initial washout during screening were ineligible for part one but could proceed directly to part two if inclusion and exclusion criteria were met. All other patients first completed part one (2 weeks) before entering part two. In part two, participants were randomized (1:1:1; Latin square design) to one of three treatment sequences (amifampridine 30 mg, 60 mg, and placebo), each administered double-blind for 5 consecutive days, separated by 2-day washout periods. Assessments were performed on day 5 of each treatment period. Following completion of part two, patients continuing amifampridine were followed up at 3 months (by phone) and 6 months.

Outcome measures

Both in part one and part two, the primary outcome was change in MGII total score compared to placebo. The MGII is a validated score and includes 22 patient-reported items and 6 items assessed through examination. Total scores range from 0 to 84, with higher scores reflecting greater severity of impairment. The MGII can be subdivided into ocular (8 items) and generalized (20 items) subscores. A change of ≥8 is considered a clinically meaningful improvement.[156] The MGII was chosen as the primary outcome measure because it provides a broader range of scores and has less floor effect compared to other commonly used outcome measures.[148] Prespecified secondary efficacy endpoints were changes on the MG-ADL, QMG, MG-QoL15r, and TSQM-9 compared to placebo. A clinically meaningful

improvement was defined as a 2-point change in the MG-ADL score and a 3-point change in the QMG score. Trial medication compliance was documented and compared between trial medication and placebo treatment.
In the PK/PD substudy, PK parameters were maximum concentration (C_{max}); area under the concentration-time curve (AUC), τ = dosing interval (AUCτ), time of maximum concentration (t_{max}), clearance (CL) and serum half-life ($t_{1/2}$). MGII was used as a PD endpoint to assess a possible dose response relationship. Safety outcomes were incidences of adverse events and side effects, vital signs, and QTc intervals.

Statistical analysis

Based on previous data with a similar population of MG patients,[156,163,164] a sample size of 17 participants was expected to have 90% power to detect a difference of 8 points on the MGII with a two-sided p value of 0.05. We assumed a standard deviation of repeated measurements of MGII between patients of 13 and a conservative intraparticipant correlation of 0.7.[156,163,164] Allowing for a 10% drop out rate, we aimed to include 19 patients in both parts of the study.
Descriptive statistics were used to summarize baseline characteristics, presented as means and standard deviations (SDs) or medians and first and third quartiles (median [Q1-Q3]). Primary analysis was performed according to the intention-to-treat principle. In addition, we performed as treated analysis for all efficacy outcomes. A mixed-effects linear regression model was used to analyze repeated measures and estimate mean differences between amifampridine MR 30 mg (30), amifampridine MR 60 mg (60), and placebo (P), with treatment and treatment period (categorical) as fixed factors and patients as a random effect. Significance was defined as a p-value lower than 0.025, to account for multiple comparisons. We analyzed safety endpoints in all participants who were exposed to at least one dose of the trial medication and summarized these data using descriptive statistics.
For the PK/PD analysis actual blood sampling times were used for PK analysis. Amifampridine concentrations were measured in plasma with a UPLC/MS-MS assay, validated according to the ICH/EMA guidelines,[175] in the ISO15189 certified clinical pharmaceutical laboratory. A population PK-model for amifampridine MR was developed in NONMEM version 7.5. For the development of the model we included the observed data for all patients including the patients not participating in the PK/PD substudy. One- and two-compartment disposition kinetics with linear and nonlinear elimination were tested to describe the structural model of amifampridine MR. Model development and inclusion of covariates followed a stepwise approach based on physiological plausibility and improvement in model diagnostics. Tested covariates were age, sex, weight, height, BMI, creatinine and NAT genotype. Estimated individual PK parameters (AUCτ, C_{max}, C_{min}, $t_{1/2}$, t_{max}, CL) were extracted from the final PK model. Associations between PK parameters and the primary outcome measure were

evaluated using Pearson correlation coefficients and linear regression models.
All statistical analyses were predefined in the study protocol. No data were missing for the primary outcome analyses and no data imputation was done to account for missing data. All statistical analyses were conducted using RStudio (version 4.4.0).

Results

Between March 22, 2023 and Feb 21, 2024 21 patients were screened for eligibility, of whom nineteen patients completed part one of the trial, and two patients failed to complete the washout period prior to randomization in part one. Of these, one did not meet the eligibility criteria for part two, and was therefore excluded from further participation. Twenty patients were enrolled and randomly assigned to one of three consecutive treatment sequences: 30-60-P (7 patients); 60-P-30 (7 patients); or P-30-60 (6 patients) (**Figure 5.2**).
Three participants discontinued treatment prematurely due to adverse events. Among them, two participants withdrew during treatment with amifampridine 60 mg on the third day of treatment, and one participant withdrew during both the amifampridine 30 mg and amifampridine 60 mg treatment periods on the second day of treatment. These patients were included in the intention-to-treat analysis but were excluded from the per-protocol analysis.

Patient demographics and clinical characteristics across treatment sequences is shown in **Table 5.1**.
Eleven patients (55%) were female and nine (45%) were male. The median age of the cohort was 60 years (IQR 41-70), and the median age at onset was 51 years (IQR 26-63). Median MGII score at inclusion was 29 (IQR 21-40). Data on daily doses of pyridostigmine and AChR antibody status for each participant is provided in **Supplementary Table 5.1** and **5.2**, respectively.

Table 5.2 provides the estimated mean values for the primary and secondary outcome variables during treatment with amifampridine 30 mg and 60 mg, as well as for the placebo treatment. The treatment differences between amifampridine 30 mg and placebo, and between amifampridine 60 mg and placebo, are also provided. The mean treatment difference for the primary outcome, the MGII score, was -1.0 point (95% CI -4.1, 2.0); p = 0.379) for amifampridine 30 mg and -1.7 (95% CI -4.7,1.4; p = 0.379) for amifampridine 60 mg when compared to placebo. Similar to the primary outcome measure, no statistically significant differences were observed across all secondary efficacy outcome measures, including MGII ocular, MGII generalized, QMG and MG-ADL, quality of life (MG-QoL15r), and

treatment satisfaction (TSQM-9). There was no evidence of a treatment-by-period interaction. (**Supplementary Table 5.3**).

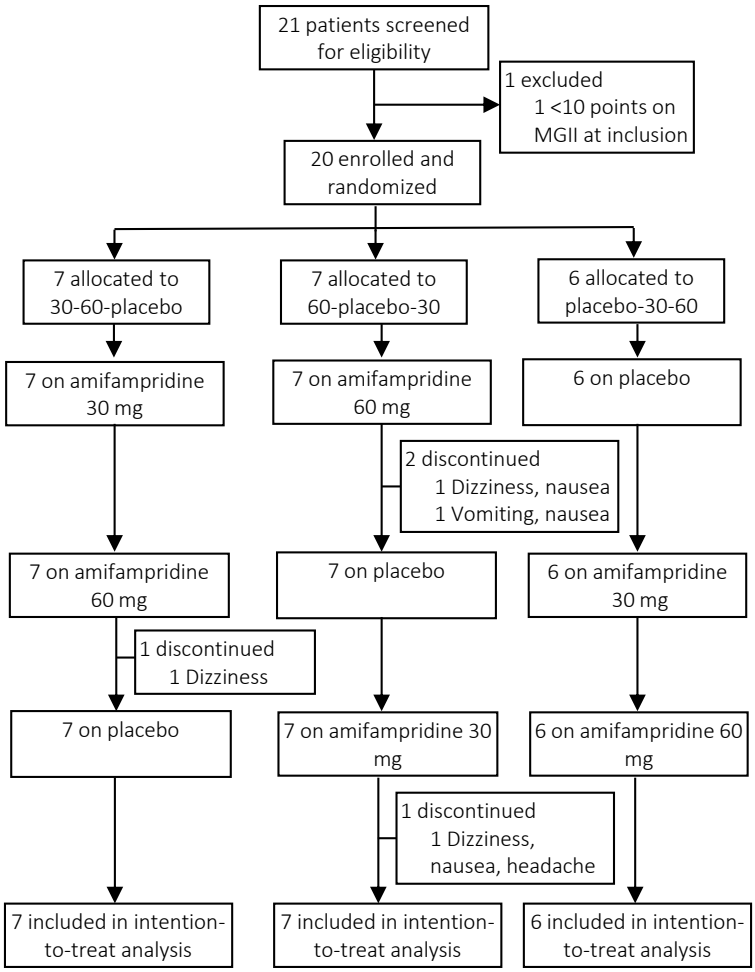

Figure 5.2 Flow diagram

[a] In two patients, primary and secondary outcome measures were obtained prematurely due to an intolerable increase in MG symptoms during the placebo treatment week.

Table 5.1 Baseline characteristics

	P-30-60[1] N = 6	**60-P-30[2] N = 7**	**30-60-P[3] N = 7**
Age, years	60 (44, 73)	60 (37, 75)	59 (47, 63)
Age at onset, years	52 (34, 63)	58 (23, 64)	45 (31, 55)
Sex, female	4 (67%)	3 (43%)	4 (57%)
BMI, kg/m2	27.4 (26.4, 28.3)	25.3 (23.7, 28.7)	28.4 (26.0, 29.4)
Disease duration, years	5 (3, 16)	8 (2, 11)	5 (4, 12)
Previous thymectomy, yes	2 (33%)	1 (14%)	4 (57%)
MGFA score at inclusion			
I	1 (17%)	2 (29%)	0 (0%)
II	4 (67%)	4 (57%)	7 (100%)
III	1 (17%)	1 (14%)	0 (0%)
MGII total score at inclusion	35 (23, 45)	30 (26, 50)	21 (21, 30)
NAT2 enzyme acetylator phenotype			
Fast	1 (17%)	4 (57%)	4 (57%)
Slow	5 (83%)	3 (43%)	3 (43%)
QTc, milliseconds	403 (400, 419)	403 (393, 404)	395 (389, 405)
Therapies in previous year			
No therapy	3 (50%)	4 (57%)	3 (43%)
Prednisolone	3 (50%)	2 (29%)	2 (29%)
Azathioprine	1 (17%)	1 (14%)	0 (0%)
Mycophenolate mofetil	2 (33%)	1 (14%)	1 (14%)
Cyclosporine	0 (0%)	1 (14%)	0 (0%)
Methotrexate	0 (0%)	0 (0%)	1 (14%)
IVIg on a regular basis	1 (17%)	0 (0%)	0 (0%)

Data are median (Q1-Q3) or n (%). Of the 20 participants, 19 had also participated in part one of the trial.
Abbreviations: MGFA, Myasthenia Gravis Foundation of America; MGII, Myasthenia Gravis Impairment Index; NAT2, N-acetyltransferase 2; QTc, corrected QT interval; IVIg, intravenous immunoglobulin therapy.
[1] Placebo-amifampridine 30 mg-amifampridine 60 mg
[2] Amifampridine 60 mg-placebo-amifampridine 30 mg
[3] Amifampridine 30 mg-amifampridine 60 mg-placebo

Table 5.2 Estimated mean scores and treatment differences on primary and secondary outcome variables

	Estimated mean scores[a] (95% CI)			Treatment difference (95% CI)					
Outcome	**Placebo**	**Amifampridine 30 mg**	**Amifampridine 60 mg**	**Amifampridine 30 mg vs. placebo**	**Unadjusted p-value**	**Adjusted p-value**	**Amifampridine 60 mg vs. placebo**	**Unadjusted p-value**	**Adjusted p-value**
Primary outcome									
MGII total	26.6 (19.2, 33.9)	25.5 (18.2, 32.8)	24.9 (17.6, 32.2)	-1.0 (-4.1, 2.0)	**0.681**	**1.000**	-1.7 (-4.7, 1.4)	**0.379**	**1.000**
Secondary outcomes									
MGII ocular	9.3 (6.3, 12.4)	9.0 (6.0, 12.0)	8.5 (5.5, 11.5)	-0.3 (-1.6, 1.0)	0.814		-0.9 (-2.2, 0.5)	0.263	
MGII generalized	17.2 (11.2, 23.2)	16.5 (10.5, 22.5)	16.4 (10.4, 22.4)	-0.7 (-3.3, 1.9)	0.784		-0.8 (-3.5, 1.8)	0.725	
QMG	10.7 (8.0, 13.4)	10.6 (7.9, 13.2)	10.2 (7.5, 12.9)	-0.1 (-1.2, 0.9)	0.943		-0.5 (-1.6, 0.6)	0.482	
MG-ADL	5.9 (4.1, 7.7)	5.3 (3.5, 7.1)	5.8 (4.0, 7.6)	-0.6 (-1.5, 0.3)	0.253		-0.1 (-1.0, 0.8)	0.975	
MG-QoL15r	11.1 (7.9, 14.3)	10.1 (6.9, 13.3)	12.2 (9.0, 15.4)	-1.0 (-3.0, 0.9)	0.402		1.1 (-0.8, 3.0)	0.351	
TSQM-9	35.8 (33.0, 38.5)	37.0 (34.2, 39.8)	36.1 (33.3, 38.9)	1.2 (-3.3, 5.8)	0.792		0.4 (-4.2, 4.9)	0.979	

[a] Values are least square means from a mixed effects linear model that includes a fixed effect for treatment period and a random effect for participant.
Significance for pairwise comparisons vs. placebo was assessed using a Bonferroni correction for two tests ($p < 0.025$ considered significant). CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication.

The number of participants who correctly guessed their treatment allocation in the preceding period was 12 (60%) during amifampridine 30 mg treatment, 9 (45%) during treatment with amifampridine 60 mg, and 12 (60%) during placebo treatment. The investigators correctly guessed the treatment allocation in 10 participants (50%) during amifampridine 30 mg treatment, 9 participants (45%) during amifampridine 60 mg treatment, and in 11 participants (55%) during placebo treatment.
Following completion of the trial, 16 out of 20 participants elected to continue treatment with amifampridine, of whom 13 participants were still using amifampridine at 6 months, with a median daily dose of 37.5 mg (range 15.0-60.0 mg). **Supplementary Table 5.4** presents the patient and disease characteristics of the subgroup that continued treatment compared to those who discontinued. Median age and age at disease onset were 59 vs. 73 years (p=0.178) and 36 vs. 62 years (p = 0.122), respectively. Immunosuppressive therapy was used by 62% of those who continued amifampridine vs. 29% who discontinued (p=0.350).
During amifampridine 30 mg treatment, 12 (60%) participants correctly identified their treatment allocation, and 9 (45%) participants did so during amifampridine 60 mg treatment. During the placebo period, 12 (60%) participants guessed their allocation correctly. Similarly, investigators correctly predicted the treatment allocation for 10 (50%) participants during amifampridine 30 mg treatment, 9 (45%) participants during amifampridine 60 mg treatment, and 11 (55%) participants during the placebo period.
Side effects were reported by 6 (30%), 12 (60%), and 15 (71%) participants treated with placebo, amifampridine 30 mg, and amifampridine 60 mg, respectively (**Table 5.3**). Most frequently reported side effects with amifampridine were paresthesia, fatigue, numbness, dizziness, and sleep disturbances. Three patients discontinued treatment during the study: two during treatment with amifampridine 60 mg, and one during treatment with both amifampridine 30 mg and 60 mg. These patients specifically reported numbness, paresthesia, anxiety, dizziness and nausea as the reason for treatment discontinuation. All participants' adverse events improved after discontinuation of the study drug. No severe adverse events were reported. There was no statistically significant difference in vital signs (blood pressure, pulse rate, and respiratory rate) and QTc interval between placebo and treatment with amifampridine 30 mg or 60 mg. Results of laboratory testing are shown in **Supplementary Table 5.5**.

Amifampridine MR pharmacokinetics were best described by a two-compartment model with linear elimination and first-order absorption. The covariate NAT genotype was significantly correlated with amifampridine clearance. Model derived PK parameters of amifampridine MR were a mean C_{max} of 18.3 ± 6.4 ng/mL for the 30 mg dose, and 38.3 ± 16.3 ng/mL for the 60 mg dose. T_{max} was 98.7 ± 43.3 min for the 30 mg dose, and 91.6 ± 29.7

min for the 60 mg dose. C_{trough} was 2.1 ± 2.3 ng/mL for the 30 mg dose, and 5.8 ± 5.0 ng/mL for the 60 mg dose. The mean steady-state AUCτ was 117.7 ± 47.8 ng·h/mL for the 30 mg dose, and 248.5 ± 126.5 ng·h/mL for the 60 mg dose. The elimination half-life (t½) was comparable between doses, with 4.4 ± 1.5 hours for 30 mg and 4.4 ± 1.4 hours for 60 mg,

Table 5.3 Adverse events by treatment condition

	Placebo	**Amifampridine 30 mg**	**Amifampridine 60 mg**
Participants with ≥1 treatment emergent adverse event	6 (30%)	12 (60%)	15 (75%)
Treatment-emergent adverse events leading to discontinuation of the study drug	0	1 (11%)	3 (50%)
Numbness	0	1 (5%)	3 (15%)
Paresthesia	0	1 (5%)	2 (10%)
Anxiety	0	1 (5%)	2 (10%)
Dizziness	0	1 (5%)	2 (10%)
Nausea	0	0	2 (10%)
Treatment-emergent adverse events occurring in any participant in any treatment group			
Paresthesia	0 (0%)	4 (20%)	10 (50%)
Fatigue	3 (15%)	4 (20%)	8 (40%)
Numbness	0 (0%)	3 (15%)	8 (40%)
Dizziness	3 (15%)	4 (20%)	7 (35%)
Sleep disturbances	1 (5%)	5 (25%)	6 (30%)
Diarrhea	1 (5%)	3 (15%)	6 (30%)
Muscle spasms or muscle twitches	1 (5%)	1 (5.0%)	6 (30%)
Headache	1 (5 %)	5 (25%)	5 (25%)
Gastric discomfort	1 (5%)	3 (15%)	5 (25%)
Nausea	1 (5%)	2 (10%)	5 (25%)
Raynaud's syndrome	1 (5%)	2 (10%)	5 (25%)
Drowsiness	1 (5%)	2 (10%)	4 (20%)
Stomach ache	0 (0%)	2 (10%)	4 (20%)
Blurred vision	1 (5%)	2 (10%)	3 (15%)
Excessive or viscous mucus in the airways	1 (5%)	2 (10%)	3 (15%)
Rapid or irregular heartbeat	0 (0%)	0 (0%)	3 (15%)
Anxiety	0 (0%)	1 (5%)	2 (10%)
Cough	1 (5%)	1 (5%)	1 (5%)
Asthma	0 (0%)	1 (5%)	1 (5%)
Chorea	0 (0%)	0 (0%)	1 (5%)
Other[a]	2 (10%)	4 (20%)	1 (5%)

Data are number of patients (%) with one or more adverse event. Seizures were queried but not reported in either treatment period. [a] Other treatment-emergent adverse events:
- During placebo treatment: urinary urgency and left foot.
- During amifampridine 30 mg treatment: urinary urgency, hyperhidrosis, muscle weakness, and bowel symptoms.
- During amifampridine 60 mg treatment: urinary urgency.

respectively. No associations were found between the mean steady-state AUCτ, and C_{max}, and the primary outcome measure (**Figure 5.3**). The estimated odds ratio for dose-limiting side effects per ng/mL increase in C_{max} was 1.05 (95% CI: 0.99-1.12, p = 0.06), and the estimated odds ratio per ng·h/mL increase in steady-state AUCτ was 1.01 (95% CI: 1.00-1.02, p = 0.06).

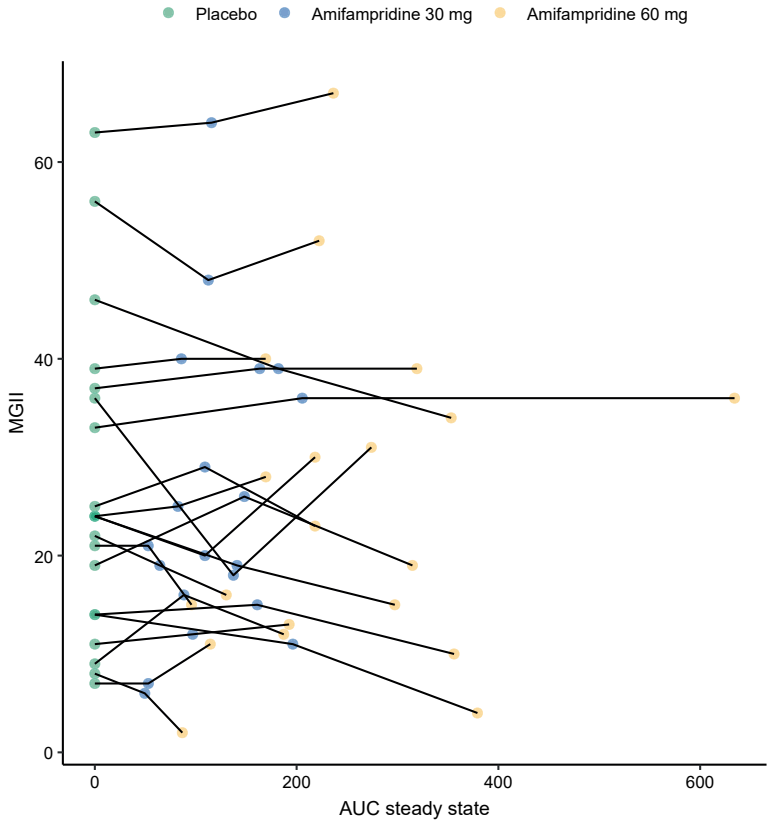

Figure 5.3 Association between area-under-the curve steady state and the primary outcome by treatment condition

Each point represents an individual patient, with lines connecting measurements from the same patient across different treatments. Colors indicate treatment conditions: placebo, amifampridine 30 mg, and amifampridine 60 mg. Data are based on a population pharmacokinetic model for amifampridine modified release, developed using a two-compartment linear approach. The dataset included 20 patients, with observed AUC data available for 8; in the remaining 12 patients, exposure was predicted using the model. This figure illustrates that AUC steady state increases with higher doses of amifampridine, with MGII scores varying independently of AUC steady state across patients.
MGII, Myasthenia Gravis Impairment Index; AUC, area under the concentration-time curve.

Discussion

IMPACT-MG, a randomized, double-blind, placebo-controlled crossover trial, evaluated the efficacy and safety of amifampridine MR in patients with AChR MG who remained symptomatic while on pyridostigmine therapy. The primary outcome, the mean change in MGII total score, showed no statically significant difference between amifampridine 30 mg, amifampridine 60 mg and placebo. Furthermore, no differences were observed across all secondary outcome measures, including the MGII ocular and MGII generalized subscores, as well as QMG, MG-ADL, MG-QoL15r, and TSQM-9, between amifampridine 30 mg, amifampridine 60 mg, and placebo. In addition, adverse events were reported more frequently with amifampridine than with placebo, with paresthesia, fatigue, numbness, dizziness, and sleep disturbances being the most commonly reported. In three patients, treatment-emergent adverse events were severe enough to necessitate discontinuation of amifampridine. These findings are supported by the additional PK/PD analyses, which did not show a statistically significant association between pharmacokinetic parameters and the primary outcome measure.

The strengths of our study include carefully controlled study procedures, e.g., all patients were evaluated throughout the study by the same investigator, and the inclusion of a comprehensive set of both patient-reported and investigator-assessed outcome measures allowing for a thorough evaluation of the impact of the treatment. Additionally, the study was marked by strong data integrity, with no drop-outs and complete data for primary outcomes (100%) and nearly complete data for secondary outcomes (>99%).

The study also had some limitations. First, the study design employed two fixed doses of amifampridine; 30 mg and 60 mg. However, some patients experienced a favorable response at 30 mg, but developed significant side effects at 60 mg, whereas others showed no response at 30 mg but did benefit from the 60 mg dose. Since it was deemed methodologically unsound to select the best individual dose post hoc and compare it to placebo, it is possible that an initial dose-titration phase to identify the optimal dose could have resulted in a demonstrable treatment effect. This could explain why such a substantial number of participants chose to continue using amifampridine once they were allowed to self-titrate their dosage. What argues against this, however, is the lack of an association between the PK parameters and the MGII. Second, the sample size in this study was relatively small. We did not account for multiple testing in the sample size calculation for this part of the trial, which in retrospect would have been appropriate. Even after applying this correction, however, 20 patients would still have been required (without adjustment for drop-out), corresponding to the number actually included. This sample size was estimated to provide sufficient power to detect a difference of 8 points on the MGII, corresponding to the established cutoff for a clinically meaningful difference at the group level. However, two

factors not accounted for in advance may have reduced its power. In part one of the IMPACT-MG trial, which assessed the efficacy of pyridostigmine in a largely similar patient population, the effect size was smaller than anticipated, with a highly significant mean improvement of 5.3 points. Therefore, the estimated minimal additional effect of amifampridine (an improvement of 8 points), was likely greater than could reasonably be expected. In addition, by the time participants were randomized into part two, they had already resumed pyridostigmine treatment, resulting in milder symptoms that made achieving an 8-point improvement more challenging compared to part one. Nonetheless, even if a significant difference were to be demonstrated with a larger sample size, the observed effect size of amifampridine in this study was small (1.7), and the clinical relevance of such an effect remains debatable.

In conclusion, the addition of amifampridine to pyridostigmine did not demonstrate a significant benefit compared to placebo on the prespecified primary and secondary outcome measures. In addition, more adverse events were observed during treatment with amifampridine. This trial provides insufficient support to justify adding amifampridine to standard treatment with pyridostigmine.

Supplements

Full list of amifampridine side effects queried

Paresthesia, numbness, gastric discomfort, nausea, diarrhea, stomach ache, cough, excessive or viscous mucus in the airways, blurred vision, rapid or irregular heartbeat, fatigue, headache, anxiety, dizziness, sleep disturbances, drowsiness, chorea, muscle spasms or muscle twitches, seizures, asthma and Raynaud's syndrome.

Supplementary Table 5.1 Pyridostigmine daily doses for each participant

Patient	Daily dose of pyridostigmine	Participation in:	
		Part one	Part two
1	30 mg four times daily	yes	yes
2	60 mg five times daily	yes	yes
3	60 mg five times daily	yes	yes
4	60 mg four times daily	yes	yes
5	60 mg five times daily	yes	yes
6	60 mg five times daily	yes	yes
7	60 mg six times daily	yes	yes
8	60 mg five times daily	yes	yes
9	60 mg five times daily	yes	yes
10	50 mg five times daily	yes	yes
11	60 mg four times daily	yes	yes
12	60 mg three times daily	yes	yes
13	1dd 90 mg and 4dd 80 mg	yes	yes
14	60 mg four times daily	yes	yes
15	60 mg four times daily	yes	yes
16	50 mg four times daily	yes	yes
17	60 mg three times daily	yes	yes
18	60 mg three times daily	yes	yes
19	60 mg four times daily	yes	yes
20	60 mg five times daily	no	yes

Supplementary Table 5.2 Acetylcholine receptor antibody status for each participant

Patient	AChR antibody titer (nmol/L) at baseline
1	11.8
2	3.3
3	5.5
4	15.8
5	17.2
6	2.7
7	15.6
8	19.6
9	2.5
10	17.4
11	13.8
12	8.1
13	1.4
14	10.1
15	1.2
16	1.2
17	21.1
18	7.0
19	9.3
20	18.4

AChR antibodies were measured by using a standard dilution of the sample in a radioimmunoassay (RSR Limited, Cardiff, UK). This assay provides a numerical value that is linearly correlated with actual serum concentrations up to a value of 20 nmol/L.

Supplementary Table 5.3 Treatment by period interaction for primary and secondary outcome measures

		Amifampridine 30 mg		Amifampridine 60 mg	
	Visit	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Primary outcome					
MGII total	Second	9.8 (-21.5, 41.1)	0.517	-15.2 (-45.3, 14.9)	0.303
MGII total	Third	21.0 (-9.2, 51.1)	0.161	9.8 (-21.5, 41.1)	0.519
Secondary outcomes					
MGII ocular	Second	8.1 (-4.0, 20.2)	0.174	-5.3 (-16.9, 6.4)	0.353
MGII ocular	Third	13.6 (1.9, 25.2)	0.025	6.9 (-5.2, 19.0)	0.244
MGII generalized	Second	1.7 (-25.4, 28.8)	0.897	-9.9 (-36, 16.2)	0.436
MGII generalized	Third	7.4 (-18.7, 33.5)	0.560	2.9 (-24.3, 30.0)	0.827
QMG	Second	0.7 (-11.6, 13.0)	0.907	-2.2 (-14.0, 9.6)	0.701
QMG	Third	1.8 (-10.0, 13.6)	0.751	1.1 (-11.2, 13.4)	0.851
MG-ADL	Second	1.8 (-5.4, 9.0)	0.609	-4.8 (-11.7, 2.2)	0.166
MG-ADL	Third	7.4 (0.5, 14.3)	0.038	2.7 (-4.5, 9.9)	0.441
MG-QoL15r	Second	7.3 (-6.2, 20.8)	0.272	-3.7 (-16.7, 9.3)	0.560
MG-QoL15r	Third	9.9 (-3.2, 22.9)	0.129	5.8 (-7.8, 19.3)	0.384
TSQM-9	Second	-0.5 (-11.0, 9.9)	0.916	6.2 (-4.0, 16.4)	0.226
TSQM-9	Third	1.8 (-8.4, 11.9)	0.728	4.7 (-5.7, 15.2)	0.364

CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication

Supplementary Table 5.4 Patient and disease characteristics of patients who continued vs. discontinued amifampridine treatment

	Discontinued amifampridine after trial completion N = 7	**Continued amifampridine after trial completion** N = 13
Age, years	73 (55, 77)	59 (36, 64)
Age at disease onset, years	62 (51, 72)	36 (25, 57)
Sex, female	3 (43%)	8 (62%)
BMI, kg/m2	26.2 (24.8, 28.7)	28.2 (26.4, 28.7)
Disease duration, years	5 (2, 8)	6 (2, 19)
Previous thymectomy, yes	2 (29%)	5 (38%)
MGFA score at inclusion		
I	2 (29%)	1 (7.7%)
II	5 (71%)	10 (77%)
III	0 (0%)	2 (15%)
MGII total score at inclusion	29 (19, 34)	30 (21, 45)
NAT2 enzyme acetylator phenotype		
Fast	2 (29%)	7 (54%)
Slow	5 (71%)	6 (46%)
QTc, milliseconds	403 (398, 415)	401 (391, 404)
Therapies in previous year		
No therapy	5 (71%)	5 (38%)
Prednisolone	1 (14%)	6 (46%)
Azathioprine	0 (0%)	2 (15%)
Mycophenolate mofetil	1 (14%)	3 (23%)
Cyclosporine	1 (14%)	0 (0%)
Methotrexate	0 (0%)	1 (7.7%)
IVIg on a regular basis	0 (0%)	1 (7.7%)

Data are median (Q1-Q3) or n(%). Abbreviations: MGFA, Myasthenia Gravis Foundation of America; MGII, Myasthenia Gravis Impairment Index; NAT2, N-acetyltransferase 2; QTc, corrected QT interval; IVIg, intravenous immunoglobulin therapy.

Supplementary Table 5.5 Laboratory results

	Baseline N = 20	**Last visit N = 19**
Hemoglobin	8.7 (8.3, 9.6)	8.4 (7.6, 8.9)
Unknown	1	0
Hematocrite	0.4 (0.4, 0.5)	0.4 (0.4, 0.4)
Unknown	1	0
Leukocytes	6.8 (5.7, 9.4)	5.9 (5.1, 8.1)
Unknown	1	0
Thrombocytes	238.0 (210.0, 291.5)	234.5 (199.5, 284.3)
Unknown	1	0
Sodium	141.0 (139.8, 143.3)	141.0 (138.0, 143.0)
Unknown	0	1
Potassium	4.6 (4.4, 4.8)	4.2 (4.1, 4.4)
Unknown	0	1
Creatinine	62.5 (56.5, 78.0)	63.0 (54.5, 76.0)
Unknown	0	1
eGFR	90.0 (88.3, 90.0)	90.0 (89.5, 90.0)
Unknown	0	1
Aspartate aminotransferase (ASAT)	21.5 (19.8, 26.5)	20.5 (18.0, 23.5)
Unknown	0	1
Alanine aminotransferase (ALAT)	22.5 (15.8, 26.3)	21.0 (16.0, 23.0)
Unknown	0	1
Alkaline phosphatase	70.0 (59.8, 80.3)	67.0 (62.5, 79.5)
Unknown	0	1
Gamma glutamyltransferase	12.5 (11.0, 22.8)	13.0 (10.5, 23.0)
Unknown	0	1
Creatinine kinase	79.5 (55.3, 102.8)	93.0 (68.5, 118.5)
Unknown	0	1
Glucose	5.0 (4.9, 5.7)	5.2 (4.8, 6.5)
Unknown	0	1
AChR antibodies	9.7 (3.2, 16.2)	8.8 (3.2, 12.4)

Data are median (Q1-Q3).