Clinvest Research, LLC.
Statistical Analysis Plan

Study Title:	**A Multicenter, Randomized, Double-blind, Placebo-controlled Study Evaluating the Efficacy and Safety of MLD10 in the Prevention of Migraine Headache in Adults**
Protocol Number:	**MLD10-002**
Sponsor:	Pharmalyte Solutions, LLC. 580 Commerce Street Suite 100 Southlake, TX 76092
Medical Monitor:	Timothy R. Smith MD, RPh, FACP 4350 S. National Ave. Suite C100 Springfield, MO 65810 Phone: (417) 883-7889 Email: timothy.smith.md@gmail.com
Trial Statistician	Jim Sly, MS 4350 S. National Ave. Suite C100 Springfield, MO 65810 Phone: (417) 841-3673 Email: jsly@clinvest.com
SAP Authors	Jim Sly, MS and Heather Manley, MS
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Approved by:	**Date:**	**Signature:**
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1 Abbreviations and Definitions

AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BOCF	Baseline Observation Carried Forward
C-SSRS	Columbia-Suicide Severity Rating Scale
EDC	Electronic Data Capture
ICHD	International Classification of Headache Disorders
IRB	Institutional Review Board
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary of Regulatory Affairs
MIDAS	Migraine Disability Assessment
mITT	Modified Intent-to-Treat
MO	Medication Overuse
MOH	Medication Overuse Headache
NSAE	Non-Serious Adverse Event
PGIC	Physician Global Impression of Change
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SGIC	Subject Global Impression of Change

2 Introduction

2.1 Study Purpose

- To evaluate the daily use of MLD10 and placebo for the treatment of migraine, as measured by the change from baseline in the number of migraine headache days of subjects treated for 3 months.
- To compare daily use of MLD10 and placebo as measured by:
  - change in the quantitative and qualitative aspects of migraine headaches from baseline, during treatment, and at the conclusion of a 3-month treatment period.
  - safety and tolerability measures in migraine headache subjects through the monitoring of adverse events (AEs) and clinical chemistry values.

2.2 Summary of Study Design

This is a multi-center, randomized, double-blind, placebo-controlled, parallel study of MLD10 for the prevention of migraine headache. The study population will consist of approximately 142 male and female subjects between 18 and 65 years of age with frequent episodic migraine as defined by International Classification of Headache Disorders beta (ICHD-3) criteria. Subjects agreeing to participate in the study and meet the entry criteria assessed at the screening visit, will begin a 28-day baseline period to confirm their diagnosis, as well as establish baseline migraine characteristics. During this baseline period, subjects will continue treating their migraines as usual, simply recording the information in a daily headache diary. Subjects who, after completing the baseline, continue to meet entrance criteria will be eligible to enter into the treatment phase and be randomized according to the Clinvest generated randomization schedule. Approximately 142 subjects (71 subjects per arm) will be randomized and enter the treatment phase receiving MLD10 or placebo in a 1:1 design at a maximum of 8 US sites. Two MLD10 (243 mg of elemental magnesium) or placebo caplets will be taken twice daily for a total daily dose of 486 mg. Diary assessments will collect study medication adherence, pain severity, headache symptoms, acute medication usage, and unusual symptoms. Serum samples will be collected and analyzed for ionized Mg, electrolytes, and creatinine.

2.3 Power Analysis

A power analysis, using GPower Version 3.1.7, indicated a total sample size of 128 subjects would be needed to detect a medium effect with 80% power using an analysis of covariance (ANCOVA) test to include fixed effects, main effects, and interactions with alpha being .05. Given an estimated 10% early termination rate, a total estimated sample size of 142 subjects is needed.

2.4 Inclusion-Exclusion Criteria

Inclusion Criteria

Subjects must meet the following inclusion criteria:

1. male or female, in otherwise good health, 18 to 65 years of age.
2. history of frequent episodic migraine (3-14 migraine days per month) (with or without aura) according to the ICHD-3 beta for at least 3 months.
3. onset of migraine before age 50.
4. stable history of migraine at least 3 months prior to screening.

5. not currently taking a migraine preventive or has been taking preventive for at least 30 days prior to screening and agrees to not start, stop, or change medication and/or dosage during the study period.
6. if female of childbearing potential, has a negative urine pregnancy test at Visits 1-5 and uses, or agrees to use, for the duration of the study, a medically acceptable form of contraception as listed:
    - complete abstinence from intercourse from 2 weeks prior to administration of study drug, throughout the study, and for 7 days after completion or premature discontinuation from the study; surgically sterile (hysterectomy or tubal ligation or otherwise incapable of pregnancy); sterilization of male partner when in a monogamous relationship; intrauterine device with published data showing lowest expected failure rate is less than 1% per year; double barrier method (i.e., 2 physical barriers OR 1 physical barrier plus spermicide) for a least 1 month prior to Visit 1 and throughout study; or hormonal contraceptives for at least 3 months prior to Visit 1 and throughout study.
7. completion of online diary must be ≥ 80% compliance, unless otherwise approved by the Sponsor and/or Clinvest.

Exclusion Criteria

Subjects must **NOT** meet any of the following exclusion criteria:

1. unable to understand the study requirements, the informed consent, or complete headache records as required per protocol.
2. pregnant, actively trying to become pregnant, or breast-feeding.
3. diagnosed with ICHD-3 beta criteria for Chronic Migraine within 3 months prior to screening, at the time of screening, and/or during the baseline period.
4. experienced the following migraine variants: basilar migraine, aura without headache, familial hemiplegic migraine, complicated migraine, ophthalmoplegic migraine and retinal migraine within the last year.
5. history of medication overuse headache (MOH) in the 3 months prior to study enrollment or during the baseline phase.
6. history of medication overuse (MO) of ergotamines, triptans, opioids, analgesics, NSAIDS and combination therapies, as defined by ICHD-3 beta criteria and/or MO during baseline period.
7. history of substance abuse and/or dependence, in the opinion of the Investigator.
8. history of impaired renal function that, in the investigator's opinion, contraindicates participation in this study.
9. unstable neurological condition or a significantly abnormal neurological examination with focal signs or signs of increased intracranial pressure.
10. suffers from a serious illness, or an unstable medical condition, one that could require hospitalization, or could increase the risk of adverse events.
11. has significant risk of suicide, defined as a "yes" answer to any of the following questions on the Columbia-Suicide Severity Rating Scale (C-SSRS), either at the screening visit (when assessing the prior 12 months) or at visit 2 (when assessing time since the screening visit):

   a. Questions 4 or 5 on the suicidal ideation section
   b. Any question on any item in the suicidal behavior section
12. any psychiatric disorder with psychotic features, and/or any other psychiatric disorder not stable or well controlled, that would interfere in their ability to complete study activities.
13. hypersensitivity, intolerance, or contraindication to the use of magnesium L-lactate dehydrate or any of its components.
14. received any investigational agents within 30 days prior to Visit 1.
15. plans to participate in another clinical study at any time during this study.

2.5 Randomization and Blinding

Eligible subjects will be randomized 1:1 to receive magnesium L-lactate dehydrate (243 mg of elemental mg++ BID for a total of 486mg daily) or placebo at Visit 2. A 2-block randomization schedule will be created prior to study initiation using www.randomization.com and uploaded into the EDC system. Randomization will not include any type of stratification. Randomization will include attesting in the EDC the subject meets inclusion/exclusion criteria and is appropriate for the study. After this determination is made, the subject will be assigned to an auto populated drug kit number which will display in the source document in the EDC.

At randomization (Visit 2), neither the subject nor the investigator will be aware to which treatment group the subject has been assigned. If needed, for safety and proper treatment of the subject, the investigator can unblind the subject's treatment assignment to determine which treatment has been assigned and institute appropriate follow-up care. When possible, Clinvest should be notified prior to unblinding study medication.

Individual unblinding envelopes are shipped with study medication. Each study medication kit has a corresponding unblinding envelope. Unblinding will include matching the kit and unblinding envelope with the subject drug number.

To unblind a subject without breaking the blind for remaining subjects' treatment the following instructions described below will be followed:

- Obtain the security envelope containing the unblinding envelopes.
- Remove the unblinding envelope with the study drug number corresponding with the study medication kit dispensed to the subject.
- Break the seal of the unblinding envelope and remove the subjects' treatment label.
- Return the subjects' treatment label to the unblinding envelope.
- Return the unblinding envelope to the security envelope.
- Document on the unblinding form located in the eAdmin Binder.
- Email copy of unblinding form to study@clinvest.com within 24 hours of unblinding.
- Complete and email or fax the Sterling Institutional Review Board (IRB), unanticipated problem report form, to Sterling IRB within 10 business days of unblinding.

2.6 Study Procedures

	Screening	Randomization	Treatment		
	Visit 1 Day 0	Visit 2 Day 29 (+/- 3)	Visit 3 Day 58 (+/- 3)	Visit 4 Day 87 (+/- 3)	Visit 5 Day 116 (+/- 3)
Informed Consent	X				
Physical/Neurological Exam	X				X
Vital Signs	X	X	X	X	X
Verify Inclusion/Exclusion	X	X			
Subject Randomization		X			
Medical History	X				
Migraine History	X				
Medication History	X				
Update Medications		X	X	X	X
Urine Pregnancy Test	X	X	X	X	X
SGIC					X
PGIC					X
Dispense Study Medication		X	X	X	
Drug Accountability			X	X	X
Headache Diary	X	X	X	X	
Review Diary		X	X	X	X
Collect Adverse Events		X	X	X	X
Administer C-SSRS	X	X	X	X	X
Administer MIDAS		X			X
Serum Electrolytes & Creatinine		X	X	X	X
Serum Ionized Mg		X	X	X	X

3 Analysis Populations

3.1 Full Analysis Population

All subjects who were randomized. The full analysis population will be used for demographic and adverse event reporting.

3.2 Modified Intent-to-Treat Population (mITT)

The modified intent-to-treat (mITT) population will include all randomized subjects who received at least one dose of study drug and obtained at least one endpoint measurement (mean change from baseline to treatment month, in the number of migraine headaches) after treating. The mITT population will be used for all efficacy analyses.

3.3 Safety Population

The safety population will include all randomized subjects who received at least one dose of study drug (active or placebo). The safety population will be used for all safety endpoints.

4 Endpoints

4.1 Primary Endpoint

To compare the mean change from baseline in the frequency of migraine headache days per 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo.

4.2 Secondary Endpoints

1. To compare change from baseline of subjects treated with MLD10 versus placebo on the following parameters during or after a 3-month treatment period:
    1. the frequency of headache days
    2. the average headache duration after 1, 2, and 3 months of treatment
    3. reduction of pain severity at the time of headache onset
    4. the use of acute medications after 1, 2, and 3 months of treatment
    5. Migraine Disability Assessment Scale (MIDAS) scores at Visits 2 and 5
    6. Subject Global Impression of Change (SGIC) at Visit 5
    7. Physician Global Impression of Change (PGIC) at Visit 5
2. To assess the safety and tolerability of magnesium-L-lacate-dihydrate versus placebo in migraine headache subjects through the comparison of adverse events.

4.3 Exploratory Endpoints

To compare the change from baseline of subjects treating with MLD10 versus placebo on the following parameters during a 3-month treatment period.

- serum ionized magnesium

4.4 Safety Endpoints

Safety assessments will include:

- adverse events

5 Collection and Derivation of Endpoint Variables

Data required for the evaluation of endpoints will be recorded for the duration of the study using electronic data capture (EDC) and include subject and clinician reported outcomes. Subjects will record data into the web-based EDC using a daily eDiary. Subjects will be provided instruction and training regarding daily eDiary entry and compliance requirements.

Clinic visits will be scheduled to occur every 28 days throughout the study; however, in practice, there may or may not be an exact 28-day duration between 2 consecutive visits. For this reason, subjects will be given access to 31 days of eDiary to complete between clinic visits. For the collection and derivation of endpoint variables and analyses, subject's eDiary information will be used as follows:

- eDiary information entered during the first 28 continuous days of the run-in period will serve as the "baseline" for calculating change from baseline for 28-day periods subsequent to each office visit. If the run-in period (starting with the screening visit

and preceding the day before visit 2) exceeds 28 days, the run-in period will include the last 28-days.

- If a subject enters more than 28 days of eDiary information between clinic visits during the treatment phase of the study, the last (most recent) 28 eDiary entries will be used for analyses. For example, if a subject has a duration of 31 days between Visit 2 and Visit 3, and the subject entered eDiary information for all 31 days, the last 28 days of eDiary information will be used to calculate and derive endpoint variables, with the first three eDiary entries being discarded from the analysis datasets.
- Each subject-entered eDiary record will be used and analyzed as a separate study day, regardless of the subject's entered diary date (value entered from the subject as the date of diary data), actual date of eDiary entry (EDC timestamp), or perceived duplicate entry.

5.1 Primary Efficacy Variable

The primary efficacy variable is the mean change from baseline in the frequency of migraine headache days per 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 3, encompassing the last 28-day period before study end. This variable is derived from the e-dairy and is based on the count of days with migraine headaches. Migraine headaches are defined as a calendar day (00:00 to 23:59) with 4 or more hours of migraine headache, fulfilling ICHD-3 beta criteria, and/or any headache of any duration with the use of migraine-specific acute medications(s) (i.e. ergot alkaloids, ergot combinations, opioids, triptans, combination analgesics [simple analgesics combined with opioids or barbiturate with or without caffeine]). Headache duration for the determination of migraine will be derived from the eDiary Yes/No question "*Did you have at least four (4) consecutive hours of headache?*".

5.2 Secondary Efficacy Variables

1. The frequency of headache days. This secondary efficacy variable is the mean change from baseline in the frequency of headache days per 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 3, encompassing the last 28-day period before study end. This variable is derived from the e-dairy and is based on the count of days with headaches. The determination of a headache day will be derived from the eDiary Yes/No question "*Did you experience a headache of any severity yesterday?*".
2. The average headache duration after 1, 2, and 3 months of treatment. This secondary efficacy variable is the mean change from baseline in the duration of headaches per each 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 1, 2, and 3. This variable is derived from the eDiary and is based on the difference (measured in minutes) between the subject-reported start and stop time for any headache days. eDiary records where there are subject-entered data errors (e.g., a headache stop time recorded as *prior* to the headache start time) will be excluded from analysis.

3. Reduction of pain severity at the time of headache onset after 1, 2, and 3 months of treatment. This secondary efficacy variable is the mean change from baseline in pain severity of headaches per each 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 1, 2, and 3. Pain severity will be determined from the eDiary question "*What was the greatest severity that your headache reached?*" and be coded as: 1 = Mild, 2 = Moderate, 3 = Severe.
4. The use of acute medications after 1, 2, and 3 months of treatment. This secondary efficacy variable is the mean change from baseline in the total number of acute headache pain medications used per each 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 1, 2, and 3. This variable is derived from the eDiary and is calculated by summing all entries from the eDiary questions "*Number of times medication was taken?*"
5. Migraine Disability Assessment Scale (MIDAS) scores at Visits 2 and 5. This secondary efficacy variable is the mean change from Visit 2 and Visit 5 (or Early Termination Visit) total MIDAS scores in subjects treated with MLD10 versus placebo. The time point for this variable is beginning of randomization to end of treatment month 3 (end of study). This variable is derived from the validated scoring of the MIDAS questionnaires at Visit 2 and end of study.
6. Subject Global Impression of Change (SGIC) at Visit 5. This variable will be derived from the subject questionnaire at the end of study, "*Since the beginning of the study, do you believe you are now*" and be coded as: -3 = Extremely Worse, -2 = Moderately Worse, -1 = Somewhat Worse, 0 = No Change, 1 = Somewhat Better, 2 = Moderately Better, 3 = Extremely Better.
7. Physician Global Impression of Change (PGIC) at Visit 5. This variable will be derived from the physician question at the end of study, "*Since the beginning of the study, do you believe the subject is now"* and be coded as: -3 = Extremely Worse, -2 = Moderately Worse, -1 = Somewhat Worse, 0 = No Change, 1 = Somewhat Better, 2 = Moderately Better, 3 = Extremely Better.

5.3 Exploratory Variables

Serum ionized magnesium levels. This exploratory variable is the mean change from baseline in serum ionized magnesium levels per 28-day period ending with the cessation of treatment period month 3 in subjects treated with MLD10 versus placebo. The time point for this variable is baseline to treatment month 1, 2, and 3. This variable is derived by taking the mean of two analyzed sample values at each visit (Visits 2, 3, 4, and 5/ET).

5.4 Safety Variables

Non-Serious adverse events (NSAEs) and Serious adverse events (SAEs) will be recorded in the EDC system by study staff and/or physicians and be coded to Medical Dictionary of Regulatory Affairs (MedDRA) preferred terms by the Clinvest Research Data Management team.

6 Missing Data

A last observation carried forward (LOCF) method will be utilized to impute missing values for all mITT population subjects with missing values for a specific analysis. This type of imputation replaces the missing value with the last observation value obtained. Baseline observation carried forward (BOCF) method will be used if a subject discontinues the study prior to receiving at least one dose of study drug and obtaining at least one endpoint measurement (mean change from baseline in the number of migraine headaches) after treating. Thus, if a subject has a missing value at month 2 (Treatment Month 1) due to discontinuation, the baseline value will be utilized and the subject will be considered a non-responder. If no prior observation is available to use for BOCF or LOCF, the mean value of all eligible subjects for that particular analysis will be imputed for the missing value.

7 Timing of Final Analyses

The final analyses will be performed after:

- the finalization and approval of this statistical analysis plan (SAP) document
- all randomized subjects have exited the study by completing Visit 5 or early terminated prior to completion
- the EDC database has been locked

8 Efficacy Analyses

All statistical tests will be two-tailed, and an alpha of .05 will be used for statistical significance. All analyses of variance (ANOVAs) will be followed by univariate post-hoc tests as appropriate. Multiple comparison adjustments will be made if needed.

8.1 Primary Efficacy Analysis

Data for the primary endpoint will be statistically analyzed via a repeated measures ANCOVA controlling for Visit 2 ionized serum Mg levels. The analysis will compare the mean change in the frequency of migraine headache days from baseline to treatment month 3 in subjects treated with MLD10 versus placebo using Visit 2 ionized serum Mg levels as the covariate.

8.2 Secondary Efficacy Analyses

8.2.1 Frequency of headache days

Data for this secondary endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in the frequency of headache days from baseline to treatment month 3 in subjects treated with MLD10 versus placebo.

8.2.2 Headache duration after 1, 2, and 3 months of treatment

Data for this secondary endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in headache duration (measured in minutes) from baseline to treatment months, 1, 2, and 3 in subjects treated with MLD10 versus placebo.

8.2.3 Pain severity at the time of headache onset

Data for this secondary endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in headache pain severity from baseline to treatment months, 1, 2, and 3 in subjects treated with MLD10 versus placebo.

8.2.4 Acute medications after 1, 2, and 3 months of treatment

Data for this secondary endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in the total number of acute headache pain medications used from baseline to treatment months, 1, 2, and 3 in subjects treated with MLD10 versus placebo.

8.2.5 Migraine Disability Assessment Scale (MIDAS) scores at Visits 2 and 5

Data for this secondary endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in total MIDAS scores from Visit 2 (randomization) to Visit 5 (end of study) in subjects treated with MLD10 versus placebo.

8.2.6 Subject Global Impression of Change (SGIC) at Visit 5

Data for this secondary endpoint will be statistically analyzed via an independent samples *t*-test. The analysis will compare the mean difference of SGIC between subjects treated with MLD10 versus placebo.

8.2.7 Physician Global Impression of Change (PGIC) at Visit 5

Data for this secondary endpoint will be statistically analyzed via an independent samples *t*-test. The analysis will compare the mean difference of PGIC between subjects treated with MLD10 versus placebo.

8.3 Exploratory Efficacy Analysis

Data for this exploratory endpoint will be statistically analyzed via a repeated measures ANOVA. The analysis will compare the mean change in serum ionized magnesium levels from baseline to treatment months, 1, 2, and 3 in subjects treated with MLD10 versus placebo.

9 Safety Analysis

9.1 Adverse Events

Data for this safety endpoint will be statistically analyzed via an independent samples *t*-test. The analysis will compare the mean difference of total adverse events between subjects treated with MLD10 versus placebo.