At The Cyprus Institute of Neurology And Genetics

By PALUPA Medical Ltd. (Incubation Company)

Project Title:

Proof-of-Concept Clinical Trial:

Novel medical nutrition formulas as possible new approach for relapsing multiple sclerosis treatment: a double-blind, randomized, placebo-controlled proof-of-concept clinical trial.

Clinical Trial Protocol and Project Design

Completed

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PALUPA Medical Ltd.

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PROTOCOL

Medical Nutrition Formulas for the Treatment of Relapsing Multiple Sclerosis

A Single-Center, Parallel-group, Proof-of-Concept, Double-blind, Randomized, Placebo-Controlled, Trial of Efficacy and Safety.

Purpose

The purpose of this proof-of-concept study is to determine the safety and efficacy of three novel interventions in the treatment of individuals who have been diagnosed with relapsing remitting multiple sclerosis (MS). We intend to evaluate the therapeutic ability of PLP10 and of two other interventions consisting of PLP10 constituent partial fractions versus placebo, when used in patients with relapsing remitting (RR) MS. It is hoped that the agents used for the composition of the interventions in general will be able to normalize structural molecules of cell membranes, normalize phospholipase function and immune system, normalize endoplasmic reticulum function/stress, specific gene expression, quench free nitro- and oxo- free radical derivatives, elevate anti-inflammatory cytokines, halt apoptosis, normalize metalloproteinases function, supply major building blocks for myelin formation/reformation, promote remyelination and neuroprotection and so halt inflammation and all related cascade of events leading to the MS pathogenesis. All these simultaneously involved pathogenic mechanisms as a result of multiple factors leading to this complex disease are thought to be the cause that can result in lesions (small areas of damage) in the brain and spinal cord, the central nervous system (CNS). These lesions are thought to be the cause of relapses and disability in MS.

Condition : Multiple Sclerosis, Relapsing-Remitting

Intervention

Drug: A (oral cocktail formula of structured lipids),

Drug: B (PLP10) (oral cocktail formula of structured molecules, lipids and vitamins),

Drug: C (antioxidant vitamin),

Drug: Placebo (virgin olive oil)

Phase : II (Proof-of-Concept)

Investigator/Trial Location: By PALUPA Medical Ltd. At The Cyprus Institute of Neurology and Genetics

Study Type: Interventional

Study Design: Allocation: Randomized

Endpoint Classification: Efficacy /Safety Study

Intervention Model: Parallel Assignment

Masking: Double Blind (Subject, Investigator, Total involved Personnel, Outcomes Assessor)

Primary Purpose: Treatment/adjuvant treatment

Investigator Specialties: Neurology, Clinical Biochemistry (Lipidology, Immunology) and Clinical Dietitian. All investigators are experienced and with more than 20 years in practice.

Enrollment: Total number of MS patients in Cyprus meeting the required criteria.

Arms	Assigned Interventions
Group 1: Experimental A, Oral Intervention: Drug: A (structured lipids)	Drug: A† Cocktail of EPA/DHA re-esterified triglycerides, triglycerides of LA/GLA, vitamins A, E as alpha tocopherol, 15.3 ml, oral, daily 30 minutes before dinner, for 30 months.
Group 2: Experimental B-PLP10, Oral Intervention: Drug: B-PLP10 (structured lipids and vitamins)	Drug: PLP10† Cocktail of EPA/DHA re-esterified triglycerides, triglycerides of LA/GLA, vitamins A, E as alpha tocopherol, E as gamma tocopherol, 16.0 ml, oral, daily 30 minutes before dinner, for 30 months.
Group 3: Experimental C, Oral Intervention: Drug: C (antioxidant vitamin)	Drug: C† Gamma tocopherol (vitamin E isoform/antioxidant), 760 mg (in pure virgin olive oil as a vehicle), 17.0 ml oral, daily 30 minutes before dinner, for 30 months.
Group 4: Placebo Comparator D-Placebo Oral Intervention: Drug: Placebo	Drug: Placebo† Placebo (Pure Virgin Olive Oil), 17.0 ml, oral, daily 30 minutes before dinner, for 30 months.

Preparations for the daily intervention formula agent dosages will be:
Intervention formula A daily dosage: EPA (1650mg) / DHA (4650mg) / GLA (2000mg) / LA (3850mg) / total other omega-3 (600mg) / total monounsaturated fatty acids (MUFA) (18:1 1300mg, 20:1 250mg, 22:1 82mg, 24:1 82mg) + total saturated fatty acids (SFA) (18:0 160mg,

16:0 650mg) / vitamin A (0.6mg) / vitamin E (22mg).

Intervention formula B (PLP10) daily dosage: EPA (1650mg) / DHA (4650mg) / GLA (2000mg) / LA (3850mg) / total other omega-3 (600mg) / total MUFA (18:1 1300mg, 20:1 250mg, 22:1 82mg, 24:1 82mg) + total SFA (18:0 160mg, 16:0 650mg) / vitamin A (0.6mg) / vitamin E (22mg) / gamma- tocopherol (γ-tocopherol) (760 mg).

Intervention formula C daily dosage: γ-tocopherol (760 mg) (in 16137 mg pure virgin olive oil as a vehicle).

Intervention formula D daily dosage**:** pure virgin olive oil (16930mg).

† Citrus aroma will be added in each intervention formula to make up a total dosage of 19.5ml of solution per day.

Stabilization and Standardization by supplier

Intervention Preparation

The specific omega-3 (re-esterified glycerides) and omega-6 (glycerides) raw materials will be purchased according to the required interventions' fraction structural, quantity/ratio and quality specification (vitamin E (alpha-tocopherol) will be used as antioxidant stabilizer by the supplier). The Vitamins and food grade masking aroma will be purchased separately. The mixing of fractions to the final required intervention composition specification will always be performed by the same team of scientists under the supervision of the involved medical biochemist and lipidology specialist, under appropriate conditions every six months. Interventions will be stored refrigerated in dark until use.

Inclusion Criteria

- Men and women
- Ages of between 18 and 65 years
- Diagnosis of relapsing remitting Multiple Sclerosis (RRMS) according to McDonald criteria
- A score of 0.0 to 5.0 on the Expanded Disability Status Scale (EDSS)
- At least one medically documented relapse within the 18 months before enrollment

- Cranial MRI scan demonstrating lesion(s) consistent with MS
- On Interferon beta (IFN-β), glatiramer acetate treatment for the last 6 continuous months or more, or on no treatment

Exclusion Criteria

- Prior immunosuppressants or monoclonal antibodies (natalizumab should be available in the market in 2006) therapy
- A recent (<30 days) relapse
- Pregnancy or nursing
- A clinically significant infectious illness within 30 days prior to randomization
- Primary progressive, secondary progressive or progressive relapsing MS
- Patients known to have a history of recent drug or alcohol abuse
- Any severe disease other than MS compromising organ function, meaning : history of, or abnormal laboratory results indicative of any significant cardiac, endocrinologic, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, gastrointestinal, dermatologic, renal and/or other major disease, that in the opinion of the investigators, would preclude the administration of PLP10 for 30 months.
- History of severe allergic or anaphylactic reactions or known specific nutritional hypersensitivity.

Note

During ***"on intervention treatment"*** the patients (depending on their pathological status) will be treated according to the normal international guidelines. A thorough record of treatment (or change of treatments) for each patient will be kept. If a patient changes therapy to immunosuppressant or monoclonal antibody or any other treatment on physicians' decision then he/she will be considered as a drop-out; but continue to be medically followed for the intention to treat analyses purposes.

If a clinical documented relapse is reported during "**normalization**" period the entry baseline EDSS for that patient will be reported as the EDSS score documented at least 4 weeks after the last relapse during this period.

"**Normalization period**" will be a 6 months period between enrolment and entry baseline (the first 6 months of the trial that patients will be on the intervention treatment for only normalization purposes). For the result analyses this 6 month period will be considered as a period before entry baseline for all four treatment arms.

Due to the nature of the intervention ingredients/agents the protocol of the clinical study is considering a normalization period for the interventions' agents to exert their beneficial effect. The normalization period is used: (a) because the incorporation/normalization of the immune cells' membranes by dietary oral agents is a long time process, (b) because the T-lymphocytes are produced in a very slow rate in adulthood and even much slower in older people, (c) because oral fatty acids need 6 months to have a neurological effect in animal models, (d) to eliminate any placebo effect and regression to the mean, and finally (e) to correct specific body lipid deficiency, and thus accurately record the efficacy as a result of the interventions. It is strongly suggested that specific lipid deficiency need to be "corrected and things be normalized as much as possible" before evaluating their effect.

Primary end points

Primary outcome will be the **annual relapse rate (ARR)**

- The study is designed to end 30 months after enrolment and neurological and clinical assessments should be scheduled at entry baseline (6 months after enrollment, the end of normalization period) and at 3, 9, 15, 21 and 24 months on-treatment. A 12 month (extension) period (free of intervention) will be followed with relapse recording.
- Patients should be examined within 48 hours after the onset of new neurological symptoms for the treating physician to confirm and record the relapse as per protocol.

Relapses are defined as new neurologic symptoms or worsening of pre-existing symptoms (that are stable for at least 1 month) not associated with fever or infection that lasts for at least 24 hours and characterized by new or worsening neurological signs on examination.

The Key Secondary end point will be the **time to confirmed disability progression**

Time to confirmed disability progression is defined as an increase of 1.0 on the EDSS from a baseline score, confirmed after 6 months, with an absence of an ongoing relapse at the time of assessment (progression cannot be confirmed during a relapse) and with no documented relapse during the 6-months period needed for the confirmation. Under the same conditions as previously discussed, the final EDSS score should also be confirmed 6 months after the end of the study.

A post-hoc analysis

A post-hoc analysis will be performed assessing the proportion of patients free from new or enlarging T2 lesions on brain MRI scans at the end of the study for the per-protocol participants of the group receiving the highest effective intervention *vs.* placebo. Comparison will be made only versus the available archival MRI scans up to three months before the enrolment date. MRI scans will be performed and blinded analyzed at an MRI evaluation center.

Additional

Diet nutritional quality control questioner (set-up by the involved clinical dietitian) will also be performed at enrolment and at every scheduled assessment for the evaluation of the patients' diet habits and adherence as well.

Blood Sample

Blood samples will be collected at enrolment, at every scheduled assessment and at every relapse incident; for

- safety and evaluation of the hematological (full blood count) and biochemical analyses at baseline, 12 months and 24months on treatment and compared to enrolment. The same tests will be performed at the end of the extension period
- membrane lipid profile quantitative analysis will be performed (after study completion to ensure the blind status of the trial) on the collected and appropriately treated and stored red blood cell membrane lipids extracts (a standard protocol will be followed); to confirm patients' adherence.

Safety adverse events

Serious adverse events are defined as those that result in admission to hospital, cause prolonged disability or death, or are judged to be life threatening or otherwise medically significant. A safety monitoring committee will be set up.

Drop Outs

The drop outs, at any time and even the drop outs that never received the assigned interventions should be followed like all other participants as required for intention to treat analyses (ITT).

Missing Data Handling

This is an ITT study with a proof-of-concept purpose. The main question that has to be answered is primarily the ability of the interventions to reduce the annual relapses rate and secondly the disability accumulation when used in RRMS patients and how safe it is vs. placebo. All patients who prematurely discontinue the study drug will be encouraged to continue in the study and follow-up until the end of the planned treatment period, regardless of the treatments received. The data collected will be included for ITT analyses. The main analysis of ARR will include all confirmed (associated with new or worsening of pre-existing neurological signs, increase of at list 0.5 score on EDSS) relapses during the study, including relapses reported after study drug discontinuation. The lost to follow missing data will be handled by an appropriate statistical

method and included in the intention to treat analysis (ITT). The overall key analysis should be based on per-protocol (excluding all protocol violations) supported by the ITT analyses.

Other:

An independent Data Monitoring Committee (DMC) will monitor and review all clinical trial data on a regular basis (by the funder).

Investigators adherence will be also monitored by DMC through CRF follow up.

Duration of Study per Patient

The clinical study period is approximately 43 months total, including a screening period up to 1 month, a 6 month normalization, 24 months treatment period and 12 months extended follow up period, after completion. The actual research period is for a total of 30 months including 6 months normalization and 24 months treatment periods.

Methods

The whole procedure follows the clinical trial guidelines as required by the USA Food and Drug Administration (FDA), European Medicines Agency (EMA) and according to the standards of the International Conference of Harmonization (ICH). The Clinical study is in agreement with the rules of Good Clinical Practice (GCP).

CING is a tertiary accredited neurological center. All patients should give a written informed consent before enrolment. The study protocol is developed by the investigators and should be approved by the National ethics committee. Patient consent forms (CFs) and case report forms (CRF) documents are set up by the investigators according to the international guidelines and the needs of the trial protocol/ design and approved by the independent committee assigned by the funder. Study data (CRFs will be completed as required per patient per assessment) will be collected by the involved neurologist and forwarded to be held and blindly analyzed centrally according to the guidelines set-up by the Helix Incubator Organization of Nicosia University (HIONU) as an independent organization assigned by the funder. A CRF copy should be kept by

the clinical site. During the study, investigators and safety/ monitoring committee representatives should meet once a year to discuss study progress.

Randomization and Masking

Patients will be randomly assigned to the four intervention groups. Randomization will be centralized and the randomization scheme will be generated, performed and securely stored by HIONU.

Sample size as a result of the absence of any previous reported data and due to the novelty of the intervention will strictly be based on the total eligible to participate relapsing remitting MS patients in Cyprus.

Responsibilities will include, but not limited to regulatory management, clinical trial management, quality control (use only of accredited laboratories) and administrative activities. External committees (ie. independent safety/ monitoring committee etc.) will be set up by the funder. Randomization scheme will be securely stored by HIONU, with the investigators, patients, all study personnel, medical monitors, clinical research associates, statisticians/bioanalysts, neuroradiologist and pharmacist being unaware of treatment assignments throughout the study.

If knowledge of the study drug will be needed to manage a patient's condition, such as in the occurrence of a serious adverse event, the patient will not be given further treatment with the study drug and should continue to be medically followed.

Study Procedures and End Points

At study site, one examining/treating neurologist will be designated (the investigator neurologist, with more than 20 years in practice with experience and training on EDSS evaluation and scoring). Neurologists will be responsible for all aspects of patient care, including the

management of adverse events and the treatment of relapses. He will perform objective evaluation with use of the EDSS. The same physician should, as best as possible, maintain the role of treating neurologist for the subject throughout the study and the same person should maintain the role of examining neurologist throughout the study. Neurologist and or any other investigator should not be in contact with patients in any other capacity, so as to reduce the possibility of being unblinded by side effects or laboratory assessments. The same neurologist must review adverse or side-effects, examine patients, and make all medical decisions. Patients should be able to contact the examining neurologist at any time if there is any adverse event, side-effect or allergic reaction.

The protocol of the clinical study is considering 6 months normalization period for the interventions' agents to exert their beneficial effects and for other reasons previously discussed. This period begins at enrolment and continues for six months with regular consumption of the assigned intervention (for all study arms). The end of this period is denoted as the entry baseline. The period between entry baseline and the 24 months of treatment until the study completion (end) is the "on- treatment" period. The interventions should be consumed orally every day approximately 30 minutes before dinner by the supplied dosage calibrated cup, continuously for 30 months.

Bottling of the interventions will be performed by a group of scientist (always by the same group of scientist) under the supervision of the involved specialist clinical biochemist at every six months. Interventions and placebo will have the same appearance and smell. The intervention will be bottled under the appropriate conditions, in dark bottle under Nitrogen bed; each coded patient will be assigned to a coded intervention or placebo according to the randomization scheme by HIONU. Both Intervention and placebo bottles will be packaged in a blank white carton box containing the dosage calibrated cup. Patients' codes and their representative intervention codes will be safely kept by HIONU. The independent safety/monitoring committee will be the only physical body that will have access on the safety codes; for use only at emergency and when absolutely necessary, when life threatening conditions such as severe site effects are unexpectedly introduced. The interventions will be handled and appropriately stored by the institution pharmacists. A new full bottle of the drug will be handled to a patient when the

previous bottle is empty returned. Pharmacists will keep a detailed record of intervention handling schedule to the patient.

The study is designed to end 30 months after enrolment and clinical assessments should be scheduled at entry baseline and at 3, 9, 15, 21and 24 months on-treatment. An extra visit is required 6 months after the end of the study to confirm final EDSS score. Patients will be followed for an extended 12 month period with relapse recording. Patients will also be seen by the neurologist at unscheduled visits within 48 hours after the onset of new neurologic symptoms. If a relapse is suspected, the patient should be referred to the neurologist for evaluation and confirmation of any changes in the EDSS score.

Primary outcomes will be the **annual relapse rate (ARR)**. **Relapses** are defined as new neurologic symptoms or worsening of pre-existing symptoms (that were stable for at least 1 month) not associated with fever or infection that lasts for at least 24 hours characterized by new or worsening neurological sign on examination as defined by previously described EDSS changes. The ARR for each treatment group is the mean of the annualized ARRs for all patients in the group calculated as the total number of confirmed relapses divided by the total number of days on study, multiplied by 365.25.

The key secondary endpoint is the **time to confirmed disability progression** defined as an increase of 1.0 on the EDSS from a baseline score, confirmed after 6 months, with an absence of an ongoing relapse at the time of assessment (progression cannot be confirmed during a relapse) and with no documented relapse during the 6-months period needed for the confirmation. The final EDSS score will also be confirmed 6 months after the end of the study.

Relapses should be treated with methylprednisolone at a dose of 1g intravenously per day, for three consecutive days followed by prednisolone by mouth at a dose of 1mg/kg of weight per day on a tapering scheme for three weeks.

Hematological (full blood count) and Biochemical analyses should be performed at enrolment, baseline, 12 months and 24months and at the end of the extended period; performed at accredited laboratory.

Blood should be collected from all randomized patients at the time of enrolment and at every scheduled clinical assessment and during relapses. Blood tubes should be appropriately labelled including date, patients' code, number of scheduled assessment, for every blood handling step. A portion of 2 ml total blood volume per patient (in anticoagulant solution) should be drawn and stored refrigerated (4^{o}C) for the lipid extraction procedure (from red blood cells) within 24 hrs according to standard protocol.

Sample Size Estimation

Power calculations could not be done before the study because of the lack of information from previous studies on potential effect sizes. Based on the population size of our country and the centre of reference (CING) we believe that we will probably be able to enroll enough patients for this proof-of-concept study. The scientific knowledge/background of the agents used, novelty of the assessed interventions and the study-protocol design/set-up will be contributing to valuable information. Hopefully the "eligible" final enrolled sample size will be enough to collect quality data able to answer the addressed proof-of-concept questioned - efficacy indication. There is no any matter of ethical conflicts since all interventions will be used as adjuvant. Sample size will strictly be based on this subjects' availability parameter.

Brief Statistical Methods

The study will be considered successful if there is a significant difference between the treatments for either of the two endpoints.

Baseline characteristics will be compared across all intervention groups by ANOVA or Kruskal-Wallis rank test for continuous variables and by an exact chi-squared test for categorical variables, as appropriate.

Each test will be performed with a significance level of 0·05 (P-values will be two-tailed).

Primary Endpoint

Annualized Relapse Rate (ARR):

A Poisson regression/ negative Binomial analysis will be used to analyze this endpoint. The analysis will allow for likely overdispersion due to within-subject correlations by basing standard errors on the empirical estimator of the parameter covariance matrix. The fixed factors in the model will include treatment and relevant pre-specified covariates.

The relapse rate will be calculated as the total number of relapses divided by the total number of patient-years followed for each treatment group. ARR differences will also be calculated among all comparable parameters and reported as percent difference (relative reduction).

Secondary Endpoint

Time to Confirmed Disability Progression:

For the secondary end-point outcome, the time to disability progression, Kaplan–Meier curves will be constructed. A Long-rank and Cox proportional-hazards regression model will be used to analyze this endpoint. The fixed factors in the model will include treatment and relevant pre-specified covariates . Additional baseline characteristics will be considered for inclusion in secondary models.

If the usual normality assumptions are not met, the endpoints will then be analyzed by either Poisson regression, using a model similar to the one to be used for the ARR, Wilcoxon Rank-Sum tests, or proportional odds logistic regression models, depending on the nature of the endpoints and whether covariates will be included in the model.

Safety

All treatment emergent signs and symptoms (those that initially occur after taking the first dose of study medication, or those that worsen after taking the first dose of study medication) will be summarized and tabulated according to their severity (mild, moderate, or severe) and relationship (definitely not related, or related) to study medication. The rates will be compared between each group and placebo using Fisher's Exact test.

Missing Data

Missing data of the lost to follow patients will be imputed by use of the last-observation-carried-forward (LOCF) approach.

We thank every and each one of you, for your total cooperation.