PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	A novel oral nutraceutical formula (PLP10) for the treatment of relapsing remitting multiple sclerosis: a randomized, double-blind, placebo-controlled proof-of-concept clinical trial.
AUTHORS	Patrikios, Ioannis; Pantzaris, Marios; Loukaides, George; Ntzani, Evangelia

VERSION 1 - REVIEW

REVIEWER	Georgios M. Hadjigeorgiou, MD Professor of Neurology Faculty of Medicine University of Thessaly Greece
REVIEW RETURNED	22-Oct-2012

GENERAL COMMENTS	for the manuscript (bmjopen-2012-002170) entitled: A novel oral nutraceutical formula (PLP10) for the treatment of relapsing remitting multiple sclerosis: a randomized, double-blind, placebo-controlled proof-of-concept clinical trial, by Pantzaris MC, et al. The authors have reported their results of a single center, randomized, double blind, placebo controlled, parallel design, phase II proof-of-concept clinical trial, in a population of relapsing remitting multiple sclerosis (RR MS). Eighty patients were randomized to four groups of 20 each. Three of the four groups received variations of the nutritional “cocktail” formulation PLP-10, representing the complete composition of the formulation, and treatments A and C representing partial formulations. The authors clearly and correctly presented enough supportive background information and references, specifically for the individual studied nutritional agents, helping readers to understand the rationale behind their use as ingredients of a cocktail formula able to synergistically and favorably modulate a variety of injury cascades that contribute to the pathobiological state of MS. Innovatively the idea of the systems medicine approach through systems nutritional biology of MS is supported by the composed regimen and the study design. As a proof-of-concept study correctly presents both the per-protocol as well as the intention to treat statistical analysis and clearly answers the main trial question of how this product affects the primary endpoint and the secondary end point when patients continuously follow the protocol (all time on study). The authors thoroughly, clearly and acceptably discuss the study limitations (i.e compliance) and explain all related parameters. Even though this is a small size clinical trial, the study design, methodology, study length, statistical analysis approach and result interpretation correctly fulfill the criteria of a scientific advance, proof-of-concept, phase II study with a very interesting and novel approach. The thrust of this study should be of substantial interest to

	the medical community in general, in relation to chronic diseases and the next generation of drugs and especially the MS community. PLP10, as clearly and correctly stated by the authors, should be tested in a larger size multicenter trial including radiographic efficacy; this is just the beginning. Rational of the study is correctly stated; the results are very interesting and clearly presented in tables and graphs and answer the trial questions (primary and secondary end points). Both tables and graphs are informative enough I support publication of this study with minor corrections believing that it presents a novel approach for the treatment of MS, with very promising results out of a small well done and well presented proof-of-concept I have only some minor comments: • The abstract is long albeit summarizes the manuscript adequately and clearly. • The unpleasant taste can be considered as a side effect since that is a major cause of patients drop out. It is suggested to eliminate the related statements and limit those to: “without any severe side effects”. • In the Methods section the length of the study should be stated more clearly. • The exact amount of the other lipids in the intervention A and PLP10 need to be stated in the main document along with the suppliers of all the ingredients and chemical. A table with the analytical compounds in each intervention is needed, as it will enhance readability. I support publication of this study with minor corrections believing that it presents a novel approach for the treatment of MS, with very promising results out of a small well done and well presented proof-of-concept trial.

REVIEWER	Makiko Mieno Research Associate Department of Medical Informatics, Jichi Medical University, Japan No competing interests.
REVIEW RETURNED	30-Oct-2012

THE STUDY	1) Information should be given in relation to epidemiological issues such as the prevalence of MS in Cyprus. 2) Does the term "exact chi-squared test" (in l.318) mean Fisher's exact test for contingency table? 3) Multiplicity for testing should be taken into account or should be mentioned in the statistical analysis for pairwise comparison.
RESULTS & CONCLUSIONS	1) Figure 3 is unclear. At least, the axis definitions should be provided in the figures. 2) In Figure 4, does the label "3a" (or "3b") mean "4A" (or "4B") ? 3) In Figure 5, the error bars of s.e.m cannot be distinguished.
GENERAL COMMENTS	I found this study interesting, although the study limitations such as small sample size and high rate of dropouts are serious. There are some typos (e.g. "allocated to intervention" boxes in Fig2). Some abbreviations are unclear, e.g. SM in l.106 and PUFA in l.115.

REVIEWER	Annette Langer-Gould, MD PhD Research Scientist and Neurologist Kaiser Permanente Southern California

	I am a site investigator for 2 drug company sponsored clinical trials (Biogen Idec; Roche). I receive research support from the National Institutes of Health and the National Multiple Sclerosis Society.
REVIEW RETURNED	06-Dec-2012

THE STUDY	This study addresses and interesting question and the manuscript is clear and honest. However there are several significant limitations primarily surrounding the lack of an a priori statistical analysis plan and some rather unsual choices in analysis that make the data very difficult to interpret. 1) a signficant number of patients dropped out before the first six months of treatment 2) the authors discount the first six months of treatment and set the beginning of the study at 6 months 3) the analyses are adjusted for potential confounders even though the study was randomized (albeit the randomization method is suspect) and there are only 9 to 12 patients in each study arm that completed the study The only realistic conclusion from this study I can take away is that it is difficult for most patients to take the dietary supplements for any length of time, that in this small study there does not appear to be a beneficial effect of the dietary supplements but the study is underpowered to detect a small but potentially clinically meaningful difference.

VERSION 1 – AUTHOR RESPONSE

Reviewer No. 1
The authors clearly and correctly presented enough supportive background information and references, specifically for the individual studied nutritional agents, helping readers to understand the rationale behind their use as ingredients of a cocktail formula able to synergistically and favorably modulate a variety of injury cascades that contribute to the pathobiological state of MS.
Innovatively the idea of the systems medicine approach through systems nutritional biology of MS is supported by the composed regimen and the study design.
As a proof-of-concept study correctly presents both the per-protocol as well as the intention to treat statistical analysis and clearly answers the main trial question of how this product affects the primary endpoint and the secondary end point when patients continuously follow the protocol (all time on study). The authors thoroughly, clearly and acceptably discuss the study limitations (i.e compliance) and explain all related parameters.
Even though this is a small size clinical trial, the study design, methodology, study length, statistical analysis approach and result interpretation correctly fulfill the criteria of a scientific advance, proof-of-concept, phase II study with a very interesting and novel approach. The thrust of this study should be of substantial interest to the medical community in general, in relation to chronic diseases and the next generation of drugs and especially the MS community.
PLP10, as clearly and correctly stated by the authors, should be tested in a larger size multicenter trial including radiographic efficacy; this is just the beginning.
Rational of the study is correctly stated; the results are very interesting and clearly presented in tables and graphs and answer the trial questions (primary and secondary end points). Both tables and graphs are informative enough
I support publication of this study with minor corrections believing that it presents a novel approach for the treatment of MS, with very promising results out of a small well done and well presented proof-of-concept.
We thank the reviewer for the time and effort dedicated to review our work. We fully acknowledge his

views as already discussed in our manuscript.
I have only some minor comments:
• The abstract is long albeit summarizes the manuscript adequately and clearly.
We acknowledge the reviewer's point as it is impossible to edit the abstract without loss of context.
• The unpleasant taste can be considered as a side effect since that is a major cause of patients drop out. It is suggested to eliminate the related statements and limit those to: "without any severe side effects".
We thank the reviewer for his point and have now restated the text as follows:
Page 4 of 62, line 79: from: "any adverse or significant side effects" changed to: "any adverse or severe side effects" (revised manuscript page 4 line 79)
Page 21 of 62, line 511: from: "where no any severe or significant side-effects" changed to: "any adverse or severe side effects" (revised manuscript page 22 line 518)
Page 36 of 62, line 737 (article summary box, key messages, first bullet): from: "without adverse or significant side effect" changed to: "without any adverse or severe side effects" (revised manuscript page 38 line 771 (in Article Summary))
• In the Methods section the length of the study should be stated more clearly.
Following the reviewer suggestion we edited the manuscript as follows:
Page 11 of 62, line 258: the paragraph continues: "More clearly the study included the "normalization period" (July 1st 2007 to Dec 31st 2007), the "on treatment" period (Jan 1st 2008 to Dec 31st 2009) and the 12-month "extended period" (Jan 1st 2010– Dec 31st 2010)." (Revised manuscript page 11 lines 259 to 261)
• The exact amount of the other lipids in the intervention A and PLP10 need to be stated in the main document along with the suppliers of all the ingredients and chemical. A table with the analytical compounds in each intervention is needed, as it will enhance readability.
As suggested we now include a Table with the analytical compounds and ingredients in each one of the interventions per treatment arm (Table 1) (revised manuscript page 28, line 662). As a result the numbers of the other tables have been changed accordingly. Having in mind the aforementioned changes in tables' numbering, there have been changes in the document when there is referral to a corresponding table.
I support publication of this study with minor corrections believing that it presents a novel approach for the treatment of MS, with very promising results out of a small well done and well presented proof-of-concept trial.

Reviewer No. 2
We thank the reviewer for the time and effort dedicated to review our work. We fully acknowledge his views as already discussed in our manuscript.
1) Information should be given in relation to epidemiological issues such as the prevalence of MS in Cyprus.
Edited as proposed: Page 13 of 62 line 312: from "Based on the population size of our country" change to: "In 2005 the prevalence of MS in Cyprus (600,000 population) was 120/100.000. Based on the aforementioned MS patients number of our country…" (revised manuscript page 14 lines 315 to 316)
2) Does the term "exact chi-squared test" (in l.318) mean Fisher's exact test for contingency table?
We thank the reviewer for the observation. Syntax corrected as proposed: Page 14 of 62line 318: "exact chi-squared test" changed to: "mean Fisher's exact test" (revised manuscript page 14 line 322)
3) Multiplicity for testing should be taken into account or should be mentioned in the statistical analysis for pairwise comparison.
We take the reviewer's point into serious consideration. The issue of multiple testing is an important issue in any clinical trial. In order to address it, we have clearly defined our primary and secondary outcomes and defined all a priori analyses.
1) Figure 3 is unclear. At least, the axis definitions should be provided in the figures.
Comment: The "axis definition in Figure 3" is provided within the Figure legends (page 44 lines 885 to

926) and in the revised manuscript (page 46 lines 919 to 960)
2) In Figure 4, does the label "3a" (or "3b") mean "4A" (or "4B")?
Corrected as proposed: In Figure 4 (page 50 of 62) the labelling 3a and 3b have been correctly changed to 4A and 4B (new Figure 4 is attached)
3) In Figure 5, the error bars of s.e.m cannot be distinguished.
Corrected as proposed: Figure 5 “page 51 of 62” has been corrected including error bars (new Figure 5 is attached)
I found this study interesting, although the study limitations such as small sample size and high rate of dropouts are serious.
We take the reviewer’s point into account. The comment on the issue of the sample size and the drop outs it is as well stated by the authors, in the manuscript, as a limitation but supplemented with a well-documented explanation (original manuscript page 13 of 62 lines 311-315; revised manuscript page 14 lines 314-319).
There are some typos (e.g. "allocated to intervention" boxes in Fig2).
Typos corrected: The typos “allocated to intervention” boxes in Figure 2 (page 48 of 62) have been corrected (new Figure 2 is attached).
Some abbreviations are unclear, e.g. SM in l.106 and PUFA in l.115.
Corrected as proposed: Abbreviation “SM” (page 5 of 62 line 106) changed to “systems medicine (SM)” revised manuscript page 5 line 106)
Abbreviation “PUFA” (page 5 of 62 line 115) changed to “polyunsaturated fatty acids (PUFA)” revised manuscript page 6 line 116)

Reviewer No. 3
We thank the reviewer for the time and effort dedicated to review our work. We fully acknowledge his views as already discussed in our manuscript.
This study addresses and interesting question and the manuscript is clear and honest.
However there are several significant limitations primarily surrounding the lack of an a priori statistical analysis plan and some rather unusual choices in analysis that make the data very difficult to interpret.
We kindly disagree with the reviewer’s point. Despite the proof-of-concept nature of the study and its exploratory aspects, the statistical analysis was planned a priori and well-defined as stated in the revised manuscript. (page 15 of 62 line 343. “After ….following the protocol.” A new sentence is added: “All statistical analyses were well defined a priori” page 15 line 347 of revised manuscript).
Regarding “rather unusual choices in analysis”, we remain at the editor’s and reviewer’s disposal and offer the opportunity to provide additional explanations should the nature of the unusual choices is defined.
1) a signficant number of patients dropped out before the first six months of treatment
This is a well known phenomenon in oil related interventions/trials that due to palatability issues a lot of patients tend to drop-out soon after first dosage (manuscript has been revised: page 21 of 62 line 494 “…trials using oily interventions…..” is followed by “...where a lot of patients tend to drop-out soon after first dosage” page 21 line 500 of revised manuscript). However the demographics between the 4 arms of the study both for all-time on-study and the intention to treat analysis have no statistical differences.
2) the authors discount the first six months of treatment and set the beginning of the study at 6 months
It is clearly stated in the manuscript that the very first 6 months of the study (before the entry baseline), according to the study design and protocol, were defined as the time period to be used for calibration, of the enrolled patients, for the specific crucial reasons clearly explained and discussed in the manuscript (original manuscript page 11 of 62 lines 251-256 including references and page 23 of 62 line 550-557; revised manuscript page 11 lines 253-257 and page 23 lines 556-563). We consider this as a new /novel point of the study and state it as such (in the lines original manuscript page23 of 62 line 550-557; revised manuscript page 23 lines 557-564).

I addition to the above according to the reviewers comment the manuscript has been revised as follow: page 23 of 62 line 552 "…..in our study design." Changed to "…..in our study design (normalization period)." page 23 line 559 in revised manuscript.

3) the analyses are adjusted for potential confounders even though the study was randomized (albeit the randomization method is suspect) and there are only 9 to 12 patients in each study arm that completed the study

We thank the reviewer for her comment and take the opportunity to discuss this point further. Adjustment for potential confounders is indeed unnecessary in large RCTs with high adherence. In our study, despite the small sample size, there were no statistically significant differences between groups for the baseline characteristics, thus ensuring that the randomization was appropriate. Yet, in small RCTs with a high drop-out rate, the per-protocol analysis could be affected by the characteristics of the patients dropping out. Again, in our study no statistically significant differences occurred between groups in the per-protocol analysis. Nevertheless, one must take into serious consideration here the considerable loss of power of the analysis that would detect differences between groups. In order to safeguard our findings in the best possible way under the circumstances, we proceeded to adjusting for confounders even at the cost of penalizing our analyses.

According to the reviewer comment a new sentence has been added in the revised manuscript page 24 of 62 line 582 "….misinterpretation." changed to "…misinterpretation. Yet, in small randomized control trials with a high drop-out rate, the per-protocol analysis could be affected by the characteristics of the patients dropping out. In order to safeguard our findings in the best possible way under the circumstances, we proceeded to adjusting for confounders." page 25 line 589-592 revised manuscript".

The only realistic conclusion from this study I can take away is that it is difficult for most patients to take the dietary supplements for any length of time, that in this small study there does not appear to be a beneficial effect of the dietary supplements but the study is underpowered to detect a small but potentially clinically meaningful difference.

We agree with the reviewer and the published literature that dietary supplements suffer variably from low adherence. Yet, it is well known that the majority of the patients suffering from Multiple Sclerosis they do use dietary supplements for a variable length of time and they prefer supplement type of "help" over conventional drugs ("The data available are insufficient to asses any potential benefit or harm that might result from PUFA supplementation. This is unfortunate since 50-75% of people with MS make use of such diets and dietary supplementations." Farinotti M., et al. Dietary interventions for multiple sclerosis (review), the Cohrane Library 2007, issue I).

Considering the reviewer's comments we revised: page 26 of 62 line 622 "…largely disregarded.57" Changed to: "…largely disregarded.57 It is well known that the majority of the patients suffering from MS they do use dietary supplements for a variable length of time and they prefer supplement type of "help" over conventional drugs. 58" page 26 lines 636-638 revised manuscript.

We also agree with the reviewer that this is a small, proof-of-concept study, by definition under-powered to detect small, but clinically meaningful treatment effects. Nevertheless, in our study, we observed a clinically meaningful difference in indices of disease progression, a finding that we ourselves treat with great caution. Yet, we cannot discard our finding as a false positive, given that this is a randomized, double-blind, placebo-controlled clinical trial and, despite its small sample size, represents a piece of evidence that only a larger RCT can replicate or refute.

As per reviewer: Page 24 of 62 line 582. A new sentence has been added "Moreover, we cannot discard our finding as a false positive, given that this is a randomized, double-blind, placebo-controlled clinical trial and, despite its small sample size, represents a piece of evidence that only a larger randomized controlled trial can replicate or refute." page 25 lines 592-595 revised manuscript.

Additional Typos Corrections
Page 2 of revised manuscript line 42
Page 6 of revised manuscript line 137
Page 10 of revised manuscript line 227

Page 17 of revised manuscript line 408
Page 18 of revised manuscript line 425
Page 18 of revised manuscript line 431
Page 25 of revised manuscript line 600
Page 25 of revised manuscript line 605
Page 25 of revised manuscript line 609
Page 26 of revised manuscript line 618
Page 34 of revised manuscript line 705 (in Table 4B)
Page 39 of revised manuscript line 771 (Article Summary, key messages)