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To:
Andrzej S Tarnawski, DSc, MD, PhD, Professor
Editor-in-Chief
World Journal of Gastroenterology

Vienna, 08 September 2022

Reply to the editor and the reviewers

Revision 1, Manuscript NO.: 78405, Randomized Controlled Trial

Manuscript title: Safety and efficacy of purified clinoptilolite tuff treatment in patients with irritable bowel syndrome with diarrhea: Randomized controlled trial

Sir,

We want to thank the editor and reviewers for their time, helpful comments, and efforts spent to improve our manuscript. Herein we provide our point-by-point response.

Comments from the Company Editor-in-Chief

"I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022. Before final acceptance, when revising the manuscript, the author must supplement and improve the

highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/."

- Answer: We would like to thank you for your consideration to publish our manuscript in your distinguished journal and for your comments on our work. We have adapted the tables, figures, and references according to the journal requirements.

Comments reviewer 1:
We thank the Reviewer 1 for the valuable feedback.

Specific comments to the authors:

1. The pathophysiology of IBS is poorly understood and is currently thought to represent a complex interplay among the gut microbiota, mucosal immune system, impaired mucosal barrier function, visceral hypersensitivity, gut motility, and alterations in the gut-brain axis. In addition to ref [4] and [5], suggest authors cite a relevant and recent review on the topic (citation: pubmed.ncbi.nlm.nih.gov/30288077).

- Answer: The recent review was added as a reference number 6.
- Changes in text, page 5, line 21: "…neuropsychological disorders, and the bidirectional brain-gut axis[4-6]."

2. "Glock Health, Science and Research GmbH acted as sponsor of this multicenter study" - more details are required to ascertain the roles and responsibilities of the sponsor, whether the sponsor was directly or indirectly involved in the design and conduct of clinical trial.

- Answer: The role of the sponsor was specified.
- Changes in text, page 6, line 18: "…sponsor of this multicenter study and was responsible for the design and report of this study."

3. "44 patients were screened" - try not to start a sentence with a number.

- Answer: The beginning of the sentence was rephrased.
- Changes in text, page 12, line 10: "We screened 44 patients in order to include 30 trial participants, of whom…"

4. The information provided in Table 1 is rather vague and hard to interpret, what exactly are "Allergies/Hypersensitivity", "Metabolism and nutrition disorders", "Psychiatric disorders" and "Gastrointestinal disorders"? These are extremely vague and broad headings, suggest zooming in to more granular and clinically meaningful conditions, e.g.

lactose intolerance, which is exceedingly common in IBS-D patients.

- Answer: We divided the original Table 1 into new Table 1 (demographic characteristics and concomitant medications) and new Table 2 (medical history) and provided more details on the respective organ system comorbidities.
- Changes in text, page 12, line 15: “Baseline patient demographics and concomitant medications are summarized in Table 1, medical history in Table 2.”
- Changes in Table 1, page 22: “Table 1. Demographic characteristics and concomitant medications at baseline.…”
- Changes in Table 2, page 23: ”Table 2. Medical history at baseline. Data are presented for patients randomized to receive G-PUR® or placebo, given as absolute numbers and percent of group. Headings represent the standard nomenclature and structure from the Medical Dictionary for Regulatory Activities (MedDRA).”

5. How was study attrition and dropout handled? This was not apparent to readers.

- Answer: We describe the patient dropouts in the Figure 1.

6. Please change "microbial architecture" to "gut microbiome".

- Answer: We rephrased "microbial architecture" to "gut microbiome"
- Changes in text, page 17, line 5: “A positive effect on the gut microbiome was in line with…”

7. Purified Clinoptilolite-Tuff has been shown to be an effective sorbent for gluten derived from food sources (citation: pubmed.ncbi.nlm.nih.gov/35563533). This could be a reason for its supposed benefit.

- Answer: We have added the potential mechanism of action in the discussion.
- Changes in text, page 16, line 15: “PCT has been shown to be an effective sorbent for gluten derived from food sources[27], which could also contribute to its supposed benefit.”

8. "…present trail" - misspelled. It should be 'trial'.

- Answer: “present trail” was rephrased to “present trial”
- Changes in text, page 15, line 28: “…rescue therapy in the present trial, is often used as first-line…”

9. "In a recent meta-analysis of established traditional therapies in IBS, tricyclic antidepressants are recommended for treatment of abdominal pain, but careful dosing is warranted based on the side-effect profile46,47" - authors should also mention that other supplements such as Vitamin D has only showed very modest effects (citation: pubmed.ncbi.nlm.nih.gov/35396764).

- Answer: We added a new paragraph concerning meta-analyses in dietary interventions and supplements in IBS.
- Changes in text, page 16, line 7: “Dietary and lifestyle changes constitute an important non-pharmacological approach in treating IBS symptoms. A recent meta-analysis

showed that patients receiving a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) in RCTs experienced a statistically significant reduction in pain and bloating compared to patients receiving a traditional diet[55]. Overall, the exclusion of foods high in FODMAP may reduce IBS symptoms and can be recommended to affected patients, but there is still a need for higher-quality evidence to guide management[56]. Similarly, supplements such as vitamin D have shown modest effects compared to placebo[57-59]."

10. "... the clinical benefit of PCT could be demonstrated in various clinical meaningful endpoints" and "In this randomized, placebo-controlled study, the purified clinoptilolite tuff product G-PUR® demonstrated safety and clinical benefit in patients with IBS-D, representing a promising novel treatment option for these patients" - given the limitations of the present trial and the fact that G-PUR did not actually perform statistically (or clinically) superior to placebo, I would suggest authors temper the study conclusions.

- Answer: We rephrased the study conclusions.
- Changes in text, page 3, line 2: "…G-PUR® demonstrated safety and clinical benefit towards some symptoms of IBS-D, representing a promising novel treatment option.."
- Changes in text, page 3, line 12: "…it reduced the use of rescue medication and tended to enrich gut microbiome diversity…"
- Changes in text, page 17, line 9: "In conclusion, this randomized placebo-controlled, double-blind pilot study provides evidence that the PCT product G-PUR® can be safely used over a prolonged period of 12 weeks, also in the treatment of patients with IBS-D. A favorable result of G-PUR® was detectable for some symptoms of IBS, i.e., abdominal pain and stool consistency. Further, a lower use of rescue medication in the G-PUR® than in the placebo group, and a trend towards greater microbial diversity generally associated with a healthy gut microbiome was observed."

11. Table 2 should include 95% confidence intervals and estimates."

- Answer: We added the 95% confidence intervals in the Table 3 (originally Table 2).
- Changes in Table 3, page 23: see Table 3 for more details on risk difference, 95% confidence intervals

<u>Comments reviewer 2:</u>

We would like to thank the Reviewer 2 for his important remarks.

Specific Comments to Authors:

The article under review is a randomized controlled trial analyzing the safety and efficacy of purified clinoptilolite tuff treatment in patients with irritable bowel syndrome and diarrhea. The authors present the results of a completed study conducted in accordance with the norms and requirements of the law. All necessary documents have been presented by the authors in full. The studies were conducted on a small sample of 30 patients. A formal

calculation of the sample size was not carried out due to the design of the pilot study. I have the following remarks on the operation and presentation of data.

1. Notes on files posted on the site: Forms, 78405-Institutional Review Board Approval Form or Document and 78405-Non-Native Speakers of English Editing Certificate contain the same information. It is desirable for the authors to clarify the correctness of the information in the attached documents.

- Answer: We have proved and uploaded the required documents again. The corrected manuscript version was reviewed by an English native speaker.

2. Introduction: it is necessary to clarify the correctness of quoting some reference, for example. 15.

- Answer: We have reviewed and corrected all references according to the journal guidelines.

3. Materials and methods; in the section The following variables were assessed as exploratory endpoints before and after 12 weeks of treatment: you must add references to sources that explain how these variables were studied.

- Answer: The references for assessments of the exploratory results were added.
- Changes in text, page 11, line 9: see references

4. Figure 1. It is unclear why, if the early withdrawal of patients from the study, why the final analysis was carried out for the primary number of patients.

- Answer: We have conducted an intention to treat analysis, all study participants were therefore included in the final study analysis.
- Changes in text, page 11, line 24: "…was compared in an intention to treat analysis between…"

5. Table 1. Clarify data on the duration of IBS since symptom onset (years) presented in the last column.

- Answer: Last column represents the median values of the total population of the study. We have rephrased two baseline characteristics for better understanding.
- Changes in Table 1, page 22: "Duration of IBS symptoms (years)", "Duration of IBS diagnosis (years)"

6. Figure 2. Numbers in the x-axis signature (exp. n=24) are unclear. Are there a numeric of patients? Why isn't it the same? If n=24, does that mean 6 people dropped out of the study? The text of the Materials and Methods section does not contain this information.

- Answer: This indicates the number of participants from whom data at the given time point was available. This was added in the figure legend.
- Changes in Figure 2, page 19: "…by time point during the 12-week treatment and 4-week withdrawal period with G-PUR® (solid squares) or placebo (open circles). "n" represents the number of patients with available data at respective time point, while patients with missing data were considered as non-responders in the intention-to-treat-analysis."

7. Figure 3. This figure does not represent real data on diversity. The real increase in

diversity is determined at the level of lower taxa - genera or phylotypes. Taking into account the obtained difference between groups at different times of sampling, shown in Figure 5, it is necessary to change Figure 3. It is necessary to present diversity at the level of genera or phylotypes, including the top 25 or 50 most represented phylotypes in the analysis. This will allow you to more clearly show the differences between patients.

- Answer: We have changed the Figure 3 for more accurate visualization of different taxa.
- Change in Figure 3, page 20: "Figure 3. Microbiome heatmap. Overall visualization of the change in the gut microbiome genera in comparison to baseline by treatment group. Microbiome studies in stool across multiple calculation methods showed an increase in diversity in the G-PUR® group, but not in the placebo group."

8. Figure 4. It is desirable to add a confidence score. It may be appropriate to provide other indices, such as Chao or the observed units (ASV or OTU).

- Answer: We have changed the Figure 4 for more detailed interpretation and added indices of the alpha diversity in gut microbiome at baseline and end of study as requested.
- Change in Figure 4, page 21: "Figure 4. Alpha diversity indices in phenotypes between timepoint by treatment group."

9. References: It is necessary to carefully review the links provided by the list for their correctness, for example, pages are not indicated in ref. 3, 12, 18, 48, 49."

- Answer: The references were proved and corrected.

<u>Comments reviewer 3:</u>

We would like to express our sincere thanks to the Reviewer 3 for the important comments.

Specific Comments to Authors:

The authors conducted a pilot randomized controlled trial to assess the safety and efficacy of purified clinoptilolite tuff treatment in IBS-D patients. Overall, the manuscript is well writen and organized with abundant data. The current report is consistent with the previously published study protocol which make it reliable. Before it could be finally published I have several comments for the authors' reference.

Major concern 1. Regarding the primary outcome, after 12 weeks of treatment the proportion of responders according to the SGA of Relief was 21% (n=3) in the G-PUR® group and 25% (n=4) in the placebo group (p=1.0; Table 2). It is obviously that G-PUR is not superiority to the placebo pill at the end of treatment from whether clinically or statistically aspect. Herein, I cannot agree with the authors who drew the current conclusion that "In this randomized, placebo-controlled study, the purified clinoptilolite tuff product G-PUR® demonstrated safety and clinical benefit in patients with IBS-D, representing a promising novel treatment option for these patients". Although you set more than 10 secondary outcomes and some of them indeed showed a positive results, it cannot change the conclusion that G-PUR is not better than placebo. Authors, please draw the conclusion again.

- Answer: We rephrased the study conclusions.
- Changes in text, page 3, line 2: "…G-PUR® demonstrated safety and clinical benefit towards some symptoms of IBS-D, representing a promising novel treatment option…"

- Changes in text, page 3, line 12: "…it reduced the use of rescue medication and tended to enrich gut microbiome diversity…"
- Changes in text, page 17, line 9: "In conclusion, this randomized placebo-controlled, double-blind pilot study provides evidence that the PCT product G-PUR® can be safely used over a prolonged period of 12 weeks, also in the treatment of patients with IBS-D. A favorable result of G-PUR® was detectable for some symptoms of IBS, i.e., abdominal pain and stool consistency. Further, a lower use of rescue medication in the G-PUR® than in the placebo group, and a trend towards greater microbial diversity generally associated with a healthy gut microbiome was observed."

Minor concenrs

1. For most RCT with moderate or more sample size, there is no necessary to provide p-value in demographic characteristics. However, as this is a pilot study with only 30 participants, I noticed that some baseline variable between groups may not comparable (such as duration of IBS, metabolism disorders, gastrointerstinal disorders, etc.). Please add p-value for table 1.

- Answer: Due to the small sample size and need for multiple testing for many demographic characteristics, we did not include p-values in order to avoid inflational error. This is unchanged in the present manuscript.

2. Current Table 2 is not acceptable. Please add more statistical information including RD (risk difference) and its 95%CI (confidence interval) for the response analysis of primary outcome and secondary outcomes that have similar data pattern. For the continuous outcomes (such as IBS-SSS, SF-12, PSQ) please provide MD (mean difference) and its 95%CI.

- Answer: We have added the Risk difference (RD) for proportional variables, and mean differences (MD) for normally distributed variables, with 95% confidence intervals (CI), respectively.

- Changes in Table 3, page 25: "…Risk difference (RD) for proportional variables, and mean differences (MD) for normally distributed variables, with 95% confidence intervals (CI) are given, respectively. Odds ratio is provided for proportional variables."

3. How you handle with the missing data, I did not locate this in the current manuscript. Five patients in total withdrew from the study before the end of treatment. Considering the small sample size, data from each person is vital for results.

- Answer: Missing data were not imputed. The numbers in the figures include all available data from the different time points of assessment.

4. Why you chose IDO as an exploratory end?"

- Answer: We chose IDO as an exploratory marker for this study, because IBS has been linked with abnormal serotonin functioning and immune activation. Tryptophan forms the substrate for serotonin biosynthesis, but it can also be catabolized to kynurenine by the enzyme IDO. In IBS patients, IDO activity has been studied in relation to IBS severity and the probability of

depression and anxiety in patients with IBS[1-4] .
- no changes in text

We feel that these refinements have improved our manuscript and hope that it is acceptable for publication in its present form. Changes in the revised text are marked.

Sincerely,

Michael Wolzt, MD

Literature

1. Clarke G, Fitzgerald P, Cryan JF, et al. Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort. BMC Gastroenterol 2009;9:6.
2. Clarke G, McKernan DP, Gaszner G, et al. A Distinct Profile of Tryptophan Metabolism along the Kynurenine Pathway Downstream of Toll-Like Receptor Activation in Irritable Bowel Syndrome. Front Pharmacol 2012;3:90.
3. Fitzgerald P, Cassidy Eugene M, Clarke G, et al. Tryptophan catabolism in females with irritable bowel syndrome: relationship to interferon-gamma, severity of symptoms and psychiatric co-morbidity. Neurogastroenterol Motil 2008;20:1291-7.
4. Kennedy PJ, Cryan JF, Dinan TG, et al. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? World J Gastroenterol 2014;20:14105-25.