Response to reviewers: Manuscript NO.: 71418 : "**Effect of *Bifidobacterium longum* 35624 on disease severity and quality of life in patients with irritable bowel syndrome"**

General response:

We thank both the Editor and reviewers for their comments on our work and the reviewers for their constructive remarks which have helped us improve the quality of this article.

Below you will find a point-by-point response to the various comments made by the two reviewers. The modications in the revised manuscript are made as described below, but in the auto-edited document generated by online submission process, only the final version with modifications appears.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

The authors described that relatively short-term treatment with B. longum 35624 reduces disease severity and improves patients' quality of life with IBS, especially patients with severe IBS in a real-world setting in France. It is a prospective, open-label, multicenter observational study. It is well-written and has good quality. However, there are some concerns about this article.

1. There is a lack of enough discussions of the mechanism of how B. longum 35624 reduces disease severity and symptoms in the article. Any cytokines or brain-gut axis are involved?

We thank the reviewer #1 for this comment, indeed in the study of O'Mahony et al. the symptom response was associated with an effect on the IL-10/IL12 cytokine ratio that was impaired a baseline suggestive of a pro inflammatory state.

The following sentence was added in the text in the discussion section in the second paragraph "The effect on digestive symptoms and disease severity reduction could be secondary to an effect on pro-inflammatory cytokines as it was shown previously by O'Mahony et al. with a normalisation of an IL10/IL-12 cytokine ratio that was impaired at baseline"

2. The authors may describe more details other medications already prescribed for IBS in the methods of this study.

Thank you for your remark, we added in the text information about previous treatment. The following sentence was added in the Result section (baseline date): “antispasmodics and transit modifiers had previously been prescribed respectively in 65.7% and 35.7% of IBS patients.”

3. Gut microbiota has racial differences. Although this is a French cohort study, please discusses this issue in the article.

Thank you for this very interesting remark because it is true that race-related differences have been published in studies on the microbiota (for exemple in breast cancer) and this could have had an influence on our results, even if in France at this time the law does not allow the collection of data on ethnicity outside of specific restricted cases. This particular point is added in the Discussion section in the weaknesses section of the article. We added the following sentence “While in some diseases there may be variations in the microbiota according to ethnicity or geography, future studies with microbiota analysis are warranted to assess microbiota changes.”

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: Thanks for inviting me to review this article 'Effect of Bifidobacterium longum 35624 on disease severity and quality of life in patients with irritable bowel syndrome'. The authors assessed the effect of B.longum 35624 on IBS patients using a real world study and the results showed that B.longum 35624 reduces IBS severity and improved the QoL particularly to those who have most severe symptoms.

Major: Although a real world, study has better generality and external validity. The term itself will not change the characteristic of a study. I still feel confused about the so-called 'real-life'.

In this study, all IBS patients after screening (who met pre-defined inclusion criteria) received B.longum 35624, which mean that this is a typical interventional study without control group rather than an observational study. This study has no placebo or usual care control to avoid the placebo effect or the natural regression processing of the disease. Moreover, the sample size is moderate compared with the real 'real world study'.

The title and methods should clearly declare the study type.

We agree with reviewer #2 and removed the reference in the text to a "real-life study” in many places (See modifications in the text in red).

This study was considered by ethics committee as non-interventional because the patients with IBS were treated according to the gastroenterologist's choice of prescribing or not in a particular patient *B. Longum 35624*.

To clarify the type of study for the reader the following sentence was added in the material and method section: "The gastroenterologist was free to prescribe or not B. longum 35624 according to his appreciation if he thought it was a good therapeutic option for the patient."

It is true, however, that only the data concerning the patients who took this probiotic are available and presented that gives the impression of an interventional study. To clarify for the reader the type of study, we change the first sentence in the Discussion section in study limitations paragraph. "This observational study, were only data concerning the patients who took treatment with *B. longum 35624* are available, had several limitations, namely the absence of a placebo or comparator group and the relatively short treatment duration.

Minor:

1. without a sample size estimation, we cannot ensure if your data have enough power.

The sample size estimation is presented in the Material and Methods section two paragraphs before statistic section "Considering a variation in the IBS-QOL score of 15, the total number of patients to be assessed was determined to be 203 to obtain a level of precision of 10%. To maintain this level of precision considering that a certain number of patients (about 10%) would not be eligible for primary endpoint analysis (missing data), 220 patients had to be included in the study".

In our manuscript we present the data of 233 patients (evaluation population, see flow-chart).

2. IBS-U has only 12 sample with an obviously skewed distribution (-66±104.2 and 4.7±10.9 for IBS-SSS and IBS-QOL, respectively). Also for IBS-M population, I wondered if the data has a normal distribution? Maybe not.

It is true that some subgroups have a small size but to overcome the hypothesis of normality, intergroup analyzes were carried out using nonparametric tests (Kruskal-Wallis).

3. too many figures illustrated same information which the tables have showed. Please re-structured figures and tables.

We moved the Figure 7 (IBS-QOL individual dimension scores at baseline and after treatment with B. longum 35624) from main manuscript to supplementary data and renamed Figure 8 as Figure 7. See modifications in the text.

In Table 1 are presented the categories of subtype, severity and overall quality of life. In the followings figures are presented different, non-redundant information useful for the readers which are the results of severity and quality of life by subtype of transit (Figure 2), of quality of life according to severity (Figure 3). It is difficult to suppress Figure 4 which presents the

main results of the study. Figures 5 and 6 are useful because they give details according to the severity items and from changes of severity categories which is meaningful information for clinicians.

4. is there any adverse events data?

There were very few adverse events, they are reported in the safety section page…" During the study, 10 AEs possibly related to the use of B. longum 35624 were reported in 4.1% (n = 10) of patients in the safety population (n = 244 patients), including flatulence (n = 3), abdominal pain (n = 2), constipation (n = 1), abdominal distension (n = 1), upper abdominal pain (n = 1), gastrointestinal motor disorder (n = 1), and increased weight (n = 1)."

5. Some subgroup analysis such as figure 8 is hard to locate each subcategory. I highly recommend the author use forest plot to present subgroup data.

Each of the items in Figure 8 shows the distribution of transit subtypes and their evolution every 10 days. It seems difficult to put this progressivity into a forest plot without loss of information.

To increase the overall readability of the figure, the relevant transit pattern subtype is now specified in each picture.