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Peer Review File

Faecalibacterium prausnitzii EXL01 for the Maintenance of Steroid-induced Clinical Response or Remission in Patients with Crohn's Disease: a first in human trial

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors present a revised version of their report on EXL01. The authors conclude the administration of EXL01 is feasible and well tolerated, although 25% (2/8) participants experienced a disease flare. There may be an impact on ileal gene expression, although small sample size and lack of controls limit this claim.

The key issue here is small sample size. Although the authors conclusions are moderate (in line with study), it is still hard to know if the data support those claims. I don't think further revisions could improve this manuscript. The paper is well written and analysis all appears thorough and valid.

The only limitation is the sample size.

Reviewer #3

(Remarks to the Author)
Most of my comments were answered, however the details provided for the linear mixed models are not satisfactory: it was not clear why clinical metadata and the number of reads were included in the model as random effects.

Also, please include in the Statistical methods section all the different methods used for the analyses reported such as PCA.

Please rephrase "BH's method was used for controlling the False-discovery rate (FDR) for each question separately" to replace "question" with "hypothesis" or "comparison" or "analysis" for example.

Reviewer #1 (Remarks to the Author):

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The key issue here is small sample size. Although the authors conclusions are moderate (in line with study), it is still hard to know if the data support those claims. I don't think further revisions could improve this manuscript. The paper is well written and analysis all appears thorough and valid.

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We agree with the reviewer about the limited sample size therefore we highlighted it in our discussion : «The small sample size, open-label, and single-arm design of Part A in this first-in-human phase 1 study limits the interpretation of results and prevents formal efficacy conclusions », « Owing to the small number of participants », « Although limited by small sample size and the absence of a control arm, »

Reviewer #3 (Remarks to the Author):

Most of my comments were answered, however the details provided for the linear mixed models are not satisfactory: it was not clear why clinical metadata and the number of reads were included in the model as random effects.

Thanks for the remarks as we indeed made a typo, as number of reads and clinical metadata were used as fixed effetcs.

Reads number were always included in the LMM models as covariable, because MaAsLin 3 identifies prevalence (presence/absence) associations. Deeper sequencing will likely increase feature detection in a way that could spuriously correlate with metadata of interest when read depth is not included in the model.
We have rewritten the method section to remove any confusing sentences

Page 15, line 557-569: *Linear mixed-effects models were fit using the R package Maaslin342 to identify microbiome abundance and prevalence associations with treatment (comparing end vs. beginning of EXL01) and with clinical metadata, separately for fecal and biopsy samples (Supplementary Datas 1-5). Taxa with prevalence below 10% were excluded, and abundances were normalized by total-sum scaling followed by log transformation. Each model took the form:*

log(Tss-abundance) ~ visit (or clinical variable) + nb_reads + (1|Subject)

where visit (end vs. beginning of EXL01) or each clinical variable of interest was included as a fixed effect, sequencing depth (number of reads) was included as a covariate, and subject ID was modeled as a random intercept to account for repeated measures.

Also, please include in the Statistical methods section all the different methods used for the analyses reported such as PCA.

We included in the 16S and Shotgun section this text : *Raw relative abundance was used for alpha-diversity indexes calculation. CLR-transformed relative abundance was used to calculate Principal Component Analysis (PCA). Total-Sum-Scaling normalization and log transformation were used on relative abundance for linear mixed models.*

Please rephrase "BH's method was used for controlling the False-discovery rate (FDR) for each question separately" to replace "question" with "hypothesis" or "comparison" or "analysis" for example.

It has been done.

p15, line 566-567: *BH's method was used for controlling the False-discovery rate (FDR) for each analysis separately and significant level was fixed at p-value < 0.2 after adjustment.*