Author's response to reviews

Title: Clinical trial: Probiotic treatment of acute distal ulcerative colitis with rectally administered Escheria coli Nissle 1917 (EcN)

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Author's response to reviews: see over
Answers to Reviewers’ reports

Reviewer 1: Per Farup

Reviewer’s report:
The paper reports the results of a randomised controlled trial (RCT) comparing different doses of a probiotic enema with placebo in patients with distal ulcerative colitis. I have several concerns about the paper, these are the major ones:

• A per protocol (PP) analysis is reported as the main result. According to international guidelines, intention to treat (ITT) analyses should always be the primary results when presenting the results of RCTs, and PP-analyses could be reported if of interest. In this study the ITT-analyses showed no effect. The lack of effect is the main result of the trial, which also is very interesting.

Reply:
The reviewer’s point (ITT as primary analysis in superiority trials) is perfectly understandable when talking about a confirmatory clinical trial, e.g. of phase III. However, EMEA states in this respect: “In practice this ideal may be difficult to achieve ... There are a limited number of circumstances that might lead to excluding randomised subjects from the full analysis set …” (ICH Topic E 9, Statistical Principles for Clinical Trials). Since the present trial is a pilot trial of phase II and has explorative character, it shall be allowed to modify analysis when required by the circumstances given. In the present trial ITT does not provide a clear read-out. This is due to the frequent self-medication of patients by using medicaments not covered by the protocol (patient’s non-compliance with the protocol), especially in the groups with low-dose EcN, thus blurring the EcN effect.
In order to give the reader all data available we included the ITT data in our manuscript (not statistically significant trend in favour of EcN) and also stated the reason for patient exclusion from PP.

• According to the abstract, the conclusion is that the probiotic is as effective as other treatments for distal ulcerative colitis. The conclusion is wrong since the trial does not compare the probiotic with other treatments. The conclusion is that there is no significant effect above placebo in the ITT-analyses, but the probiotic seems to be superior to placebo in the PP-analyses. Comparisons with other treatments could be discussed in the paper but not presented as the conclusion.

Reply:
We addressed to this point in the manuscript. The Abstract’s conclusion now reads: “In contrast to ITT analysis, efficacy of rectal application of EcN was significant in PP and points to EcN as a well tolerated treatment alternative in moderate distal UC.”

• The analyses of the trial are incorrect. Again, according to international guidelines, two-sided tests (not one-sided) should be used.

Reply:
The reviewer’s point (two-sided test) is perfectly understandable when talking about a large confirmatory clinical trial, e.g. of phase III. Since the present trial
is a pilot trial of phase II and comprises a relatively small number of patients, it shall be allowed to modify analysis. Even with one-sided test statistics any clear trend can be made out.

A lot of the results are poorly or not documented. For example, it is reported that time to remission was shorter in the 40 ml and 20ml groups than in placebo. This is presented in one figure but no statistical calculation was performed.

Reply:

This point should be addressed to already by the existing manuscript. The Materials-and-Methods section reads: “Data on secondary objectives were compared descriptively.” Descriptive statistical methods should be sufficient to analyse secondary objectives. In our opinion, further calculations would increase the size of the manuscript inappropriately.

• In the method section it is said to be a randomised controlled trial with four groups. Later it is written that the placebo group was pooled from the three groups.... What does that mean?

Reply:

We addressed to this point in the manuscript. In fact, there were 4 patient groups receiving different doses of EcN, 3 different verum doses and 1 placebo. The verum doses were applied by applying different volumes of the same verum (= EcN) solution. The placebo group was a group of 20 patients receiving no active ingredient, but all 3 different volumes of placebo solution. Of the 20, 6 patients received 10-ml enemas, 7 received 20-ml enemas, and 7 received 40-ml enemas. The respective sentence in the Materials-and-Methods section now reads: “The placebo group was pooled from three groups that matched the three different enema volumes used in the EcN groups (6 patients received 10-ml enemas, 7 received 20-ml enemas, and 7 received 40-ml enemas).”

• Please describe the DAI, without a description it is impossible to understand the scoring system. The same applies to the histological classification, how was is made and what was significant inflammation? Without a description it is impossible to understand what “disappearance of histological signs of significant inflammation” means.

Reply:

The “DAI” as mentioned in the manuscript was defined by Sutherland et al. (Gastroenterology 1987). The “Clinical DAI” in the manuscript was the DAI without histology. This was necessary because it was unethical to subject the patients to colonoscopy as often as every 2 weeks. Histological data of each patient were gathered at the beginning and end of the study period only.

• Histological abnormalities of the descending colon were observed in 20/88 patients. These patients had left-sided colitis and not UC proctitis /proctosigmoiditis which was the inclusion criterion. Two patients had remission (histological?) on baseline. Were patients in remission included? Inclusion criteria
were mild and moderate disease activity.

**Reply:**
Patients with (minor) histological signs of left-sided colitis remained in the PP population indeed while no patient was included in the study without disease symptoms at baseline. Figure 4, e.g., shows patients with no rectal disease activity at baseline. These patients had inflammation in the sigma only.

• The text refers to table 1 which is not included in the version I have received, and the figures are not the most appropriate ones.

**Reply:**
We will make sure to include all Tables and Figures that are referred to in the manuscript.

• The presentation is mixed-up.

**Reply:**
We will make sure to keep to the standard manuscript structure as is required by the Instructions for Authors.

• English is not my native language, but I think the written language deserves improvement.

**Reply:**
We will check on our language use.

It is likely that the trial contains interesting findings. The results might be negative, but that is also of interest. The study deserves new and correct analyses and a better scientific presentation.

**Reply:**
Hopefully, we could meet an acceptable scientific standard now with our corrections and explanations.

**Reviewer 2:** Mario Guslandi

**Reviewer's report:**
This is an interesting paper, with only minor marginal flaws. In the Introduction, line 10 "Cytoprotective agents" are mentioned for IBD therapy. Please explain which drugs you mean to put under this label.

**Reply:**
We addressed to this point in the manuscript. The words “cytoprotective agents” were omitted.

At lines 17-18 some studies employing probiotic agents for ulcerative colitis are mentioned. Please distinguish between treatment of active disease and maintenance treatment of UC in remission (e.g. the study with S.boulardii in UC is NOT a maintenance study)
Reply:
We addressed to this point in the manuscript. The Introduction section now contains the following sentence (includes induction of remission for *S. boulardii*): “Successful oral application of microorganisms in UC remission induction or maintenance, such as EcN [8], *S. boulardii* [9], bifidobacteria [10], and others [11, 12] as well as the role of probiotic functional foods or probiotic therapies in inflammatory bowel disease was reviewed by [13, 14, 15, 16].”

Please double-check your English, there are some small mistakes here and there in the Discussion section.

Reply:
We will check on our language use.