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**Randomised clinical trial: Bifidobacterium bifidum
MIMBb75 significantly alleviates irritable bowel
syndrome and improves quality of life: a double-blind,
placebo-controlled study**

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Randomised clinical trial: Bifidobacterium bifidum MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life: a double-blind, placebo-controlled study

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2
3 1 **Randomised clinical trial:** *Bifidobacterium bifidum* MIMBb75

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6 2 significantly alleviates irritable bowel syndrome and improves quality

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9 3 of life: a double-blind, placebo-controlled study

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57 23 787979 031.

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25 Abstract

26 Background. Recent research suggests that an imbalance of the intestinal microbiota
27 and a dysfunctional intestinal barrier might trigger irritable bowel syndrome (IBS). As
28 probiotics have been reported to restore the intestinal microbiota and the gut barrier,
29 the therapeutic potential of probiotics within IBS became of strong interest. Aim. To
30 assess the efficacy of *Bifidobacterium bifidum* MIMBb75 in IBS. Methods. 122
31 patients were randomized to receive either placebo (N=62) or MIMBb75 (N=60) once
32 a day for four weeks. The severity of IBS symptoms was recorded daily on a 7 point
33 Likert scale. Results. MIMBb75 significantly reduced the global assessment of IBS
34 symptoms by -0.88 points [95%CI: -1.07; -0.69] as compared to only -0.16 [95%CI: -
35 0.32; 0.00] points in the placebo group ($p < 0.0001$). MIMBb75 also significantly
36 improved the IBS symptoms pain/discomfort, distension/bloating, urgency and
37 digestive disorder. The evaluation of the SF12 sum scores showed a significant gain
38 in quality of life within the bifidobacteria group. Furthermore, adequate relief was
39 reported by 46.7% of the patients in the bifidobacteria and only by 11.3% of the
40 patients in the placebo group ($p < 0.0001$). Overall responder rates were 56.7% in the
41 bifidobacteria group but only 21.0% in the placebo group ($p = 0.0001$). MIMBb75 was
42 well tolerated and adverse events were not different from placebo. Conclusion. *B.*
43 *bifidum* MIMBb75 effectively alleviates global IBS and improves IBS symptoms
44 simultaneously with an improvement of quality of life. Considering the high efficacy of
45 MIMBb75 in IBS along with the good side effect profile, MIMBb75 is a promising
46 candidate for IBS therapy.

47
48 Keywords: Irritable Bowel Syndrome, IBS, Colon irritable, Intestinal microbiota,

49 Probiotics, Bifidobacteria

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2 56 1. Introduction
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4 57 Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder,
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6 58 diagnosed through the Rome III criteria (1). Abdominal pain, flatulence and bloating
7
8 59 are common IBS symptoms for which no endoscopic, biochemical or radiological
9
10 60 cause is verifiable. IBS affects 15-20 % of the population, with a highly increasing
11
12 61 tendency in industrial nations (2, 3, 4). The pathophysiology of IBS is yet only partly
13
14 62 understood. Recent research suggests that an imbalance of the intestinal microbiota
15
16 63 with a significant reduction of bifidobacteria and a dysfunctional intestinal barrier with
17
18 64 subsequent bacterial translocation may contribute to the development of IBS and its
19
20 65 symptoms (5, 6, 7, 8, 9).

21 66 As probiotics have been reported in several studies to restore the intestinal
22
23 67 microbiota and gut barrier as well as hinder bacterial translocation, the therapeutic
24
25 68 potential of probiotics in IBS has become of strong interest (10, 11). However the
26
27 69 efficacy of probiotics is strongly strain specific and only certain strains might be able
28
29 70 to improve IBS and its symptoms (12, 13, 14). The ability of specific strains to adhere
30
31 71 well to intestinal cells may play a pivotal role in altering the intestinal microbiota and
32
33 72 increasing the intestinal barrier, which might be of significant value especially in the
34
35 73 treatment of IBS. Guglielmetti et al. were able to show in a Caco-2 cell line that the
36
37 74 adherence of *B. bifidum* MIMBb75, which was isolated from a fecal sample of a
38
39 75 healthy adult, was significantly better than that of well-studied commercial probiotics
40
41 76 (15, 16). Taking into consideration the pathophysiology of IBS and the role of
42
43 77 adherence of probiotics regarding the restoration of the intestinal microbiota as well
44
45 78 as of the gut barrier, *B. bifidum* MIMBb75 might be effective for IBS treatment. As the
46
47 79 conventional medical treatment of IBS is unsatisfactory, we have evaluated the
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49 80 efficacy of *B. bifidum* MIMBb75 in IBS in a double blind, placebo-controlled multi-
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51 81 center study.
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7 84 2. Patients and Methods8
9 85 2.1 Study Population

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11 86 Patients were recruited in several physician centers in Bavaria, Germany from
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13 87 principal investigators and by advertisements. The nutritional study protocol has been
14
15 88 presented to the Ethics Committee of the Bavarian Chamber of Physicians. For
16
17 89 inclusion, subjects aged between 18 and 68 years with mild to moderate IBS (Rome
18
19 90 III criteria) have been considered. Individuals with inflammatory organic
20
21 91 gastrointestinal disease, systemic diseases, cancer, autoimmune diseases, diabetes,
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23 92 known lactose intolerance or immunodeficiency, known further abdominal surgery
24
25 93 except appendectomy, being older than 50 years and having had a positive
26
27 94 sigmoidoscopy or colonoscopy within the last five years, diagnosed hyperthyroidism,
28
29 95 use of antipsychotics or systemic corticosteroids for at least 3 months prior to study
30
31 96 start, major psychiatric disorder, celiac disease or pregnancy had been excluded.
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39 98 2.2 Study Design

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41 99 This study was performed as a prospective, multi-center, randomized, double-blind,
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43 100 placebo-controlled, two-arm nutritional study.

44
45 101 Throughout the study, patients recorded their global IBS symptoms on a daily basis
46
47 102 as well as individual IBS symptoms using a patient diary. Additionally, patients have
48
49 103 been questioned at a physician site for IBS symptoms (visit 2-4) and quality of life
50
51 104 (visit 3 and 4).

52
53 105 Physician visits took place at screening, after two weeks (run-in phase), after 6
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55 106 weeks (end of treatment) and after 8 weeks (end of wash-out phase) (Figure 1).

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1
2 108 After **the** patients have given their written informed consent, they qualified for the
3
4 109 screening examination **on** day 1 (visit 1), which included a complete medical history
5
6 110 and physical examination. A blood sample was taken for analysis in a central
7
8 111 laboratory, including a pregnancy test. At the screening visit patients were instructed
9
10 112 to maintain their eating and life style habits throughout the study. A patient diary **was**
11
12 113 handed out.

13
14 114 At the second visit (day 15) diaries were reviewed. Patients who had at least 2 days
15
16 115 with mild to moderate pain during the second week of run-in and who fulfilled all
17
18 116 inclusion criteria and who did not violate any of the exclusion criteria were 1:1
19
20 117 randomized to receive either *B. bifidum* MIMBb75 or placebo. The treatment was
21
22 118 allocated according to a computer-generated blocked randomization list with a block
23
24 119 size of 4. The block size was not disclosed to the investigators. During the
25
26 120 intervention period, patients received either one probiotic capsule daily **over a 4 week**
27
28 121 **period** or an identical appearing placebo. The allocation was blinded to both patients
29
30 122 and site staff.

31
32 123 At the end of the treatment phase (visit 3, day 43), investigators collected **the** unused
33
34 124 study product and empty sachets in order to confirm compliance. Diaries were
35
36 125 collected and reviewed.

37
38 126 After the nutritional supplement-free wash-out phase (visit 4, day 57), a complete
39
40 127 physical examination was performed and a blood sample was taken.

41
42 128 Bisacodyl and Loperamid were allowed as rescue medication. Other probiotics and
43
44 129 medications that **might** influence the efficacy of the study product were not allowed.

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48 131 2.3 Probiotic Preparation

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50 132 *Bifidobacterium bifidum* MIMBb75 was isolated from the fecal sample of a healthy
51
52 133 **adult. *Bifidobacterium bifidum* is a species that is commonly detected in the feces of**

1
2 134 healthy adults and infants. Several strains of *B. bifidum* have been observed to
3
4 135 adhere very well to human intestinal cell lines. In a human Caco-2 model, the
5
6 136 adhesion ability of *B. bifidum* MIMBb75 was even significantly better than that of well-
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8
9 137 studied commercial probiotics.

10
11 138 Guglielmetti et al. showed that BopA, a surface protein, which functions as a protein
12
13 139 promoter, is directly involved in the adhesion to Caco-2 cells (15). BopA has been
14
15 140 found in several strong adhesive *B. bifidum* strains, indicating that BopA might be an
16
17 141 indicator of strong adherence. Additionally *B. bifidum* MIMBb75 showed considerably
18
19 142 more adhesion to the Caco-2 monolayer in the presence of fucose and mannose and
20
21 143 less when *B. bifidum* MIMBb75 grew in Oxgall bile salts compared to standard
22
23 144 environmental conditions. The colonization strategy of this bacterium could be
24
25 145 influenced by several factors varying along the gastrointestinal tract, such as the
26
27 146 presence of specific sugars and bile salts and the pH, likely supporting adhesion of
28
29 147 *B. bifidum* MIMBb75 to distal sites of the gut (16).

30
31 148 Nutritional supplement was prepared under good manufacturing process (GMP)
32
33 149 conditions. *B. bifidum* MIMBb75 was grown in a protein-rich liquid growth medium,
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35 150 harvested through centrifugation, stabilized, freeze-dried, milled and sieved. The dry
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37 151 powder bacteria were mixed with an excipient and filled into uncoated capsules of 1x
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39 152 10⁹ cfu. Placebo capsules appeared identical and contained maltodextrin.

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42 154 2.4 Endpoint definitions

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44 155 The prospectively defined primary efficacy variable was the subject's global
45
46 156 assessment of IBS symptoms using a 7-point Likert scale. Patients were asked to
47
48 157 answer the daily question "If you consider your IBS symptoms (e.g. abdominal
49
50 158 pain/discomfort, distension/bloating, urgency, bowel habit) in general, how have you
51
52 159 been affected by these symptoms during the last 24 hours?" Possible answers

1
2 160 ranged from 0 (not at all), 1 (very mild), 2 (mild), 3 (moderate), 4 (strong), 5 (very
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4 161 strong) to 6 (intolerable).

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6 162 Secondary efficacy variables included “abdominal pain/discomfort”,
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9 163 “distension/bloating”, and “urgency”, recorded on the same 7-point Likert scale. The
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11 164 individual symptom scores were additionally combined into a composite symptom
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13 165 score as the arithmetic mean of three individual symptom scores. Furthermore, the
14
15 166 number of bowel movements, feeling of incomplete bowel evacuation and intake of
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17 167 other medications were reported daily in the diary.

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21 168 At the end of the treatment and again at the end of the study, physicians questioned
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23 169 the patients regarding the global assessment of efficacy, tolerability as well as
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25 170 digestive disorder (“bowel movement satisfaction”). Efficacy was assessed by the
26
27 171 following question: “Please consider how you felt during the 4 week treatment
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29 172 regarding your overall well-being, and symptoms of abdominal discomfort/pain and
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31 173 altered bowel habit. Compared to the way you usually felt before taking the study
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33 174 medication, how would you rate your relief of symptoms during the last 4 weeks?”
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35 175 Possible answers were: “completely relieved (1), considerably relieved (2), somewhat
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37 176 relieved (3), unchanged (4) or worse (5)”. Both “completely relieved” and
38
39 177 “considerably relieved” were defined as “adequate relief”.

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43 178 Health related quality of life was assessed by the use of the SF-12 questionnaire
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45 179 prior to the treatment and at the end of the treatment.

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49 180 Adverse events were recorded throughout the study and the global assessment of
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51 181 tolerability was questioned at physician visit 3 and 4. Laboratory values and vital
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53 182 signs were examined at the screening visit and at the end of the study.

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59 184 2.5 Statistical methods

60 185 Sample size estimation

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2 186 A reduction of the **subject's global assessment (SGA)** of at least 20% on the 7-point
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4 187 Likert scale was considered **as** a relevant treatment effect. Based on published data
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6 188 (17), a difference of 0.6 points in the SGA of IBS symptoms between *B. bifidum*
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8 189 MIMBb75 and placebo on the 7-point Likert scale was anticipated (e.g. 3.0 in the
9
10 190 placebo group and 2.4 in the bifidobacteria group). Standard deviation was estimated
11
12 191 with 1.0 using the same data. With these assumptions, a Wilcoxon-Mann-Whitney
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14 192 test with a two-sided significance level of $\alpha=0.05$ and a power of $1 - \beta = 0.8$, a sample
15
16 193 size of 47 patients per group was required. With an estimated drop-out rate of 15-
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18 194 20% after randomization, 110 randomized patients were planned and 132 patients
19
20 195 were recruited to account for possible withdrawals prior to **the start of the** study.
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28 197 Statistical analysis

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30 198 The primary objective of this study was to prove a significant reduction of the SGA of
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32 199 general IBS symptoms at the end of **the** treatment in the bifidobacteria group vs.
33
34 200 placebo. The SGA was calculated for each subject as arithmetic mean at baseline,
35
36 201 during the treatment period and during the wash-out phase. To account for possible
37
38 202 differences in the baseline values, the change from baseline calculated as mean
39
40 203 score during 4 weeks of treatment minus mean score during the run-in phase (week
41
42 204 1-2) was defined as primary target criterion. The non-parametric Van Elteren test
43
44 205 stratified by study centers was used for the comparison of treatment arms. $P < 0.05$
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46 206 was considered statistically significant.

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51 207 The primary analysis was based on the intent-to-treat population where all
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53 208 successfully randomized patients were included. Missing post-baseline values were
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55 209 imputed by the baseline value for the primary target criterion and these patients were
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57 210 evaluated as non-responders (**n=1 during treatment, n=3 during wash-out phase**). An
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59 211 additional per protocol analysis was performed for supportive purposes.
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2 212 Descriptive analyses of secondary target criteria were based on available data.
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4 213 Treatment differences were tested by the use of the non-parametric Wilcoxon test for
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6 214 continuous variables or by Fishers exact test for binary variables. All p-values are
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8
9 215 two-sided.

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11 216 Secondary efficacy variables included response based on a 50% rule of symptom
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13 217 relief during the treatment (at least improvement in two out of four weeks within the
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15 218 treatment period and improvement defined as at least one point reduction from
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18 219 baseline).

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21 220 All statistical analyses were performed using SAS version 9.1.3 for windows, SAS
22
23 221 Institute Inc., Cary, NC.

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29
30 224 3. Results

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33 225 Subjects

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35 226 A total of 132 patients were included into the study and 122 patients were
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37 227 successfully randomized to receive either placebo (N=62) or *B. bifidum* MIMBb75
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39 228 (N=60). All randomized patients were analyzed for intent to treat (N=122). One
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41 229 patient with no post randomization visit was excluded from the analysis of adverse
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44 230 events. A total of 103 patients (49 placebo, 54 *B. bifidum* MIMBb75) were examined
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47 231 as per protocol (Fig. 2).

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52 233 Baseline Characteristics

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54 234 In terms of baseline characteristics, there were no significant differences between the
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56 235 groups. 21.3% were classified as diarrhea-predominant IBS (23.3% in the
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58 236 bifidobacteria group, 19.4% in the placebo group), 19.7% as constipation-
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60 237 predominant IBS (15% in the bifidobacteria group, 24.2% in the placebo group) and

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2 238 58.2% as alternators (61.7% in the bifidobacteria group, 54.8% in the placebo group)
3
4 239 with no significant differences between the bifidobacteria and the placebo group.
5
6 240 Demographics were well balanced between the treatment groups with about 67%
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8 241 female patients and mean weight of 71 kg corresponding to a BMI of 24. The
9
10 242 average age of patients was 41 years in the placebo group and 37 years in the
11
12 243 bifidobacteria group (Table 1).
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18 245 Subject's global assessment (SGA) of IBS symptoms

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20 246 The primary endpoint was the reduction of the SGA of IBS symptoms on the
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22 247 subject's global assessment diary. *B. bifidum* MIMBb75 significantly improved global
23
24 248 IBS symptoms by -0.88 points [95%CI: -1.07; -0.69] (from 2.95 in the run in phase to
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26 249 2.07 in the treatment phase) compared to only -0.16 points [95%CI: -0.32; 0.00] (from
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28 250 2.79 in the run in phase to 2.63 in the treatment phase) in the placebo group
29
30 251 (p<0.0001) using the 7-point Likert scale. The evaluation of the SGA on a weekly
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32 252 basis showed a significant benefit for patients within the bifidobacteria group for
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34 253 every single week starting the second week of treatment till the end of the study (Fig.
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36 254 3).
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44 256 Secondary endpoints

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46 257 Secondary endpoints included changes in IBS symptoms – “pain/discomfort”,
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48 258 “distension/ bloating”, “urgency”, “number of bowel movements” and “feeling of
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50 259 incomplete evacuation” - on a seven-point Likert scale. *B. bifidum* MIMBb75 showed
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52 260 a significant reduction of pain/discomfort by
53
54 261 -0.82 points [95%CI: -1.01; -0.63] vs. -0.18 [95%CI: -0.35; -0,01] in the placebo group
55
56 262 (p<0.0001), and distension/bloating by -0.92 points [95%CI: -1.15; -0.69] vs. -0.21
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58 263 [95%CI: -0.37; -0.05] in the placebo group (p<0.0001) during the treatment. The
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2 264 reduction persisted during the wash-out phase. Urgency was significantly reduced by
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4 265 -0.67 points [95%CI: -0,86; -0,48] vs. -0.21 [95%CI: -0,35; -0.07] in the placebo group
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6
7 266 (p=0.0001) during the treatment but not during the wash-out phase. No effects could
8
9 267 be detected for frequency of bowel movement and feeling of incomplete bowel
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11 268 evacuation (Fig. 4).

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16 270 The evaluation of the IBS symptoms pain/discomfort and distension/bloating on a
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18 271 weekly basis showed a significant benefit for patients within the bifidobacteria group
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20 272 compared to placebo for every single week beginning the second week of treatment
21
22 273 till the end of the study. A significant difference in urgency between the bifidobacteria
23
24 274 and the placebo group was shown between week four and six (Fig. 5 and Fig. 6).

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29
30 276 Bowel movement satisfaction (digestive disorder) decreased from 3.89 to 2.44 in the
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32 277 bifidobacteria group vs. 3.69 to 3.47 in the placebo group (p=0.0002) after treatment.
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34 278 The reduction persisted during wash-out phase (2.33 in the bifidobacteria group vs.
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36 279 3.47 in the placebo group, p<0.0001).

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41 281 Composite score

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43 282 A composite score was calculated for the IBS symptoms pain/discomfort,
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45 283 distension/bloating and urgency. During the run in phase, the score was comparable
46
47 284 in both groups. The patients within the bifidobacteria group significantly benefited
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49 285 from the consumption of *B. bifidum* MIMBb75 vs. placebo. The composite score
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51 286 improved from 2.68 at baseline to 1.88 after treatment in the bifidobacteria group and
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53 287 from 2.56 at baseline to 2.37 after treatment in the placebo group (-0.80 in the
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55 288 bifidobacteria group; -0.20 in the placebo group; p<0.0001). This improvement was
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1
2 289 also preserved during the wash-out phase (-0.85 in the bifidobacteria group; -0.31 in
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4 290 the placebo group; $p < 0.0001$).

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9 292 Treatment responders

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11 293 Overall responders were defined as patients experiencing an improvement of the
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13 294 average weekly score of at least 1 point on the Likert scale for the primary parameter
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15 295 (SGA of IBS symptoms) in at least two out of the 4 weeks treatment period (50%
16
17 296 rule). Abdominal pain responders were defined using the same 50% rule for at least
18
19 297 one point average improvement for the assessment of "pain/discomfort". Overall
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21 298 responder rates were 56.7% in the bifidobacteria group and only 21.0% in the
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23 299 placebo group ($p = 0.0001$). The difference between the treatment arms was only a
24
25 300 little bit less pronounced when considering only the symptom "pain/discomfort" where
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27 301 responder rates were calculated to be 48.3% in the bifidobacteria and only 24.2% in
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29 302 the placebo group ($p = 0.008$) (Fig. 7).

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37 304 Global efficacy at physician site

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39 305 The overall assessment of efficacy was significantly better in the bifidobacteria group
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41 306 compared to placebo. At the end of treatment 43.3% of the patients in the
42
43 307 bifidobacteria group achieved adequate relief compared to only 8.1% in the placebo
44
45 308 group ($p < 0.0001$). At the end of the study adequate relief was reported for 46.7% in
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47 309 the bifidobacteria and 11.3% of the patients in the placebo group ($p < 0.0001$; Fig. 8).

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54 311 Health related quality of life

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56 312 The evaluation of the SF12 sum scores showed a significant gain in quality of life
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58 313 within the bifidobacteria group. Physical health sum improved from 47.89 at baseline
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60 314 to 51.80 after treatment in the bifidobacteria group and from 47.33 to only 48.85 in

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2 315 the placebo group. Physical health sum significantly changed by 3.99 in the
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4 316 bifidobacteria group and by only 1.08 in the placebo group compared to baseline
5
6 317 (p=0.0185). Mental health sum improved from 45.53 at baseline to 51.11 after
7
8 318 treatment in the bifidobacteria group and from 47.01 to only 48.29 in the placebo
9
10 319 group. Mental health sum significantly changed by 5.78 in the bifidobacteria group
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12 320 and by only 1.58 in the placebo group compared to baseline (p=0.0083).

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18 322 Adverse events

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20 323 Only 36 adverse events were reported with suspected relation to the study product,
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22 324 13 in the placebo and 23 in the treatment group, but no significant differences could
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24 325 be detected in the side effects profile of *B. bifidum* MIMBb75 vs. placebo.
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26 326 Additionally, no severe adverse events have been recorded in either group.
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34 331 4. Discussion and Conclusion

35 332

36 333 This randomized, double blind, placebo-controlled study indicates that *B. bifidum*
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38 334 MIMBb75 has beneficial effects in the treatment of IBS. In this study, *B. bifidum*
39
40 335 MIMBb75 significantly improved global IBS as well as its related symptoms such as
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42 336 pain/discomfort, distension/bloating and digestive disorders compared to placebo.
43
44 337 Moreover, *B. bifidum* MIMBb75 also significantly improved the quality of life. These
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46 338 benefits persisted within the consumption-free wash-out phase. Overall responder
47
48 339 rates were predominantly high with 56.7% in the bifidobacteria group compared to
49
50 340 only 21.0% in the placebo group (p=0.0001). At the end of the study adequate relief

1
2 341 was reported for 46.7% in the bifidobacteria and only 11.3% of the patients in the
3
4 342 placebo group ($p < 0.0001$).

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6
7 343 Up to date, several studies have examined the effects of probiotics on IBS and its
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9 344 symptoms (13, 14, 18, 19). However, only a few could show a significant benefit.
10
11 345 Additionally, to our knowledge, no probiotic strain could show to significantly alleviate
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13 346 irritable bowel syndrome and simultaneously improve the quality of life. While some
14
15 347 studies might have missed to show efficacy due to small sample size and
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17 348 randomization errors, several different probiotic strains did repeatedly show no
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19 349 significant improvement in IBS (20, 21, 22, 23, 24). Recently, Brenner et al. (2009)
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21 350 published a systematic review of randomized controlled trials (RCTs) aimed at the
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23 351 evaluation of the efficacy, safety, and tolerability of probiotics in the treatment of IBS.
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25 352 A total of 16 RCTs were included in the analysis. Of those, exclusively one
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27 353 bifidobacteria strain showed efficacy for improvement of IBS symptoms in two
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29 354 appropriately designed studies (4). Apart from inappropriate study design, in our
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31 355 opinion, these findings could be attributed to the fact that the efficacy of probiotics is
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33 356 strongly strain specific and that only few strains might be able to show efficacy in
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35 357 IBS.

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37 358 No study to date could prove unambiguously the mode of action of probiotics, which
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39 359 can be clearly linked to the improvement of IBS and its symptoms. Several studies
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41 360 have observed altered intestinal microbiota with a significant lack of bifidobacteria
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43 361 (25, 26) and a dysfunctional intestinal barrier in IBS patients. These studies have
44
45 362 been supported by the fact that a normalization of the lactulose breath test, which
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47 363 suggests the presence of small intestinal bacterial overgrowth or an increased
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49 364 number of enteric microorganisms, is highly correlated with a significant reduction of
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51 365 IBS symptoms (27). It has been stated that the imbalance of the microbiotic
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53 366 composition may lead to a different fermentation pattern, especially with increased
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2 367 hydrogen production resulting in bloating (28, 29). Additionally, gut mucosal barrier
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4 368 disruption has been proven to be associated with bacterial translocation and
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6 369 inflammatory conditions (4,5,6). It seems likely that only specific strains are able to
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8 370 restore an imbalanced intestinal microbiota and gut barrier, however further research
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10 371 is needed to investigate if the positive effects of *B. bifidum* MIMBb75 can be
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12 372 attributed to this mode of action.

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16 373 There are several limitations of this study. First of all, the study population was not
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18 374 large enough for a sub-group analysis of IBS subtypes. We could therefore not show
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20 375 whether some IBS-subtypes would benefit more from the consumption of *B. bifidum*
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22 376 MIMBb75 than others. In order to prove whether some subgroups might benefit
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24 377 more, a larger study might be useful. The study population has mild to moderate IBS
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26 378 symptoms as evidenced by the indicated SGA-baseline values. A further study
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28 379 should be aimed at the examination whether the patients with severe IBS symptoms
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30 380 will profit in the same way as those with mild to moderate symptoms or whether these
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32 381 patients are more refractory to this treatment.

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37 382 Additionally, the duration of the study was perhaps short at 4 weeks. It would be of
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39 383 specific value to explore whether patients benefit more from a longer consumption of
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41 384 *B. bifidum* MIMBb75. Finally, the observation that patients still benefit during the
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43 385 consumption-free wash-out phase is of significant value and requires further
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45 386 investigation (for longer follow-up).

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49 387 In conclusion, the beneficial effects of *B. bifidum* MIMBb75 in improving global IBS as
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51 388 well as its symptoms along with the good side effect profile suggests that this
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53 389 probiotic strain has the potential as an effective alternative to current treatment
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55 390 options.

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4 394 **5. References**

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22 508 **Statement of Interests**

23
24 509 **Author's declaration of personal interest: none.**

25
26 510 **Declaration of funding interests:**

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28
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31 512 **München, Germany**

32
33 513 - **Initial data analyses were undertaken by Dr. Karl Fehnle who is employee of**

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35 514 **Algora Gesellschaft für Medizinstatistik und Vertriebssysteme mbH and**

36
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516 **Tables**

517 Table 1: Demographic characteristics of the ITT-population

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519 **Table 2: Summary of reported adverse events with suspected relationship by system**
520 **organ class (SOC)**

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522 **Table 3: Incidence of adverse events at visit 3-4 by preferred term (occurrence of**
523 **more than 2 %)**

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For Peer Review

542 Table 2

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	Placebo	<i>Bifidobacterium bifidum</i> MIMBb75	
	N(%) or Mean ± SD	N(%) or Mean ± SD	
N=122 (62+60)			544
Age	40.98 ± 12.80	36.65 ± 12.42	545
Female sex	41 (66.1)	41 (68.3)	546
Height (cm)	169.50 ± 8.75	170.78 ± 9.47	547
Weight (kg)	70.79 ± 15.54	70.45 ± 16.02	548
BMI	24.60 ± 5.19	24.02 ± 4.45	549
IBS Type (N=122 (61+60))			550
Diarrhea predominant	12 (19.4)	14 (23.3)	551
Constipation predominant	15 (24.2)	9 (15.0)	552
Alternating type	34 (54.8)	37 (61.7)	553
			554
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			557
			558

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561 Table 2

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Visit	SOC	Group					
		Placebo		Verum		All	
		N	PctN	N	PctN	N	PctN
3	Gastrointestinal disorders	7	53.85	12	52.17	19	52.78
	General disorders and administration site conditions	1	7.69	.	.	1	2.78
	All	8	61.54	12	52.17	20	55.56
4	SOC						
	Gastrointestinal disorders	5	38.46	8	34.78	13	36.11
	General disorders and administration site conditions	.	.	1	4.35	1	2.78
	Investigations	.	.	1	4.35	1	2.78
	Skin and subcutaneous tissue disorders	.	.	1	4.35	1	2.78
	All	5	38.46	11	47.83	16	44.44
All		13	100.00	23	100.00	36	100.00

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565 Table 3

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	Treatment		Control		Total		P
	N	(%)	N	(%)	N	(%)	
Abdominal distension	3	5.00	4	6.56	7	5.79	1.0000
Abdominal pain	2	3.33	2	3.28	4	3.31	1.0000
Abdominal pain upper	3	5.00	1	1.64	4	3.31	0.3645
Constipation	2	3.33	1	1.64	3	2.48	0.6187
Diarrhoea	3	5.00	1	1.64	4	3.31	0.3645

	Treatment N	(%)	Control N	(%)	Total N	(%)	P
Frequent bowel movements	3	5.00	0	0.00	3	2.48	0.1188
Nausea	3	5.00	0	0.00	3	2.48	0.1188

All p values by Fishers exact test.

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For Peer Review

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2 569 **Figures**
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5 570

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7 571 Fig. 1. Study schematic.
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11 573 Fig. 2. Diagram of study flow.
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15 575 Fig. 3. Comparison of effects of placebo and *B. bifidum* MIMBb75 on global IBS
16 symptoms (by SGA, recorded on a 0-6 scale) on a weekly basis. Significant
17 576 improvement of global IBS symptoms in the bifidobacteria group vs. placebo.
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21 579 Fig 4.. Comparison of the reduction of IBS symptoms (*B. bifidum* MIMBb75 vs.
22 placebo) on mean score changes from baseline to treatment phase.
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26 582 Fig. 5. Comparison of effects of placebo and *B. bifidum* MIMBb75 on pain/discomfort
27 (recorded on a 0-6 Likert scale) on a weekly basis. Significant improvement in the
28 583 bifidobacteria group vs. placebo group.
29 584
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32 586 Fig. 6. Comparison of effects of placebo and *B. bifidum* MIMBb75 on
33 distension/bloating (recorded on a 0-6 scale) on a weekly basis. Significant
34 587 improvement in the bifidobacteria group vs. placebo group.
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38 590 Fig. 7. Overall responders during treatment (ITT).
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41 592 Fig. 8. Adequate relief after treatment (ITT).
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Fig.1

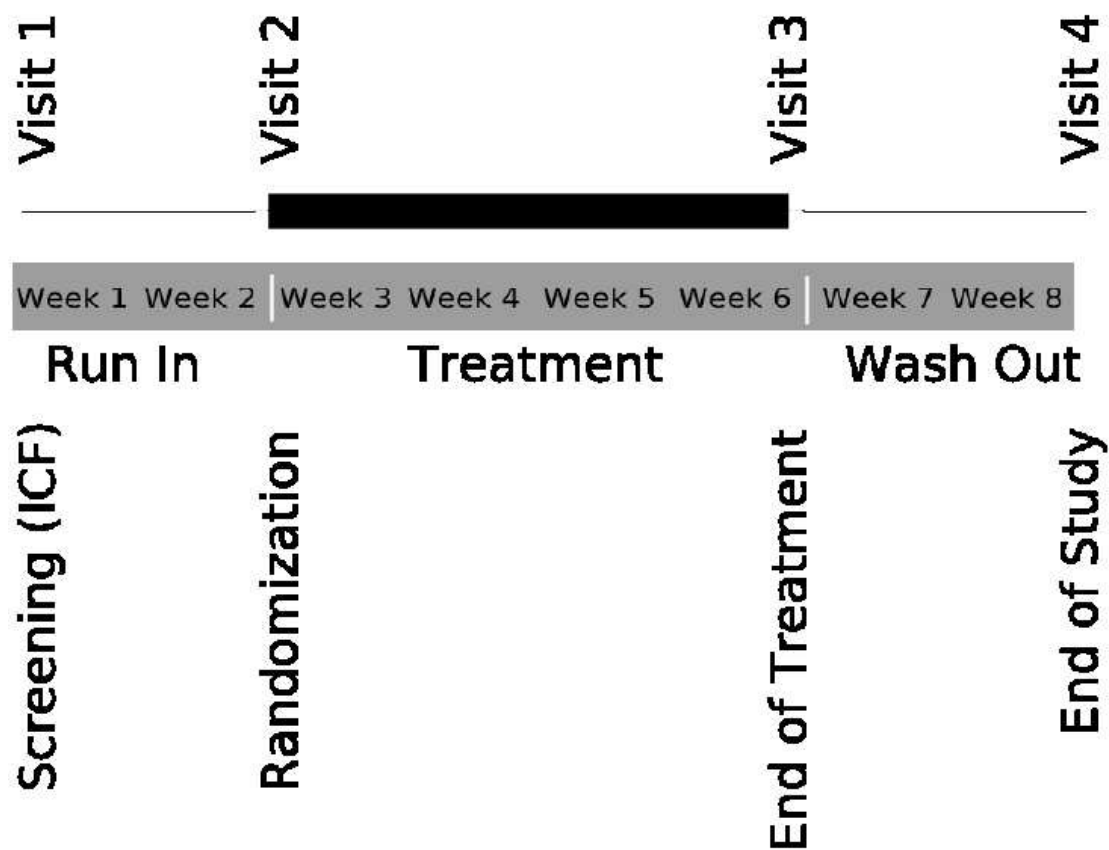


Fig.2.

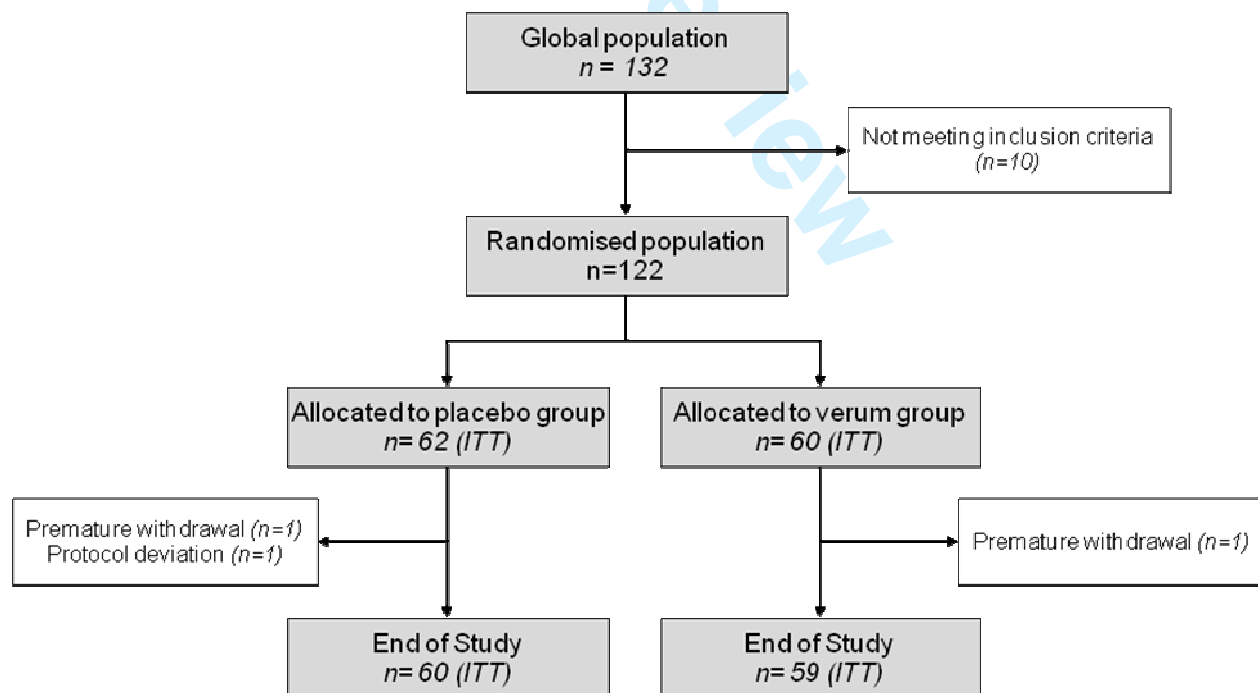


Fig. 3

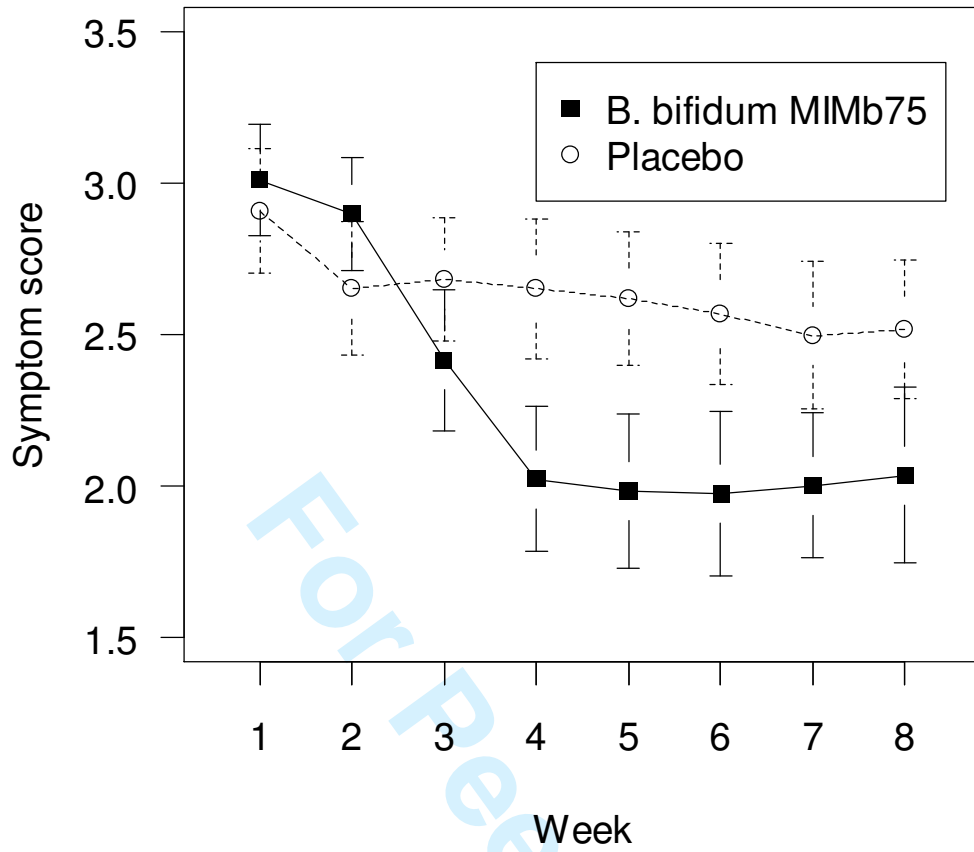


Fig.4

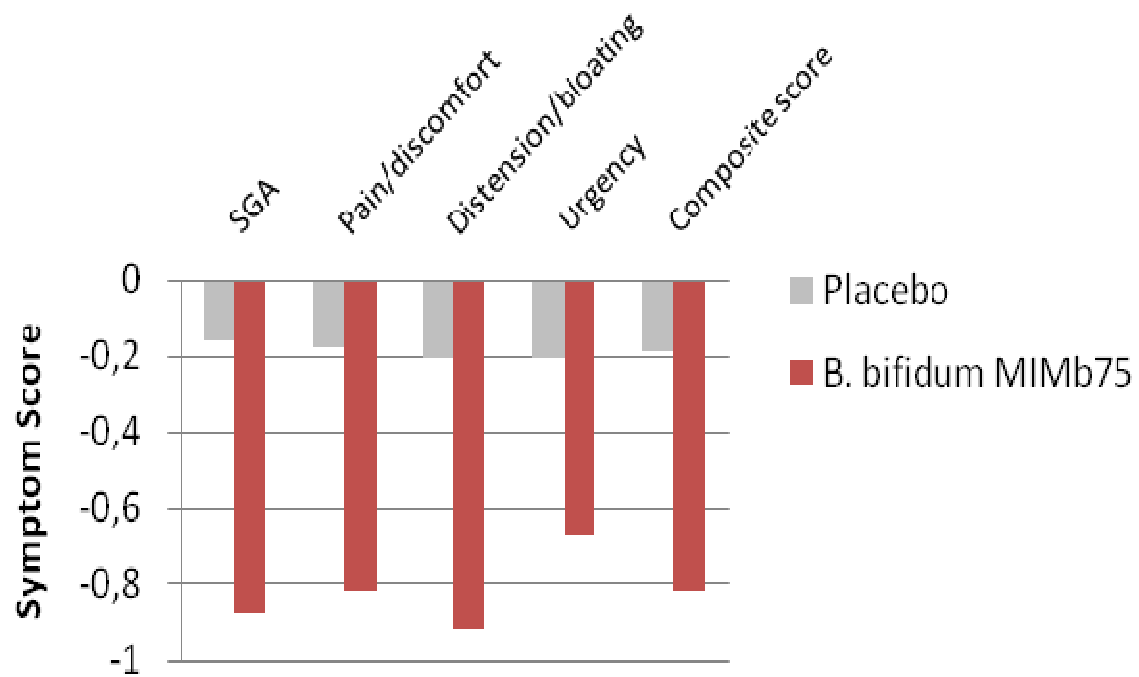


Fig. 5

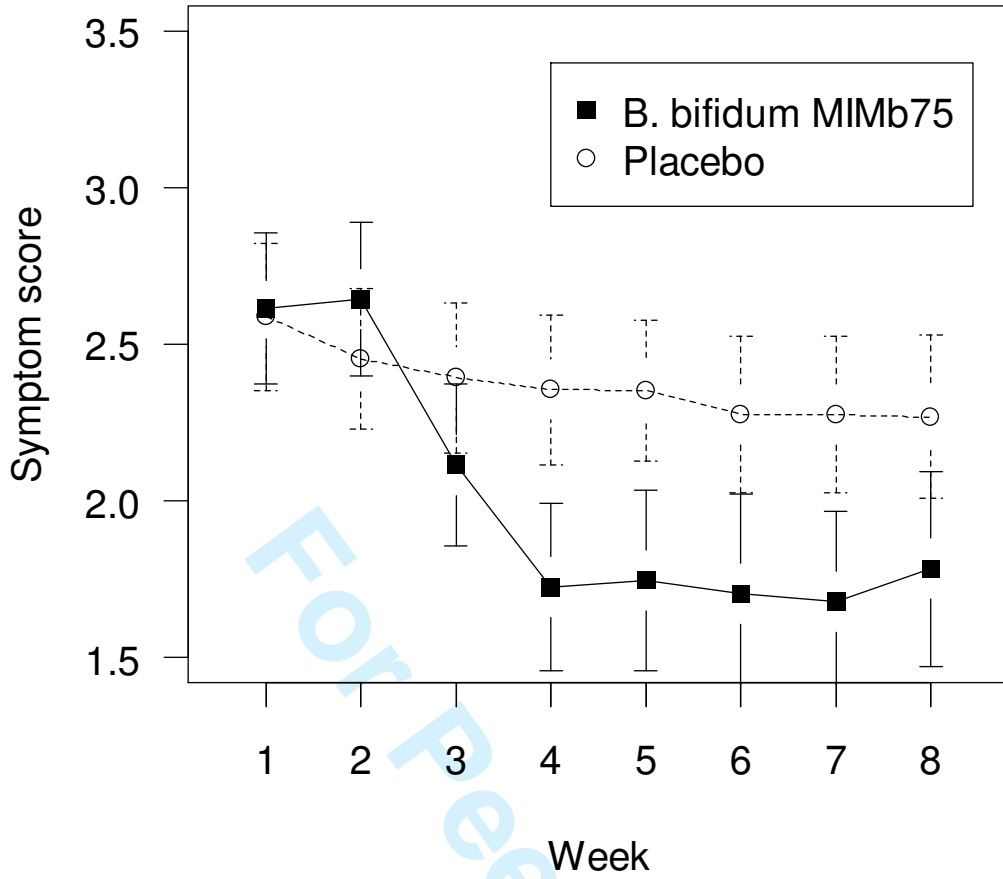


Fig. 6

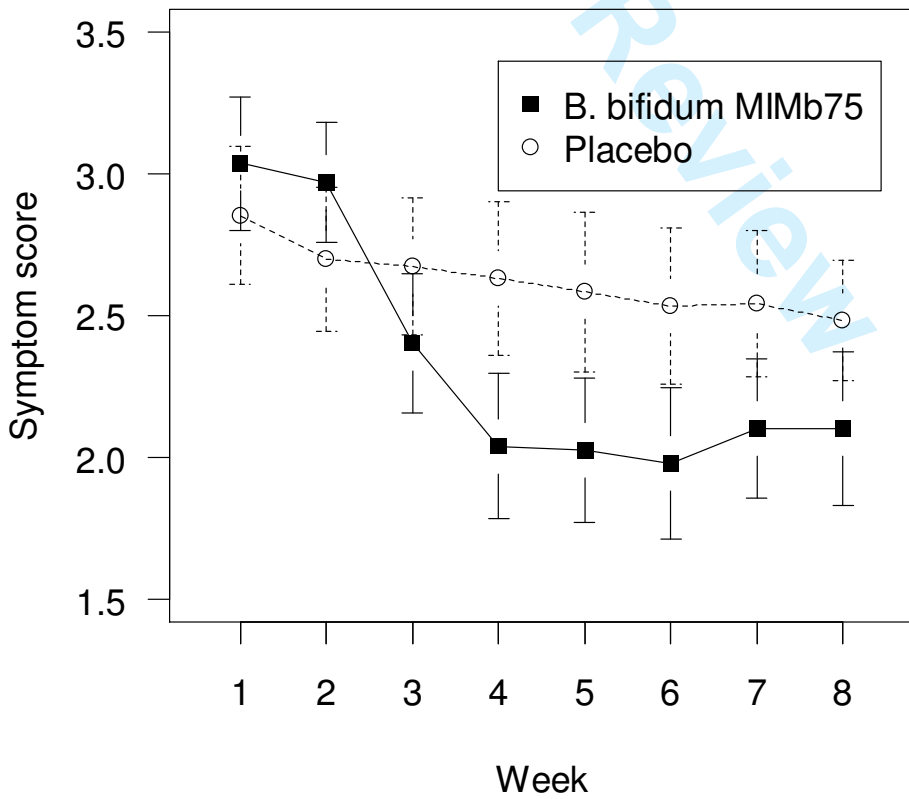


Fig. 7

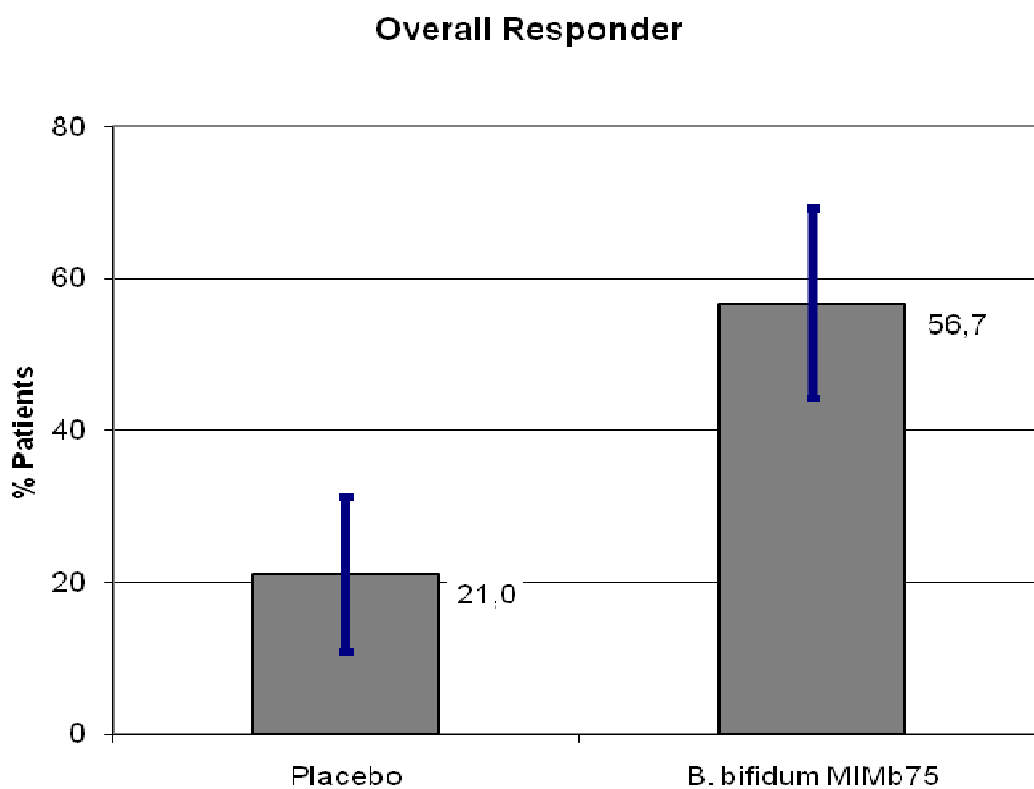
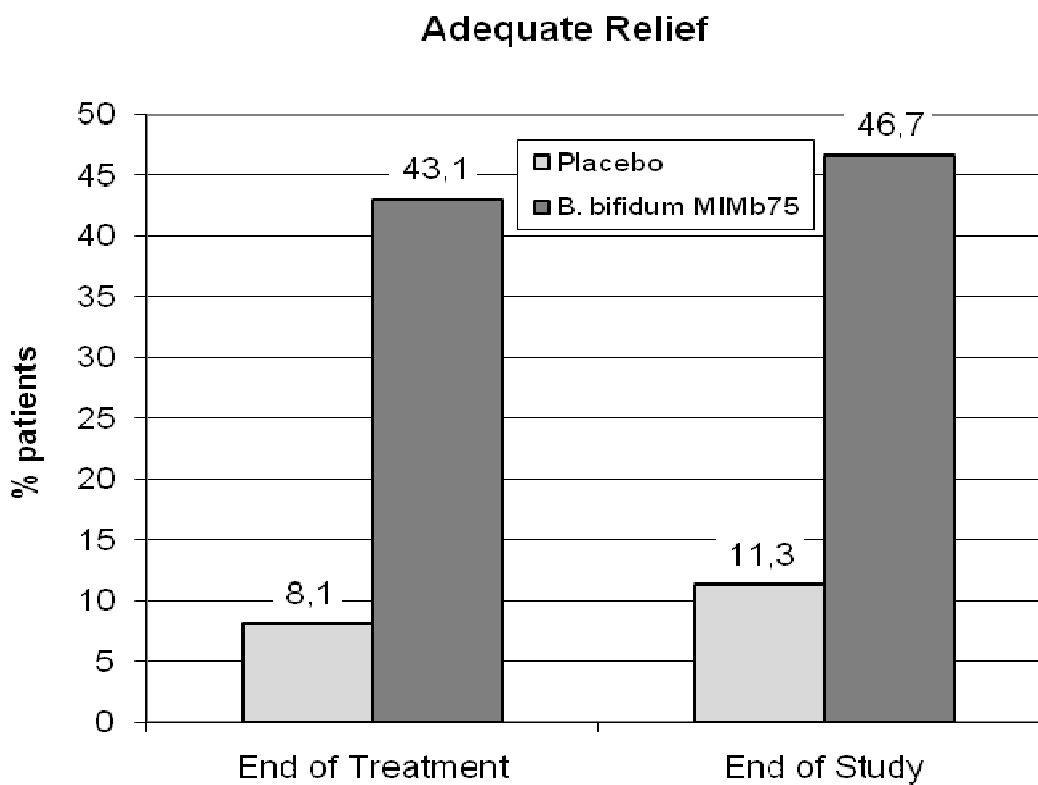
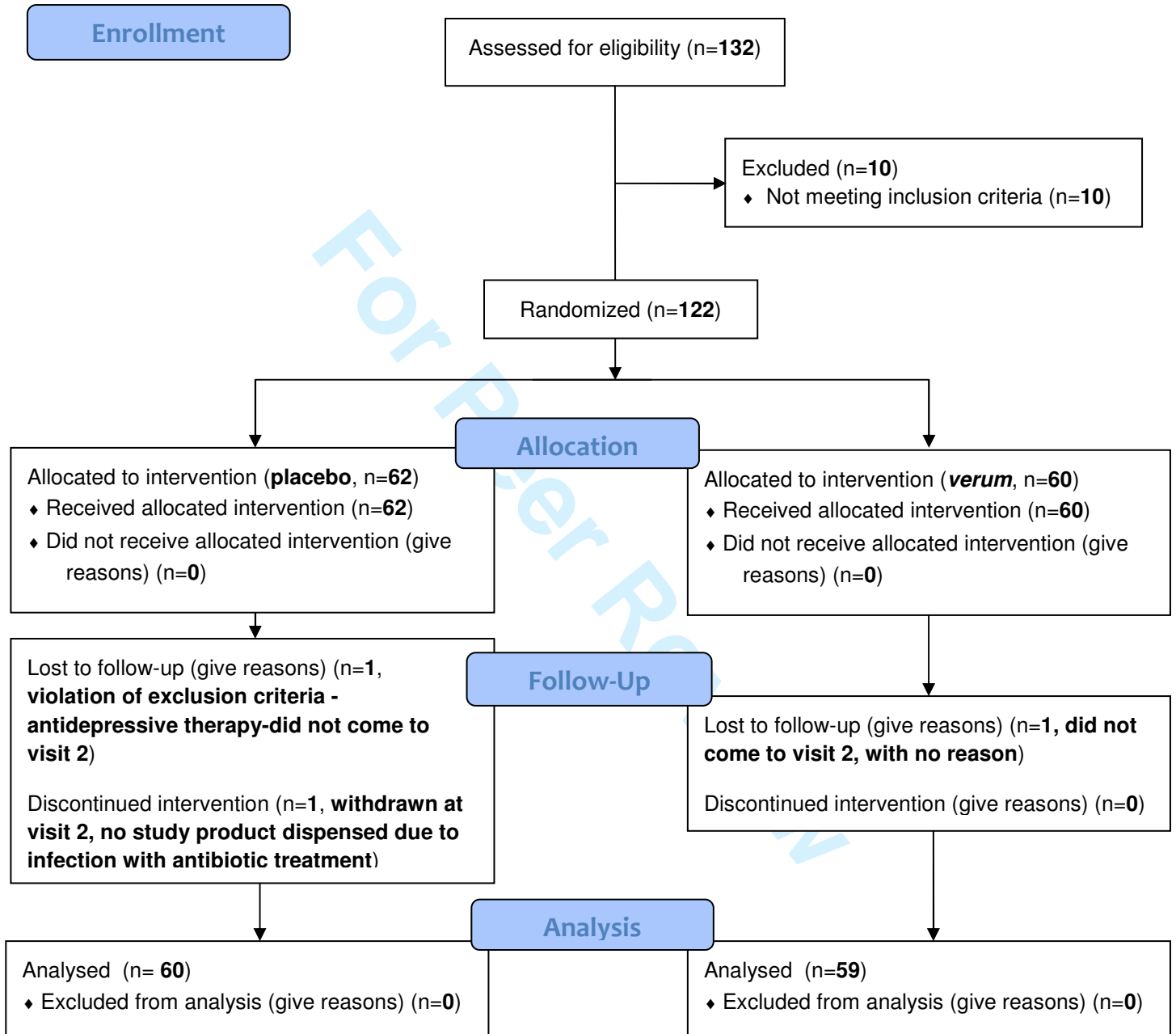


Fig. 8



CONSORT 2010 Flow Diagram





CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4, 5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	/
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	/
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	/
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	/
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

1		assessing outcomes) and how	
2	11b	If relevant, description of the similarity of interventions	/
3	Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes
4		12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses
5			7, 8
6			8, 9
7	Results		
8	Participant flow (a	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and
9	diagram is strongly		were analysed for the primary outcome
10	recommended)	13b	For each group, losses and exclusions after randomisation, together with reasons
11			9
12			CONSORT
13	Recruitment	14a	Flow Diagram
14		14b	4, 5
15	Baseline data	15	/
16	Numbers analysed	16	A table showing baseline demographic and clinical characteristics for each group
17			20
18	Outcomes and	17a	For each group, number of participants (denominator) included in each analysis and whether the analysis was
19	estimation		by original assigned groups
20		17b	For each primary and secondary outcome, results for each group, and the estimated effect size and its
21			precision (such as 95% confidence interval)
22	Ancillary analyses	18	For binary outcomes, presentation of both absolute and relative effect sizes is recommended
23			Fig. 1-8
24			/
25	Harms	19	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing
26			pre-specified from exploratory
27			/
28	Discussion		13
29	Limitations	20	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)
30	Generalisability	21	15
31	Interpretation	22	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses
32			13, 14
33	Other information		13-15
34	Registration	23	Registration number and name of trial registry
35	Protocol	24	/
36	Funding	25	Where the full trial protocol can be accessed, if available
37			/
38			18

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.