

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

Journal:	<i>Alimentary Pharmacology & Therapeutics</i>
Manuscript ID:	APT-0148-2011.R1
Wiley - Manuscript type:	SuperFast
Date Submitted by the Author:	01-Mar-2011
Complete List of Authors:	Guglielmetti, Simone; Università degli Studi di Milano, Department of Food Science and Microbiology Mora, Diego; Università degli Studi di Milano, Department of Food Science and Microbiology Gschwender, Martin Popp, Katja Hug, Sandra; Naturwohl Pharma GmbH, Bauerstraße
Keywords:	Irritable bowel syndrome < Disease-based, Probiotics/prebiotics < Topics, X keyword = no topic , Y keyword = no topic

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

4
5
6 2 significantly alleviates irritable bowel syndrome and improves quality

7
8
9 3 of life: a double-blind, placebo-controlled study

10
11
12 4
13
14
15 5 Dr. Simone Guglielmetti^{a,*}, Prof. Dr. Diego Mora^b, Dr. med. Martin Gschwender^c,
16
17 6 Katja Popp^d, Sandra Hug^{e,**}

18
19
20
21 7
22
23 8 ^a Department of Food Science and Microbiology (DiSTAM), Università degli Studi di
24
25 9 Milano, Milan, Italy.

26
27
28 10 ^b Department of Food Science and Microbiology (DiSTAM), Università degli Studi di
29
30 11 Milano, Milan, Italy.

31
32
33 12 ^c Director of the nutritional study, practising physician, Munich, Germany

34
35 13 ^d Master of Science in Molecular Biotechnology

36
37 14 ^e Naturwohl Pharma GmbH, Munich, Germany

38
39
40 15
41
42 16
43
44 17
45
46 18
47
48 19 * Mailing address: Via Celoria 2, 20133, Milan, Italy. E-mail:

49
50 20 simone.guglielmetti@unimi.it. Tel: +39 02 503 19136. Fax: +39 02 503 19191.

51
52
53 21 ** Mailing address: Naturwohl Pharma GmbH, Bauerstraße 22, 80796 München,
54
55 22 Germany. E-Mail: sh@naturwohl-pharma.de. Tel: +49 89 787979 013. Fax: +49 89
56
57 23 787979 031.

58
59
60
24

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

50

51

52

53

54

55

1
2 56 1. Introduction
3

4 57 Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder,
5
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
48
49 80 efficacy of *B. bifidum* MIMBb75 in IBS in a double blind, placebo-controlled multi-
50
51
52
53
54
55
56
57
58
59
60 81 center study.

1
2 82

3
4 83

5
6
7 84 2. Patients and Methods

8
9 85 2.1 Study Population

10
11 86 Patients were recruited in several physician centers in Bavaria, Germany from
12
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,
22
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.
32
33
34
35
36

37 97

38
39 98 2.2 Study Design

40
41 99 This study was performed as a prospective, multi-center, randomized, double-blind,
42
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
54
55 106 weeks (end of treatment) and after 8 weeks (end of wash-out phase) (Figure 1).

56
57
58
59
60 107

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.

45
46 130

47 131 2.3 Probiotic Preparation

48
49 132 *Bifidobacterium bifidum* MIMBb75 was isolated from the fecal sample of a healthy
50
51 133 **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-
7
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,
34
35 150 harvested through centrifugation, stabilized, freeze-dried, milled and sieved. The dry
36
37 151 powder bacteria were mixed with an excipient and filled into uncoated capsules of 1x
38
39 152 10⁹ cfu. Placebo capsules appeared identical and contained maltodextrin.

40
41 153

42 154 2.4 Endpoint definitions

43
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
3
4 161 strong) to 6 (intolerable).

5
6 162 Secondary efficacy variables included “abdominal pain/discomfort”,
7
8 163 “distension/bloating”, and “urgency”, recorded on the same 7-point Likert scale. The
9
10 164 individual symptom scores were additionally combined into a composite symptom
11
12 165 score as the arithmetic mean of three individual symptom scores. Furthermore, the
13
14 166 number of bowel movements, feeling of incomplete bowel evacuation and intake of
15
16 167 other medications were reported daily in the diary.

17
18 168 At the end of the treatment and again at the end of the study, physicians questioned
19
20 169 the patients regarding the global assessment of efficacy, tolerability as well as
21
22 170 digestive disorder (“bowel movement satisfaction”). Efficacy was assessed by the
23
24 171 following question: “Please consider how you felt during the 4 week treatment
25
26 172 regarding your overall well-being, and symptoms of abdominal discomfort/pain and
27
28 173 altered bowel habit. Compared to the way you usually felt before taking the study
29
30 174 medication, how would you rate your relief of symptoms during the last 4 weeks?”
31
32 175 Possible answers were: “completely relieved (1), considerably relieved (2), somewhat
33
34 176 relieved (3), unchanged (4) or worse (5)”. Both “completely relieved” and
35
36 177 “considerably relieved” were defined as “adequate relief”.

37
38 178 Health related quality of life was assessed by the use of the SF-12 questionnaire
39
40 179 prior to the treatment and at the end of the treatment.

41
42 180 Adverse events were recorded throughout the study and the global assessment of
43
44 181 tolerability was questioned at physician visit 3 and 4. Laboratory values and vital
45
46 182 signs were examined at the screening visit and at the end of the study.

47
48 183

49
50 184 2.5 Statistical methods

51
52 185 Sample size estimation
53
54
55
56
57
58
59
60

1
2 186 A reduction of the **subject's global assessment (SGA)** of at least 20% on the 7-point
3
4 187 Likert scale was considered **as** a relevant treatment effect. Based on published data
5
6 188 (17), a difference of 0.6 points in the SGA of IBS symptoms between *B. bifidum*
7
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
13
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-
17
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.
21
22
23
24
25
26
27

28 197 Statistical analysis

29
30 198 The primary objective of this study was to prove a significant reduction of the SGA of
31
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. $P < 0.05$
45
46 206 was considered statistically significant.

47
48
49
50
51 207 The primary analysis was based on the intent-to-treat population where all
52
53 208 successfully randomized patients were included. Missing post-baseline values were
54
55 209 imputed by the baseline value for the primary target criterion and these patients were
56
57 210 evaluated as non-responders (**n=1 during treatment, n=3 during wash-out phase**). An
58
59 211 additional per protocol analysis was performed for supportive purposes.
60

1
2 212 Descriptive analyses of secondary target criteria were based on available data.
3
4 213 Treatment differences were tested by the use of the non-parametric Wilcoxon test for
5
6 214 continuous variables or by Fishers exact test for binary variables. All p-values are
7
8
9 215 two-sided.

10
11 216 Secondary efficacy variables included response based on a 50% rule of symptom
12
13 217 relief during the treatment (at least improvement in two out of four weeks within the
14
15 218 treatment period and improvement defined as at least one point reduction from
16
17
18 219 baseline).

19
20
21 220 All statistical analyses were performed using SAS version 9.1.3 for windows, SAS
22
23 221 Institute Inc., Cary, NC.

24
25
26 222

27
28 223

29
30 224 3. Results

31
32 225 Subjects

33
34
35 226 A total of 132 patients were included into the study and 122 patients were
36
37 227 successfully randomized to receive either placebo (N=62) or *B. bifidum* MIMBb75
38
39 228 (N=60). All randomized patients were analyzed for intent to treat (N=122). One
40
41 229 patient with no post randomization visit was excluded from the analysis of adverse
42
43 230 events. A total of 103 patients (49 placebo, 54 *B. bifidum* MIMBb75) were examined
44
45
46 231 as per protocol (Fig. 2).

47
48
49 232

50
51 233 Baseline Characteristics

52
53 234 In terms of baseline characteristics, there were no significant differences between the
54
55 235 groups. 21.3% were classified as diarrhea-predominant IBS (23.3% in the
56
57 236 bifidobacteria group, 19.4% in the placebo group), 19.7% as constipation-
58
59 237 predominant IBS (15% in the bifidobacteria group, 24.2% in the placebo group) and
60

1
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%
7
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).
13

14
15
16 244

17
18 245 Subject's global assessment (SGA) of IBS symptoms

19
20 246 The primary endpoint was the reduction of the SGA of IBS symptoms on the
21
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
25
26 249 2.07 in the treatment phase) compared to only -0.16 points [95%CI: -0.32; 0.00] (from
27
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
31
32 252 basis showed a significant benefit for patients within the bifidobacteria group for
33
34 253 every single week starting the second week of treatment till the end of the study (Fig.
35
36 254 3).
37
38
39
40
41

42 255

43
44 256 Secondary endpoints

45
46 257 Secondary endpoints included changes in IBS symptoms – “pain/discomfort”,
47
48 258 “distension/ bloating”, “urgency”, “number of bowel movements” and “feeling of
49
50 259 incomplete evacuation” - on a seven-point Likert scale. *B. bifidum* MIMBb75 showed
51
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
57
58 263 [95%CI: -0.37; -0.05] in the placebo group (p<0.0001) during the treatment. The
59
60

1
2 264 reduction persisted during the wash-out phase. Urgency was significantly reduced by
3
4 265 -0.67 points [95%CI: -0,86; -0,48] vs. -0.21 [95%CI: -0,35; -0.07] in the placebo group
5
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
10
11 268 evacuation (Fig. 4).

12
13 269

14
15
16 270 The evaluation of the IBS symptoms pain/discomfort and distension/bloating on a
17
18 271 weekly basis showed a significant benefit for patients within the bifidobacteria group
19
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).

25
26 275

27
28
29
30 276 Bowel movement satisfaction (digestive disorder) decreased from 3.89 to 2.44 in the
31
32 277 bifidobacteria group vs. 3.69 to 3.47 in the placebo group (p=0.0002) after treatment.
33
34 278 The reduction persisted during wash-out phase (2.33 in the bifidobacteria group vs.
35
36 279 3.47 in the placebo group, p<0.0001).

37
38 280

39
40
41 281 Composite score

42
43 282 A composite score was calculated for the IBS symptoms pain/discomfort,
44
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
48
49 285 from the consumption of *B. bifidum* MIMBb75 vs. placebo. The composite score
50
51 286 improved from 2.68 at baseline to 1.88 after treatment in the bifidobacteria group and
52
53 287 from 2.56 at baseline to 2.37 after treatment in the placebo group (-0.80 in the
54
55 288 bifidobacteria group; -0.20 in the placebo group; p<0.0001). This improvement was
56
57
58
59
60

1
2 289 also preserved during the wash-out phase (-0.85 in the bifidobacteria group; -0.31 in
3
4 290 the placebo group; $p < 0.0001$).

5
6
7 291

8
9 292 Treatment responders

10
11 293 Overall responders were defined as patients experiencing an improvement of the
12
13 294 average weekly score of at least 1 point on the Likert scale for the primary parameter
14
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
20
21 298 responder rates were 56.7% in the bifidobacteria group and only 21.0% in the
22
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
26
27 301 responder rates were calculated to be 48.3% in the bifidobacteria and only 24.2% in
28
29 302 the placebo group ($p = 0.008$) (Fig. 7).

30
31
32
33
34
35 303

36
37 304 Global efficacy at physician site

38
39 305 The overall assessment of efficacy was significantly better in the bifidobacteria group
40
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
46
47 309 the bifidobacteria and 11.3% of the patients in the placebo group ($p < 0.0001$; Fig. 8).

48
49
50
51
52 310

53
54 311 Health related quality of life

55
56 312 The evaluation of the SF12 sum scores showed a significant gain in quality of life
57
58 313 within the bifidobacteria group. Physical health sum improved from 47.89 at baseline
59
60 314 to 51.80 after treatment in the bifidobacteria group and from 47.33 to only 48.85 in

1
2 315 the placebo group. Physical health sum significantly changed by 3.99 in the
3
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
11
12 320 and by only 1.58 in the placebo group compared to baseline (p=0.0083).

13
14
15
16 321

17
18 322 Adverse events

19
20 323 Only 36 adverse events were reported with suspected relation to the study product,
21
22 324 13 in the placebo and 23 in the treatment group, but no significant differences could
23
24 325 be detected in the side effects profile of *B. bifidum* MIMBb75 vs. placebo.
25
26 326 Additionally, no severe adverse events have been recorded in either group.

27
28
29
30 327

31
32 328

33
34 329

35
36 330

37 331 **4. Discussion and Conclusion**

38
39 332

40
41 333 This randomized, double blind, placebo-controlled study indicates that *B. bifidum*
42
43 334 MIMBb75 has beneficial effects in the treatment of IBS. In this study, *B. bifidum*
44
45 335 MIMBb75 significantly improved global IBS as well as its related symptoms such as
46
47 336 pain/discomfort, distension/bloating and digestive disorders compared to placebo.
48
49 337 Moreover, *B. bifidum* MIMBb75 also significantly improved the quality of life. These
50
51 338 benefits persisted within the consumption-free wash-out phase. Overall responder
52
53 339 rates were predominantly high with 56.7% in the bifidobacteria group compared to
54
55 340 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 ($p < 0.0001$).

5
6
7 343 Up to date, several studies have examined the effects of probiotics on IBS and its
8
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
12
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
16
17 348 randomization errors, several different probiotic strains did repeatedly show no
18
19 349 significant improvement in IBS (20, 21, 22, 23, 24). Recently, Brenner et al. (2009)
20
21 350 published a systematic review of randomized controlled trials (RCTs) aimed at the
22
23 351 evaluation of the efficacy, safety, and tolerability of probiotics in the treatment of IBS.
24
25 352 A total of 16 RCTs were included in the analysis. Of those, exclusively one
26
27 353 bifidobacteria strain showed efficacy for improvement of IBS symptoms in two
28
29 354 appropriately designed studies (4). Apart from inappropriate study design, in our
30
31 355 opinion, these findings could be attributed to the fact that the efficacy of probiotics is
32
33 356 strongly strain specific and that only few strains might be able to show efficacy in
34
35 357 IBS.

36
37 358 No study to date could prove unambiguously the mode of action of probiotics, which
38
39 359 can be clearly linked to the improvement of IBS and its symptoms. Several studies
40
41 360 have observed altered intestinal microbiota with a significant lack of bifidobacteria
42
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
46
47 363 suggests the presence of small intestinal bacterial overgrowth or an increased
48
49 364 number of enteric microorganisms, is highly correlated with a significant reduction of
50
51 365 IBS symptoms (27). It has been stated that the imbalance of the microbiotic
52
53 366 composition may lead to a different fermentation pattern, especially with increased
54
55
56
57
58
59
60

1
2 367 hydrogen production resulting in bloating (28, 29). Additionally, gut mucosal barrier
3
4 368 disruption has been proven to be associated with bacterial translocation and
5
6 369 inflammatory conditions (4,5,6). It seems likely that only specific strains are able to
7
8 370 restore an imbalanced intestinal microbiota and gut barrier, however further research
9
10 371 is needed to investigate if the positive effects of *B. bifidum* MIMBb75 can be
11
12 372 attributed to this mode of action.

13
14
15
16 373 There are several limitations of this study. First of all, the study population was not
17
18 374 large enough for a sub-group analysis of IBS subtypes. We could therefore not show
19
20 375 whether some IBS-subtypes would benefit more from the consumption of *B. bifidum*
21
22 376 MIMBb75 than others. In order to prove whether some subgroups might benefit
23
24 377 more, a larger study might be useful. The study population has mild to moderate IBS
25
26 378 symptoms as evidenced by the indicated SGA-baseline values. A further study
27
28 379 should be aimed at the examination whether the patients with severe IBS symptoms
29
30 380 will profit in the same way as those with mild to moderate symptoms or whether these
31
32 381 patients are more refractory to this treatment.

33
34
35
36
37 382 Additionally, the duration of the study was perhaps short at 4 weeks. It would be of
38
39 383 specific value to explore whether patients benefit more from a longer consumption of
40
41 384 *B. bifidum* MIMBb75. Finally, the observation that patients still benefit during the
42
43 385 consumption-free wash-out phase is of significant value and requires further
44
45 386 investigation (for longer follow-up).

46
47
48
49 387 In conclusion, the beneficial effects of *B. bifidum* MIMBb75 in improving global IBS as
50
51 388 well as its symptoms along with the good side effect profile suggests that this
52
53 389 probiotic strain has the potential as an effective alternative to current treatment
54
55 390 options.

56
57
58
59 391

60
392

1
2 3933
4 394 **5. References**

5 395

6 396 [1] Longstreth GF et al Functional bowel disorders. *Gastroenterology* 2006;
7 397 130:1480-1491.

8 398

9 399 [2] Madden JAJ, Hunter JO. A review of the role of the gut microflora in irritable
10 400 bowel syndrome and the effects of probiotics. *British Journal of Nutrition*, 2002; 88:
11 401 67-72.

12 402

13 403 [3] Silk DBA et al. Clinical trial: the effects of a trans-galactosaccharide prebiotic on
14 404 faecal microbiota and symptoms in irritable bowel syndrome. *Alimentary*
15 405 *Pharmacology & Therapeutics*, 2009; 29: 508-518.

16 406

17 407 [4] Brenner DM et al. The Utility of probiotics in the treatment of irritable bowel
18 408 syndrome: a systematic review. *The American Journal of Gastroenterology*, 2009;
19 409 104: 1033-1049.

20 410

21 411 [5] Pimental M et al. Eradication of small intestinal bacterial overgrowth reduces
22 412 symptoms of irritable bowel syndrome. *The American Journal of Gastroenterology*,
23 413 2000; 95: 3503-3506.

24 414

25 415 [6] Zeng J et al. Clinical trial: effect of active lactic acid bacteria on mucosal barrier
26 416 function in patients with diarrhea-predominant irritable bowel syndrome. *Alimentary*
27 417 *Pharmacology & Therapeutics*, 2008; 28: 994-1002.

28 418

29 419 [7] Marshall JK et al. Intestinal permeability in patients with irritable bowel syndrome
30 420 after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Alimentary*
31 421 *Pharmacology & Therapeutics*, 2004; 20: 1317-1322.

32 422

33 423 [8] Kassinen A et al. The fecal microbiota of irritable bowel syndrome patients differs
34 424 significantly from that of healthy subjects. *Gastroenterology*, 2007; 133: 24-33.

35 425

36 426 [9] Mättö J. Composition and temporal stability of gastrointestinal microbiota in
37 427 irritable bowel syndrome – a longitudinal study in IBS and control subjects.
38 428 *Immunology and Medical Microbiology*, 2005; 43:213-222.

39 429

40 430 [10] Alvares-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: a
41 431 modern perspective on a traditional therapy. *Clinical Infectious Diseases*, 2001; 32:
42 432 1567-1576.

43 433

44 434 [11] Salminen S. Clinical use of probiotics for stabilizing the gut mucosal barrier:
45 435 successful strains and future challenges. *Antonie van Leeuwenhoek*, 1996; 70: 347-
46 436 358.

47 437

48 438 [12] Fanigliulo L et al. Role of gut microflora and probiotic effects in the irritable bowel
49 439 syndrome. *Acta Biomed*, 2006; 77: 85-89.

50 440

51
52
53
54
55
56
57
58
59
60

- 1
2 441 [13] O'Mahony L et al. Lactobacillus and Bifidobacterium in irritable bowel syndrome:
3 442 symptom responses and relationship to cytokine profiles. Gastroenterology, 2005;
4 443 128: 541-551.
5 444
- 6 445 [14] Kajander K et al. A probiotic mixture alleviates symptoms in irritable bowel
7 446 syndrome patients: a controlled 6-month intervention. Alimentary Pharmacology &
8 447 Therapeutics, 2005; 22: 387-394.
9 448
- 10 449 [15] Guglielmetti S et al. Implication of an outer surface lipoprotein in adhesion of
11 450 bifidobacterium bifidum to caco-2 cells. Applied and Environmental Microbiology,
12 451 2008; 74(15): 4695-4702.
13 452
- 14 453 [16] Guglielmetti S et al. Study of the adhesion of bifidobacterium bifidum MIMBb75
15 454 to human intestinal cell lines. Current Microbiology, 2009; 59: 167-172.
16 455
- 17 456 [17] Whorwell PJ. Efficacy of an Encapsulated Probiotic Bifidobacterium infantis
18 457 35624 in Women with Irritable Bowel Syndrome. American Journal of
19 458 Gastroenterology, 2006; 101: 1581-1590.
20 459
- 21 460 [18] Williams EA et al. Clinical trial: a multistrain probiotic preparation significantly
22 461 reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled
23 462 study. Alimentary Pharmacology & Therapeutics, 2008; 29: 97-103.
24 463
- 25 464 [19] Guyonnet D et al. Effect of fermented milk containing Bifidobacterium animalis
26 465 DN-173 010 on the health-related quality of life and symptoms in irritable bowel
27 466 syndrome in adults in primary care: a multicentre, randomized, double-blind,
28 467 controlled trial. Alimentary Pharmacology & Therapeutics, 2007; 26: 475-486.
29 468
- 30 469 [20] Marteau P. Probiotics in functional intestinal disorders and IBS: proof of action
31 470 and dissecting the multiple mechanisms. Gut, 2010; 59: 285-286.
32 471
- 33 472 [21] Bausserman M, Michail S. The use of Lactobacillus GG in irritable bowel
34 473 syndrome in children: a double-blind randomized control trial. Journal of Pediatrics,
35 474 2005; 147(2): 197-201.
36 475
- 37 476 [22] Niv E, Naftali T, Hallak R, Vaisman N. The efficacy of Lactobacillus reuteri ATCC
38 477 55730 in the treatment of patients with irritable bowel syndrome—a double blind,
39 478 placebo-controlled, randomized study. Clinical Nutrition, 2005; 24: 925-931.
40 479
- 41 480 [23] Kim HJ et al. A randomized controlled trial of a probiotic combination VSL#3 and
42 481 placebo in irritable bowel syndrome with bloating. Neurogastroenterology and
43 482 Motility, 2005; 17: 687-696.
44 483
- 45 484 [24] Sen S. Effect of Lactobacillus plantarum 299v on colonic fermentation and
46 485 symptoms of irritable bowel syndrome. Digestive Diseases and Sciences, 2002;
47 486 47(11): 2615-2620.
48 487
- 49 488 [25] Kerckhoffs APM et al. Lower Bifidobacteria counts in both duodenal mucosa-
50 489 associated and fecal microbiota in irritable bowel syndrome patients. World Journal
51 490 of Gastroenterology, 2009; 15(23): 2887-2892.
52 491

1
2 492 [26] Si JM, Yu YC, Fan YC, Chen SJ. Intestinal microecology and quality of life in
3 493 irritable bowel syndrome patients. World Journal of Gastroenterology, 2004; 10(12):
4 494 1802-1805.

5 495
6
7 496 [27] Pimental M et al. Normalization of lactulose breath testing correlates with
8 497 symptom improvement in irritable bowel syndrome: a double-blind, randomized,
9 498 placebo-controlled study. The American Journal of Gastroenterology, 2003; 98(2):
10 499 412-419.

11 500
12 501 [28] Keith LE, Marinos E, Hunter JO. Do interventions which reduce colonic bacterial
13 502 fermentation improve symptoms of irritable bowel syndrome? Digestive Diseases and
14 503 Sciences, 2005; 50(4): 758-766.

15 504
16 505 [29] Quigley EMM. Probiotics in irritable bowel syndrome: an immunomodulatory
17 506 strategy. Journal of the American Journal of Nutrition, 2007; 26(6): 684S-690S.
18 507

19
20
21
22 508 **Statement of Interests**

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

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

27
28
29 511 - **This study was funded by Naturwohl Pharma GmbH, Bauerstraße 22, 880796**

30
31 512 **München, Germany**

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

34
35 514 **Algora Gesellschaft für Medizinstatistik und Vertriebssysteme mbH and**

36
37 515 **received funding from Naturwohl Pharma GmbH**
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

516 **Tables**

517 Table 1: Demographic characteristics of the ITT-population

518

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

521

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

524

525

526

527

528

529

530

531

532

533

534

535

536

537

538

539

540

541

For Peer Review

542 Table 2

543

	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
			555
			556
			557
			558

559

560

561 Table 2

562

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

563

564

565 Table 3

566

	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.

567

568

For Peer Review

1
2 569 **Figures**
3
4

5 570

6
7 571 Fig. 1. Study schematic.
8
9

10 572

11
12 573 Fig. 2. Diagram of study flow.
13
14

15 574

16 575 Fig. 3. Comparison of effects of placebo and *B. bifidum* MIMBb75 on global IBS
17 576 symptoms (by SGA, recorded on a 0-6 scale) on a weekly basis. Significant
18
19 577 improvement of global IBS symptoms in the bifidobacteria group vs. placebo.
20
21
22

23 578

24
25
26 579 Fig 4.. Comparison of the reduction of IBS symptoms (*B. bifidum* MIMBb75 vs.
27
28 580 placebo) on mean score changes from baseline to treatment phase.
29
30

31 581

32
33 582 Fig. 5. Comparison of effects of placebo and *B. bifidum* MIMBb75 on pain/discomfort
34 583 (recorded on a 0-6 Likert scale) on a weekly basis. Significant improvement in the
35
36 584 bifidobacteria group vs. placebo group.
37
38
39

40 585

41
42 586 Fig. 6. Comparison of effects of placebo and *B. bifidum* MIMBb75 on
43 587 distension/bloating (recorded on a 0-6 scale) on a weekly basis. Significant
44
45 588 improvement in the bifidobacteria group vs. placebo group.
46
47
48

49 589

50
51
52 590 Fig. 7. Overall responders during treatment (ITT).
53

54 591

55
56 592 Fig. 8. Adequate relief after treatment (ITT).
57
58
59
60

Fig.1

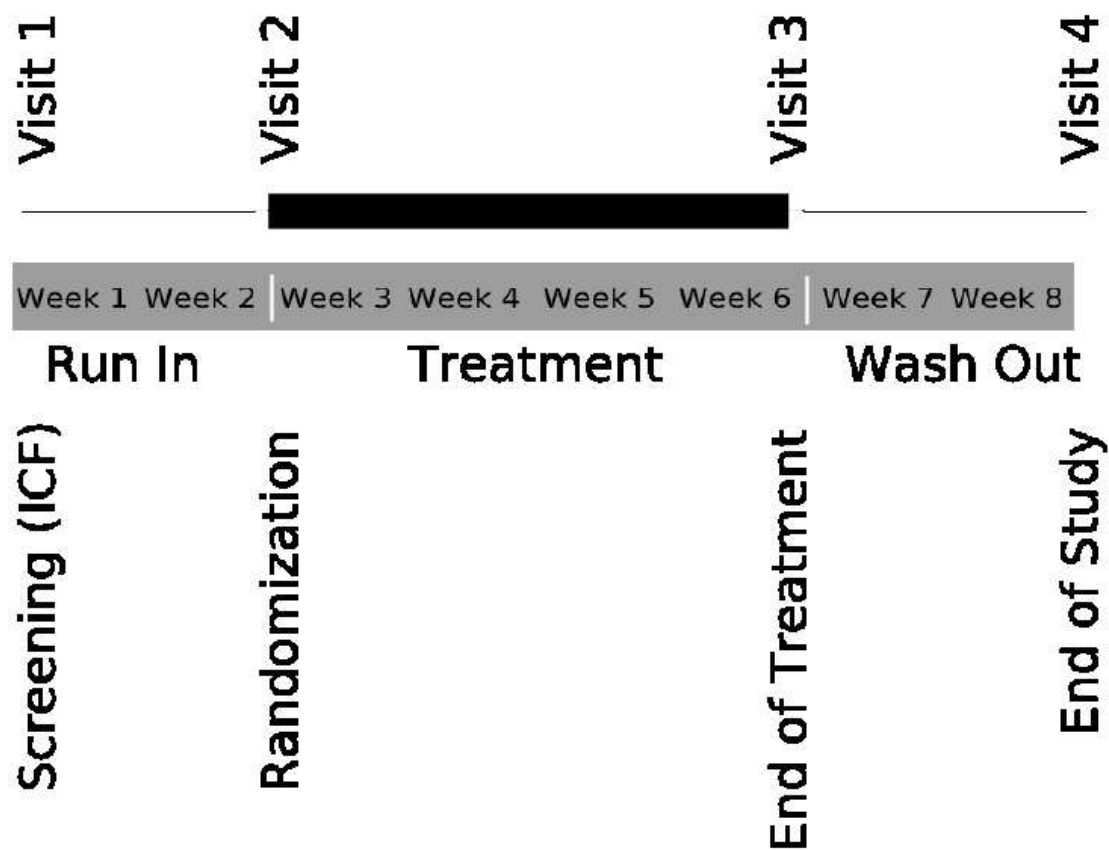


Fig.2.

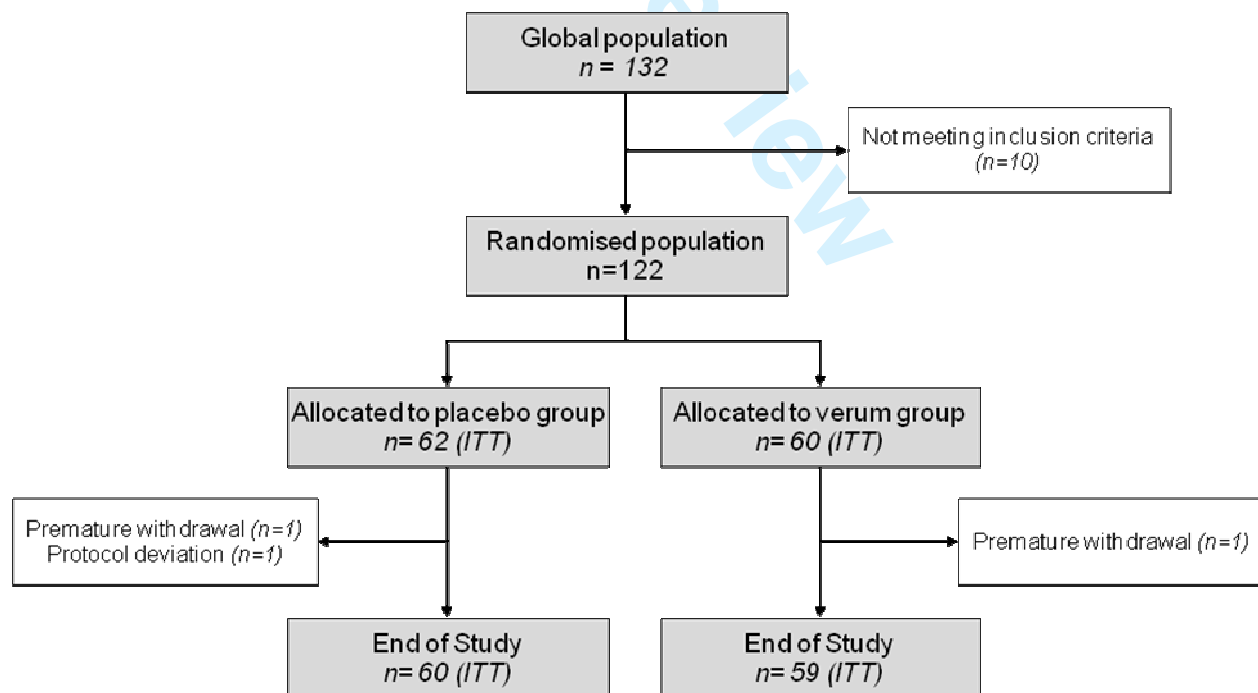


Fig. 3

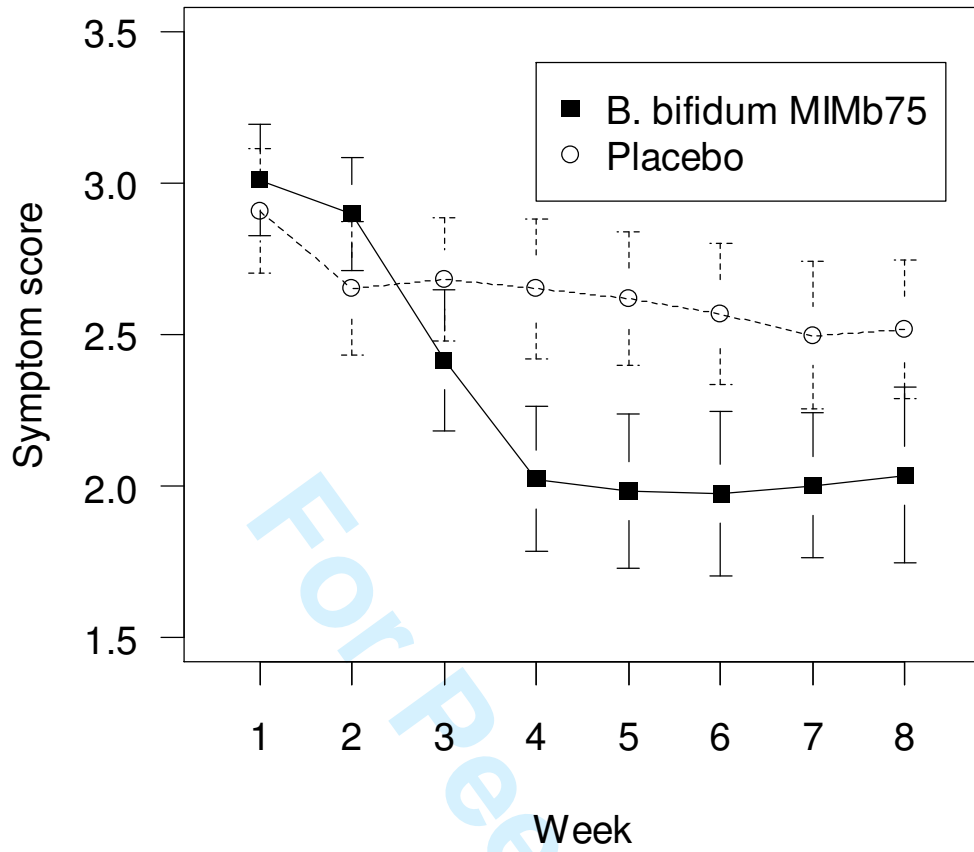


Fig.4

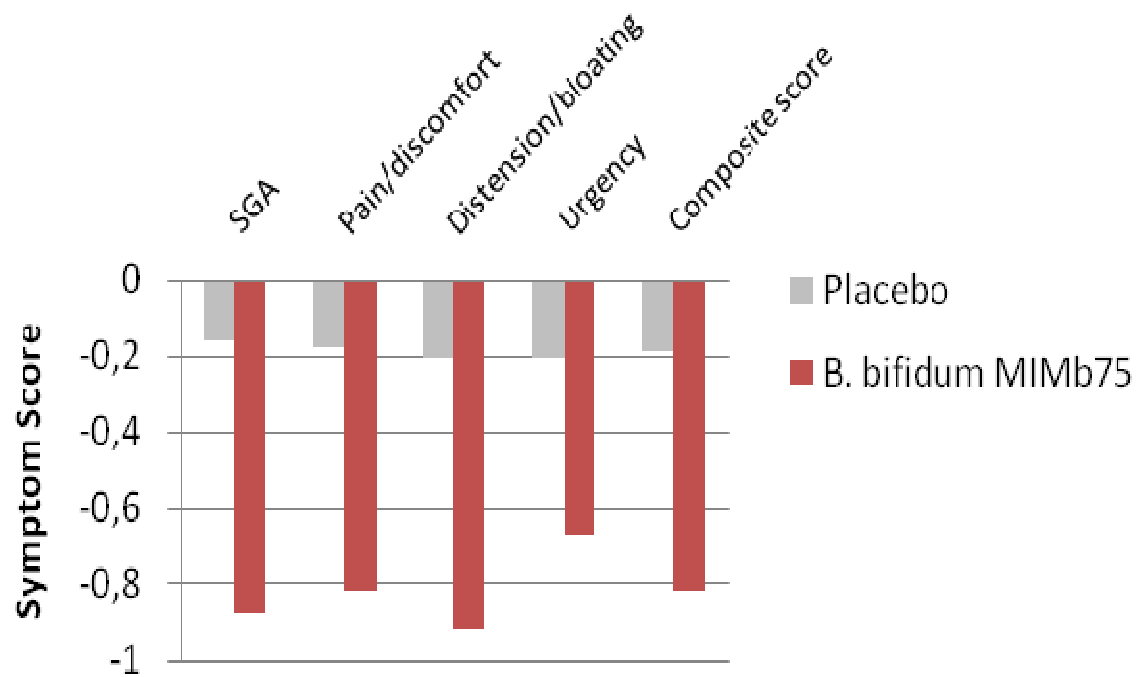


Fig. 5

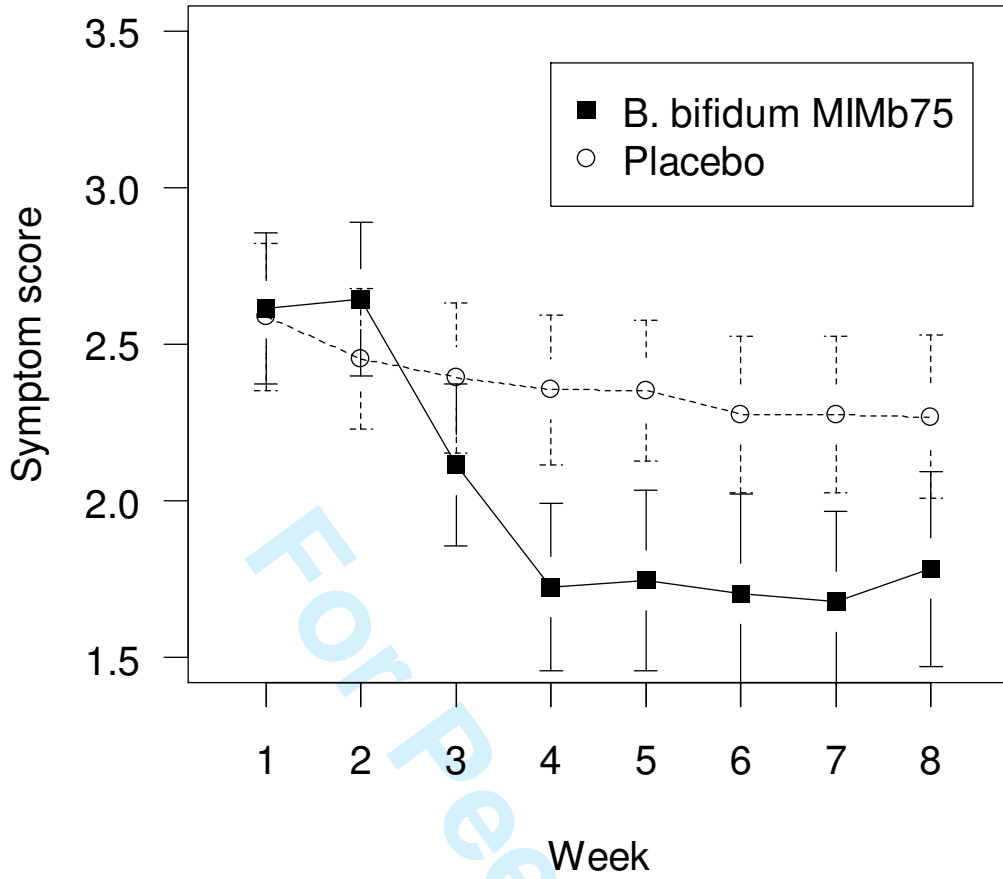


Fig. 6

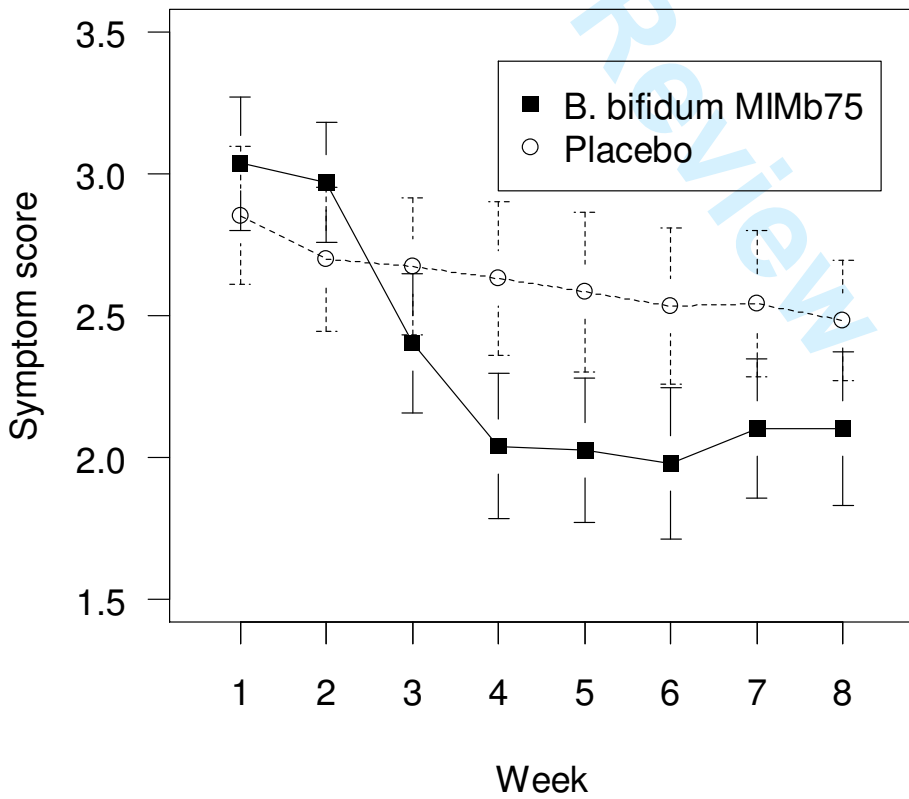


Fig. 7

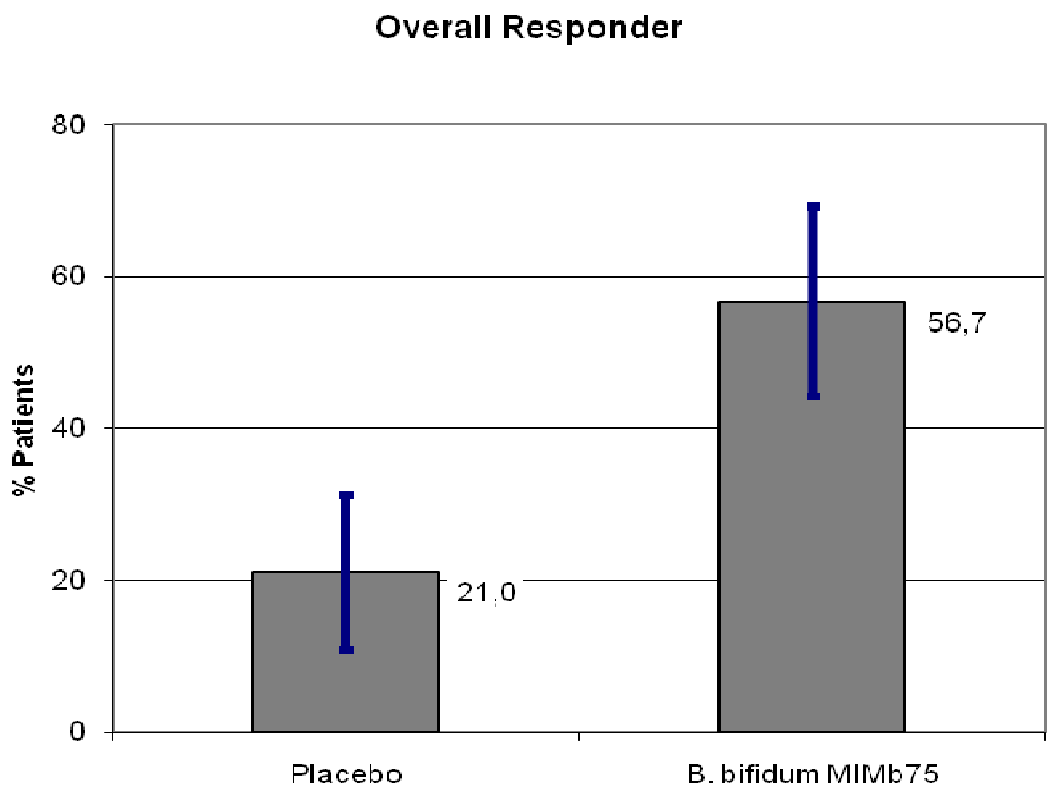
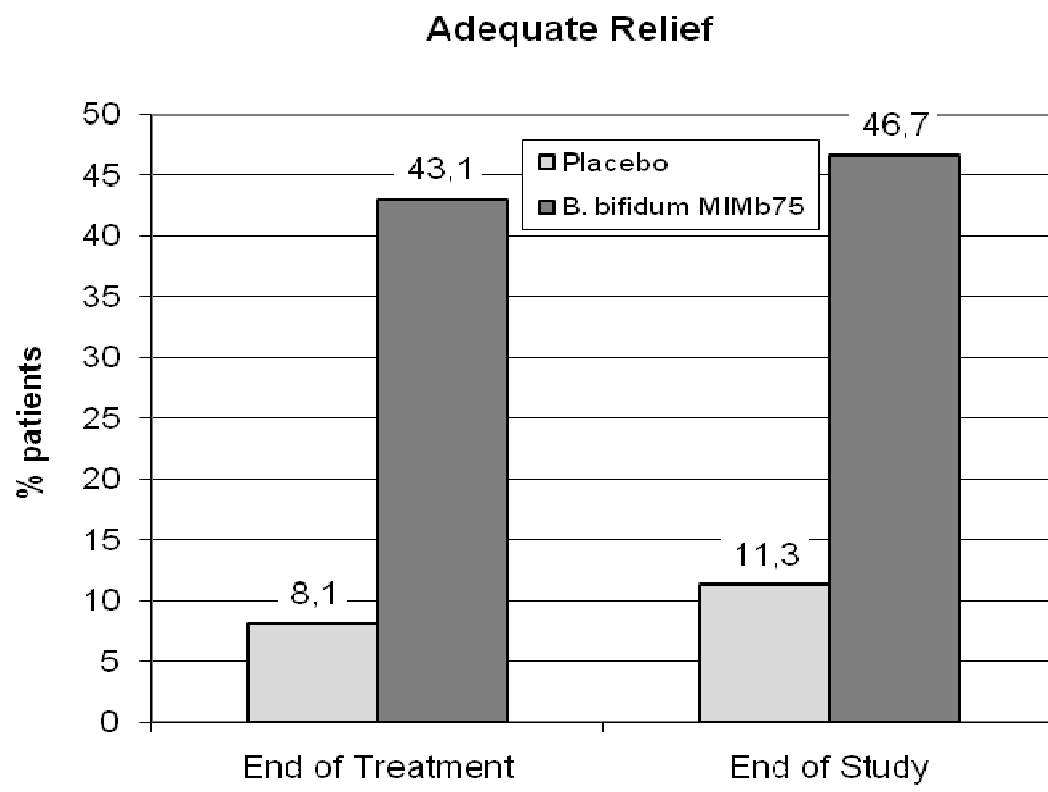
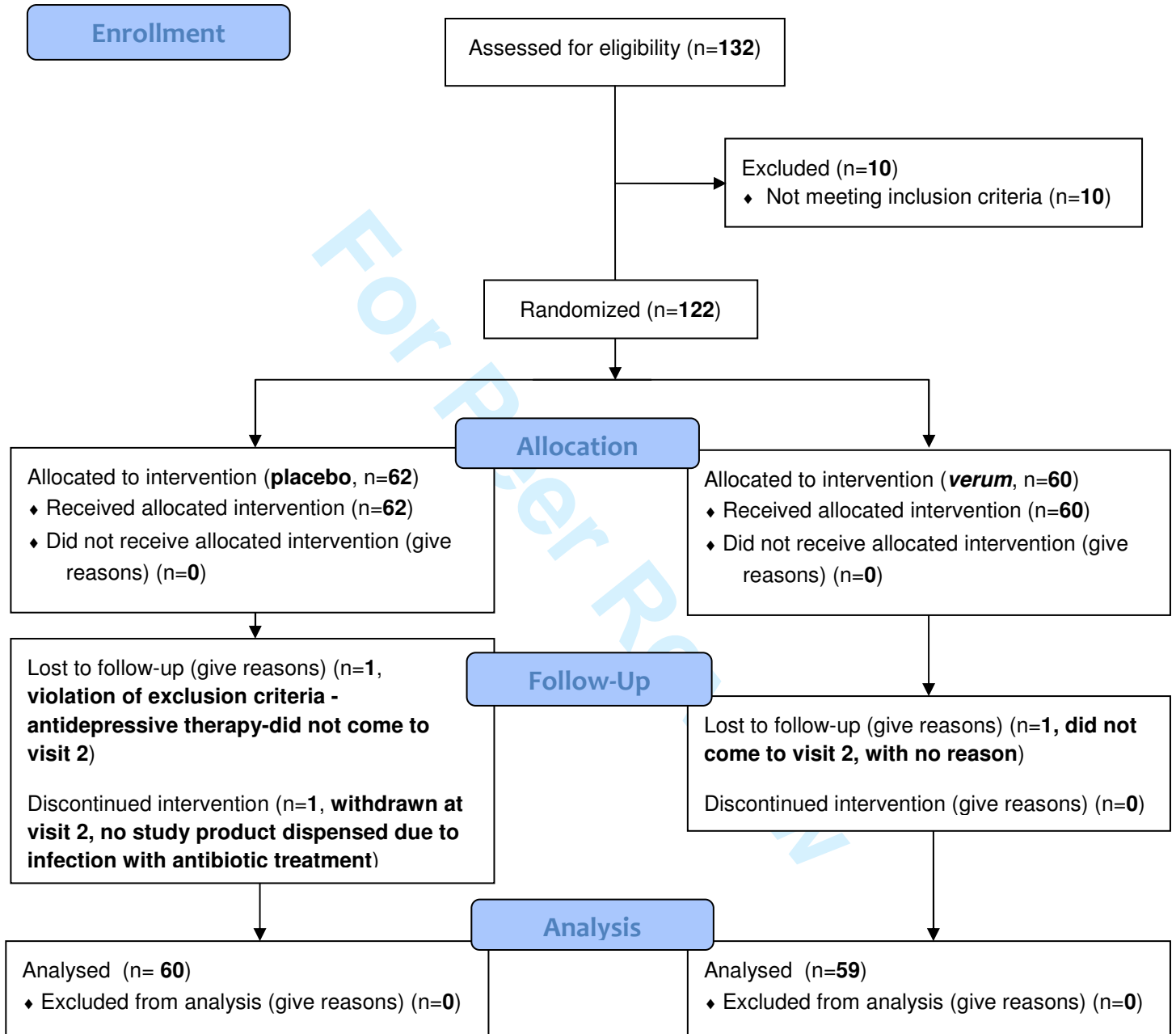


Fig. 8



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

39
40 *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
41 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.
42 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.
43