Supplementary File

Inclusion and Exclusion Criteria

Inclusion criteria comprised a positive diagnosis of all IBS subtypes, at least 18 and no more of 65 years of age, negative colonoscopy or barium enema examination within the previous 5 years, negative relevant additional screening or consultation whenever appropriate. Patients were excluded if they were pregnant, breast-feeding, or not using reliable methods of contraception. Exclusion criteria also included the current use of non-steroidal anti-inflammatory drugs, corticosteroids, and mast cell stabilizers, the use of topic or systemic antibiotics in the last month, treatment with lactulose or with any compound that lowered the colonic pH and could prevent the release of the active moiety, major abdominal surgery, a history of inflammatory bowel disease or diverticular disease, celiac disease (by detection of anti-transglutaminase and anti-endomysial antibodies), allergic diseases, including asthma (excluded by family and personal history and specific anti-IgE antibodies), and other organic or psychiatric disorders as assessed by medical history, appropriate consultations and laboratory tests.

Protocol Violations

The most common protocol violations were the following: 18 patients in the mesalazine group and 19 in the placebo group did not complete the entire treatment period of 12 weeks, and 14 patients in the mesalazine group and 13 in the placebo group had a compliance of <70% or >120%.

Rescue medications

During the study the following rescue therapies were allowed: butylscopolamine (no more than two tablets/day according to need, in case of severe abdominal pain); a single lukewarm water enema (if needed in case of severe constipation); loperamide (no more than one tablet once a day according to need, in case of severe diarrhoea). Weekly number of rescue medications taken was computed and described with summary statistics by treatment group and by week.

Primary and secondary endpoints according to IBS subtypes and effects on bowel functions

According to the prevalence approach, for the primary endpoint (i.e., satisfactory relief of abdominal discomfort or pain), 66.7% of patients with IBS-C were responder patients in the mesalazine group *vs*. 83.3% in the placebo group (P=0.611; difference=16.7%; 95% CI: -20.7 to 54.0), 72.7% of patients with IBS-D were responder patients in the mesalazine group *vs*. 57.6% in the placebo group (P=0.391; difference= -15.5%; 95% CI: -40.3 to 10.0), and 83.3% of patients with IBS-M were responder patients in the mesalazine group *vs*. 50.0% in the placebo group (P=0.333; difference=33.3%; 95% CI: -100.0 to 19.3). The Breslow-Day test showed no homogeneity differences in the odds ratio among IBS subtypes (P=0.193).

According to the prevalence approach, for the key secondary endpoint (i.e., satisfactory relief of overall IBS symptoms), 66.7% of patients with IBS-C were responder patients in the mesalazine group *vs*. 58.3% in the placebo group (P=1.000; difference= -8.3%; 95% CI: -49.9 to 33.2), 63.6% of patients with IBS-D were responder patients in the mesalazine group *vs*. 51.5% in the placebo group (P=0.418; difference= -12.12% ; 95% CI: -38.5 to 14.2), and 83.3% of patients with IBS-M were responder patients in the mesalazine group *vs*. 50.0% in the placebo group (P=0.333; difference= -33.3% ; 95% CI: -100.0 to 19.3). The Breslow-Day test showed no homogeneity differences in the odds ratio among IBS subtypes (P=0.309).

Stool consistency was described by treatment group and by day. Summary descriptive statistics of weekly stool frequency were provided by treatment group and by week. No relevant difference between treatment groups was detected for stool consistency and stool frequency. According to the Bristol Stool Form Scale, in exploratory analyses, patients with IBS-C were considered responders if stool consistency improved from 1-2 to 3-5, while patients with IBS-D were considered responders if stool consistency improved from 6-7 to 3-5. With these definitions, 50.0% of patients with IBS-C were responder patients in the mesalazine group *vs*. 42.9% in the placebo group, while 47.1% of patients with IBS-D were responder patients in the mesalazine group *vs*. 57.1% in the placebo group. Weekly stool frequency did not differ between the screening

period and at the end of treatment either in the mesalazine group (10.23±5.90 *vs*. 10.54±5.06) or in the placebo group (11.22±6.43 *vs*. 10.26±6.77).