INFLAMMATORY BOWEL DISEASE

Modified-Release Phosphatidylcholine (LT-02) for Ulcerative Colitis: Two Double-Blind, Randomized, Placebo-Controlled Trials

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BACKGROUND & AIMS: **The aim of this study was to evaluate the efficacy of LT-02, a novel modified-release phosphatidylcholine (PC) formulation, for induction and maintenance of remission in patients with mild to moderate ulcerative colitis (UC) and inadequate response to mesalamine.**

METHODS: **LT-02 was evaluated in a multicenter double-blind, randomized, placebo-controlled study comprising a 12-week induction trial (PCG-2), followed by a 48-week maintenance trial (PCG-4). In PCG-2, patients were randomized 1:1:1 to treatment with 0.8 g LT-02 4 times daily (QID), 1.6 g LT-02 twice daily (BID), or placebo, respectively. All patients continued to take a standard dose of oral mesalamine (≥2.4 g/day). The primary end point in PCG-2 was deep remission. Patients achieving remission at week 12 were randomly assigned 2:1:1 to 1.6 g LT-02 BID, placebo, or 500 mg mesalamine (3 times daily), respectively, in PCG-4; the primary end point was remission at 48 weeks.**

RESULTS: **PCG-2 was terminated early for futility after a prespecified interim analysis; 466 patients (of 762 planned) were randomized. There was no statistically significant difference in deep remission at week 12 (placebo, 13.5%; LT-02 BID, 14.2%; LT-02 QID, 9.7%). In PCG-4, 150 patients (of approximately 400 planned) were randomized. There was no statistically significant difference in remission rates at week 48 (LT-02 BID, 49.3%; mesalamine, 50.0%; placebo, 43.2%). LT-02 was safe.**

CONCLUSIONS: **Despite prior evidence of beneficial effects of PC in phase 2 trials, our induction study with LT-02 in patients with mild to moderate UC was terminated prematurely for futility. Signals of efficacy in maintenance therapy require confirmation in an adequately powered maintenance trial. LT-02 was safe and well-tolerated. ClinicalTrials.gov: NCT02280629, NCT02142725.**

Keywords: Ulcerative Colitis; Phosphatidylcholine; Mucus; Mesalamine.

Abbreviations used in this paper: **BID, twice daily; CAI, Clinical Activity Index; CRP, C-reactive protein; EI, Endoscopic Index; FAS, full analysis set; HI, Histological Index; IDMC, independent data monitoring committee; mDAI, Modified Disease Activity Index; PC, phosphatidylcholine; QID, 4 times daily; SHS, Short Health Scale; TEAE, treatment-emergent adverse event; UC, ulcerative colitis.**

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Ulcerative colitis (UC) is an idiopathic, chronic relapsing and remitting inflammatory bowel disease of unknown etiology and pathogenesis affecting the colonic and/or rectal mucosa. The primary goal of treatment in UC is to induce and maintain remission and to normalize patients' quality of life.[1,2] The choice of treatment depends on the severity of the disease as well as on its localization and course. Some patients with mildly to moderately active UC do not respond to first-line therapy with mesalamine, lose response to therapy, or in rare cases develop intolerable side effects. In this setting, other treatment options would be desirable before escalating to steroids or advanced therapies. Thus, there is still a need for novel and safe treatments for patients with mild to moderate UC.

Phosphatidylcholine (PC) is the predominant phospholipid species present in the intestinal mucus.[3–6] It is essential for the hydrophobic property of the mucus surface and is thought to be a key player in the mucosal barrier function.[7] The PC concentration in ileal and colonic mucus from patients with UC is lower than in healthy controls.[6,8] This results in a reduced barrier function of the mucus,[9] allowing commensal colonic bacteria to penetrate the membrane, which leads to inflammation of the mucus and eventually ulceration. In addition, PC exerts anti-inflammatory effects in vitro.[10] The low PC content in the intestinal mucus of UC patients together with the cytoprotective and anti-inflammatory properties of PC suggest that exogenous PC may be beneficial in the treatment of UC. Preliminary results from 3 small placebo-controlled clinical studies using a modified-release preparation of soy lecithin containing 30% PC (named LT-01) suggested that exogenous PC may be effective in patients with UC.[9,11–15] A subsequent phase 2 study in 156 patients with mesalamine-refractory UC using 3 different doses of an optimized PC formulation containing >94% PC (named LT-02) showed a significant improvement in disease activity at the highest dose of LT-02 (3.2 g) versus placebo.[16] On the basis of these findings, a series of phase 3 trials was initiated in patients with inadequate response to mesalamine: first in a 12-week induction trial (PCG-2), which evaluated LT-02 as add-on treatment to underlying oral mesalamine, followed by a 48-week maintenance trial (PCG-4) in patients who achieved remission in the PCG-2 trial, which compared LT-02 monotherapy with placebo and mesalamine.

Methods

Study Design and Treatments

PCG-2 was conducted at 79 sites in 14 countries and PCG-4 at 41 sites in 10 countries. Both studies were randomized, double-blind, double-dummy, placebo-controlled trials performed from July 2014 (first patient in, PCG-2) through October 2018 (last patient out, PCG-4).

PCG-2 evaluated LT-02 versus placebo for induction of remission over a treatment period of 12 weeks in patients with inadequate response to mesalamine. Patients were randomized at a 1:1:1 ratio to 0.8 g LT-02 four times daily (QID), 1.6 g LT-02 twice daily (BID) plus placebo BID, or placebo QID. Randomization was stratified by prior stable mesalamine dosage (≥2.4 g/day oral treatment for ≥6 weeks vs "other" (eg, combination treatment (oral [≥2.4 g/day] or therapeutic equivalent [≥2.4 g/day olsalazine, ≥5.6 g/day balsalazide, or ≥6.2 g/day sulfasalazine plus rectal (any approved dose)]) for ≥10–14 days). The PCG-2 trial included a 7- to 10-day screening period, a 12-week treatment period with visits at 2, 4, 6, 8, and 12 weeks, and a 4-week follow-up visit. Non-responders had the option to continue in an open-label phase with 1.6 g LT-02 BID for 12 weeks (Supplementary Figure 1). Patients' baseline regimen of ≥2.4 g/day oral mesalamine (or other therapeutic equivalent) had to be stably continued throughout the entire trial. Treatment adherence was monitored at each visit.

Patients who achieved remission in the PCG-2 trial (double-blind or open-label phase) had the option to continue in the PCG-4 trial, in which case they were required to stop concomitant treatment with mesalamine just before randomization into the PCG-4 trial. Patients were randomized 2:1:1 to 1.6 g LT-02 BID, placebo, or 500 mg mesalamine (3 times daily), respectively, stratified by baseline remission status (deep remission vs remission). The trial consisted of a screening period of up to 10 days, a 48-week treatment period with visits at day 0 (baseline), weeks 4, 12, 24, 36, and 48, and a 4-week follow-up period.

Both trials were approved by all relevant ethics committees and were performed in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable national regulations. All patients provided written informed consent before inclusion. All authors had access to the study data and reviewed and approved the final manuscript.

Patients

The PCG-2 trial enrolled patients fulfilling the following key inclusion criteria: aged 18–70 years with a histologically and endoscopically confirmed diagnosis of active UC, ie, a score of at least 1 in each subscore of the modified Disease Activity Index (mDAI), and disease extending ≥15 cm from the anus. Patients were required to exhibit inadequate response to mesalamine, defined as a total mDAI score of ≥4 and ≤10 with a ≤2-point decrease in the sum of the mDAI subscores for stool frequency, rectal bleeding, and physician's global assessment at baseline compared with screening (details of

pretreatment with mesalamine [Supplementary Methods]). Other criteria were stool calprotectin ≥250 μg/g at screening, or ≥100 μg/g and <250 μg/g if the Riley Histological Index (HI)[17] was >1 (at least mild activity). Patients who achieved either deep remission or remission had the option to continue in the maintenance trial PCG-4.

Major exclusion criteria for both trials included other inflammatory or infectious bowel diseases, prior colon surgery, prior or concomitant treatment with steroids within 4 weeks, or immunosuppressive or biologic agents within 8 weeks before randomization. Before transitioning into maintenance trial PCG-4, patients had to discontinue the mesalamine regimen taken during the induction trial.

What You Need to Know

Background

New drugs are needed for patients with mild to moderate ulcerative colitis (UC) who do not respond to mesalamine or who develop intolerable side effects. Phosphatidylcholine (PC) is an essential constituent of the intestinal mucus previously shown to be beneficial in phase 2 studies.

Findings

Oral LT-02 (gastro-resistant granules containing PC) plus mesalamine was not superior to placebo in inducing or maintaining remission. LT-02 was safe and well-tolerated.

Implications for patient care

This study did not demonstrate a benefit of oral PC in patients with mild to moderate UC and inadequate response to first-line treatment with mesalamine and could not confirm the promising results of previous phase 2 studies.

End Points

The primary end point in the PCG-2 trial was deep remission, defined as an mDAI score ≤1 with a score of 0 points for rectal bleeding and stool frequency and ≥1-point decrease in the mucosal appearance score from baseline to week 12. Key secondary end points were remission defined as an mDAI score ≤2 with no subscore >1, clinical improvement defined as ≥3-point decrease in mDAI score from baseline, mucosal healing defined as mDAI mucosal appearance score of ≤1 plus ≥1-point decrease in mDAI from baseline, and changes in total mDAI score, total Clinical Activity Index (CAI) score,[18] Endoscopic Index (EI),[18] HI, fecal calprotectin, and C-reactive protein (CRP) from screening or baseline.

In the maintenance trial (PCG-4), the primary end point was maintenance of remission during the double-blind 48-week treatment period, meaning not experiencing any clinical relapse and not being a treatment failure. Clinical relapse was defined as an mDAI rectal bleeding score of ≥1 plus mucosal appearance score of ≥2. Treatment failure was defined as premature withdrawal for any reason. Key secondary end points were endoscopic remission (defined as EI ≤3), time to clinical relapse or discontinuation (baseline visit until relapse or premature withdrawal, whichever occurred first), change in total mDAI score from baseline, individual mDAI subscores (stool frequency, rectal bleeding, and mucosal appearance), CAI score, HI, and fecal calprotectin. Quality of life was assessed by using the Short Health Scale (SHS) questionnaire, including the 4 health dimensions of symptom burden, social function, disease-related worry, and general well-being, which were assessed on a visual analogue scale (range of 0–100 mm for each dimension, with lower scores reflecting better quality of life).[19]

Procedures

The complete mDAI[20] was assessed at baseline (PCG-2 trial) and at the end of the double-blind treatment periods (PCG-2 and PCG-4), whereas the partial mDAI without the mucosal appearance score was assessed at each visit. Efficacy was further assessed by using the CAI,[18] the EI,[18] and Riley HI.[17] A full endoscopy with biopsy collection was performed at screening in the PCG-2 trial and at the end of the double-blind treatment period (PCG-2 and PCG-4). Endoscopies were evaluated locally, whereas biopsies were read centrally and blinded to treatment. Concomitant medications, vital signs, routine laboratory parameters, and adverse events were documented at each visit.

Patients recorded clinical outcomes and medication use daily in a diary. Data from the 7 days preceding a visit were used to calculate mean scores for the mDAI and CAI.

Statistical Analysis and Sample Size

All randomized patients were included in safety and efficacy analyses (full analysis set [FAS]). The primary efficacy variable in the PCG-2 trial was evaluated by pairwise comparisons of the active treatment arms against placebo within a closed testing procedure using a Simes intersection test at a one-sided α of 0.025. In the PCG-4 trial, confirmatory testing was only performed for the comparison between LT-02 and placebo by applying the same methodology as for the primary efficacy variable in the PCG-2 trial, using the deep remission criterion as a stratification factor in the test statistics. Supplementary Methods describes the statistical procedures used for secondary end points.

Calculation of the sample size is described in the Supplementary Methods. Both trials included a prespecified interim analysis at which a sponsor-independent data monitoring committee (IDMC) reviewed and evaluated unblinded interim results.

Results

Patients

The PCG-2 trial was terminated early because of futility per recommendation of the IDMC at the prespecified interim analysis. Up to the time of termination, 647 patients had been screened, and 465 patients were randomized and treated (Supplementary Figure 2). Baseline characteristics were generally comparable across all treatment groups (Table 1). Overall, 330 patients (71.0%) completed the trial: 109 (70.3%) in the placebo group, 109 (70.3%) in the LT-02 BID group, and 112 patients (72.3%) in the LT-02 QID. The predominant reason for early discontinuation in all treatment groups was lack of efficacy for all prespecified end points. Baseline characteristics were generally comparable across all treatment groups (Table 1).

After termination of PCG-2, the IDMC recommended at an ad hoc interim analysis that only patients already enrolled in PCG-4 continue as per protocol. Overall, 151 patients entered the PCG-4 trial. Of these, 150 patients were randomized and treated (Supplementary Figure 2). A total of 76 patients completed the trial: 16 patients (43.2%) in the placebo group, 40 patients (53.3%) in the LT-02 group, and 20 patients (52.6%) in the mesalamine group. The most common reason for early discontinuation in all treatment groups was lack of efficacy. Baseline characteristics were similar across all treatment groups with few exceptions (Table 2). Fecal calprotectin concentrations were slightly lower in the LT-02 group than the other 2 groups. Eighty-nine patients (59.3%) were in deep remission at the start of the PCG-4 trial.

Efficacy

Induction trial (PCG-2). The proportion of patients achieving deep remission at week 12 was similar in the placebo group (13.5%) and LT-02 BID group (14.2%) but lower in the LT-02 QID group (9.7%; Figure 1). Subgroup analyses for the primary end point demonstrated a high variability across subgroups (Supplementary Figure 3 and Supplementary Table 1). Although there were no relevant differences between active treatment groups and placebo in any subgroup, patients receiving the BID regimen tended toward better outcomes than patients receiving the QID regimen.

Secondary end points displayed similar results. The proportion of patients achieving clinical remission was similar in all 3 treatment groups (placebo, 23.2%; LT-02 BID, 24.5%; LT-02 QID, 23.2%; Figure 1). The clinical improvement rate was numerically slightly higher in the LT-02 BID group than the placebo group (47.4% vs 43.2%; Figure 1). Mucosal healing rates were similar in the placebo and LT-02 BID groups (36.1% and 36.8%) and numerically lower in the LT-02 QID group (32.9%; Figure 1). There were also no relevant differences between the LT-02 groups and the placebo group in total mDAI, total CAI, EI, HI, fecal calprotectin, and CRP (Table 3).

Maintenance trial (PCG-4). *Interim analysis.* At the interim analysis, 23.1% of patients treated with placebo, 38.7% with LT-02 BID, and 30.8% with mesalamine remained in remission through week 48. The difference (95% confidence interval) between placebo and LT-02 BID treatment was 21.0% (–6.6 to 48.6), with the overall inverse normal test statistics providing a value of 1.260. Although this value was below the critical value of 3.188, the IDMC recommended continuing the trial.

Final analysis. Among patients who achieved deep remission or remission in the PCG-2 trial and continued into the PCG-4 trial, the percentage maintaining remission through week 48 (primary end point) was numerically slightly higher in the LT-02 BID group (49.3%) than in the placebo group (43.2%) and similar to the percentage observed in the mesalamine group (50.0%). Statistical significance was not achieved (Figure 2*A*). Similar results were also observed in prespecified subgroup analyses as presented in Supplementary Table 2.

The results of the secondary end points also pointed to numerically slightly greater efficacy of LT-02 than placebo. Total mDAI and CAI increased in all treatment groups, but least in the LT-02 BID group (Table 4). Fecal calprotectin levels decreased with LT-02 BID treatment, whereas they increased with placebo and mesalamine treatment (Table 4). In addition, LT-02 BID–treated patients experienced clinical relapse considerably later than placebo-treated patients (338 days vs 167 days, mesalamine: 343 days; Table 4). Quality of life in the LT-02 BID group improved versus placebo to a similar extent as mesalamine, as indicated by greater decreases in all 4 dimensions of the SHS questionnaire compared with placebo (Figure 2*C*). However, endoscopic remission was achieved by more patients treated with placebo than LT-02 (59.5% vs 49.3%, mesalamine: 57.9%; Figure 2*B*).

Safety. A total of 245 patients (52.7%) in the PCG-2 and 96 patients (64%) in the PCG-4 trial experienced treatment-emergent adverse events (TEAEs). No notable differences in frequencies or types of adverse events were observed across treatment groups in both trials (Supplementary Table 3). All serious TEAEs were unrelated to treatment except for 1 event of vomiting experienced by 1 patient in the LT-02 BID group in the PCG-2 trial. This event was treated with antiemetic therapy and resolved on continued LT-02 BID treatment. No deaths

Table 1. Baseline Characteristics, PCG-2 (FAS, n = 465)

Parameter	Placebo (n = 155)	LT-02 1.6 g BID (n = 155)	LT-02 0.8 g QID (n = 155)
Male, n (%)	85 (54.8)	89 (57.4)	91 (58.7)
Age (y), mean (SD)	40.6 (12.0)	39.6 (13.8)	39.4 (12.7)
BMI (kg/m^2), mean (SD)	24.5 (4.1)	23.8 (4.1)	24.5 (4.9)
White, n (%)	153 (98.7)	154 (99.4)	155 (100.0)
Smoking habits, n (%)			
Current	8 (5.2)	7 (4.5)	8 (5.2)
Former	38 (24.5)	25 (16.1)	33 (21.3)
Non-smoker	109 (70.3)	123 (79.4)	114 (73.5)
Time since diagnosis (y), median (IQR)	5.5 (2.2–11.5)	5.4 (1.8–10.8)	5.1 (1.8–11.6)
Time since first symptoms of disease (y), mean (SD)	8.6 (7.17)	8.3 (7.94)	8.9 (7.53)
Course of disease, n (%)			
Newly established	1 (0.6%)	0	1 (0.6%)
Continuous	52 (33.5%)	55 (35.5%)	50 (32.3%)
Recurrent	102 (65.8%)	100 (64.5%)	104 (67.1%)
No. of previous acute episodes, mean (SD)[a]	6.6 (7.91)	5.1 (4.86)	5.6 (6.55)
mDAI at baseline[b]			
Mean (SD)	7.4 (1.90)	7.1 (2.00)	7.3 (1.81)
≤6, n (%)	43 (27.7%)	54 (34.8%)	44 (28.4%)
>6, n (%)	112 (72.3%)	100 (64.5%)	109 (70.3%)
CAI at baseline, mean (SD)[c]	7.5 (2.44)	7.3 (2.68)	7.4 (2.68)
HI at baseline, mean (SD)	2.6 (0.71)	2.6 (0.71)	2.5 (0.72)
EI at baseline, mean (SD)	7.7 (2.03)	7.5 (1.90)	7.5 (2.04)
Fecal calprotectin			
Median (IQR), (mg/L)	826 (307–1800)	712 (320–1560)	898 (334–1650)
≥5% of ULN, n (%)	121 (78.1%)	123 (79.4%)	127 (81.9%)
CRP (mg/L), median (IQR)	4.5 (1.4–11.0)	3.3 (0.9–10.3)	3.7 (1.2–9.7)
Dose of concomitant mesalamine[d]			
<3.0 g/day	24 (15.5%)	25 (16.1%)	28 (18.1%)
3.0 g/day	74 (47.7%)	75 (48.4%)	67 (43.2%)
>3.0 g/day	57 (36.8%)	54 (34.8%)	57 (36.8%)
Pretreatment with mesalamine (stratum)[e]			
≥2.4 g/day for ≥6 weeks	130 (83.9%)	124 (80.0%)	125 (80.6%)
Other pretreatment	25 (16.1%)	30 (19.4%)	30 (19.4%)
Disease localization, n (%)			
Left-sided[f]	84 (54.2)	84 (54.2)	90 (58.1)
Extended[f]	71 (45.8)	71 (45.8)	65 (41.9)

BMI, body mass index; IQR, interquartile range; SD, standard deviation; ULN, upper limit of normal.
[a]n = 99 (placebo), n = 102 (LT-02 QID), n = 96 (LT-02 BID).
[b]n = 153 (LT-02 QID), n = 154 (LT-02 BID).
[c]n = 152 (placebo), n = 149 (LT-02 QID), n = 151 (LT-02 BID).
[d]n = 152 (LT-02 QID), n = 154 (LT-02 BID).
[e]n = 154 (LT-02 BID).
[f]Left-sided includes proctosigmoiditis and left-sided colitis; extended includes subtotal colitis and pancolitis.

occurred. Colitis ulcerative (ie, deterioration of ulcerative colitis) was the most common TEAE in both trials. The second-most common TEAEs were headache and nasopharyngitis in the PCG-2 trial and nasopharyngitis in the PCG-4 trial (Supplementary Table 3).

There was no evidence of clinically important changes in laboratory values, blood pressure, or heart rate with a suspected relationship to LT-02 treatment. The majority of patients assessed the tolerability as good or very good.

Table 2. Baseline Characteristics, PCG-4 (FAS, n = 150)

Parameter	Placebo (n = 37)	LT-02 BID (n = 75)	Mesalamine (n = 38)
Male, n (%)	24 (64.9)	43 (57.3)	20 (52.6)
Age (y), mean (SD)	42.0 (13.0)	39.5 (11.3)	40.7 (11.7)
BMI (kg/m^2), mean (SD)	23.6 (3.5)	24.6 (4.0)	23.2 (3.7)
White, n (%)	37 (100.0)	74 (98.7)	38 (100.0)
Smoking habits, n (%)			
Current	0	3 (4.0%)	3 (7.9%)
Former	10 (27.0%)	13 (17.3%)	6 (15.8%)
Non-smoker	27 (73.0%)	59 (78.7%)	29 (76.3%)
mDAI at baseline, mean (SD)	1.1 (1.03)	1.2 (0.88)	1.1 (0.88)
Remission status, n (%)			
Deep remission	24 (64.9%)	43 (57.3%)	22 (57.9%)
No deep remission	13 (35.1%)	32 (42.7%)	16 (42.1%)
CAI at baseline			
Mean (SD)[a]	0.9 (1.59)	1.0 (1.65)	0.8 (1.29)
≤6, n (%)[b]	9 (24.3%)	26 (34.7%)	16 (42.1%)
>6, n (%)[b]	28 (75.7%)	48 (64.0%)	22 (57.9%)
HI at baseline, mean (SD)[b]	1.4 (0.72)	1.4 (0.61)	1.4 (0.60)
EI at baseline, mean (SD)[a]	1.4 (1.83)	1.5 (1.93)	1.3 (1.73)
Fecal calprotectin[c]			
Median (IQR), (mg/L)	452 (207–944)	765.5 (279–1565)	639 (327–1355)
≥5× ULN, n (%)	19 (67.9%)	37 (77.1%)	19 (79.2%)
CRP (mg/L), median (IQR)[c]	3.8 (1.6, 8.3)	2.8 (0.8–6.2)	1.9 (0.8–8.7)
Dose of concomitant mesalamine, n (%)[c]			
<3.0 g/day	4 (10.8%)	17 (22.7%)	6 (15.8%)
3.0 g/day	23 (62.2%)	29 (38.7%)	15 (39.5%)
>3.0 g/day	10 (27.0%)	29 (38.7%)	17 (44.7%)
Pretreatment with mesalamine[c]			
≥2.4 g/day for ≥6 wk	34 (91.9%)	64 (85.3%)	30 (78.9%)
Other pretreatment	3 (8.1%)	11 (14.7%)	8 (21.1%)
Time since first symptoms of disease (y), mean (SD)[c]	9.1 (9.34)	9.9 (7.50)	7.6 (6.29)
Disease localization, n (%)[c]			
Left-sided[d]	20 (54.1%)	43 (57.3%)	25 (65.8%)
Extended[d]	17 (45.9%)	32 (42.7%)	13 (34.2%)

IQR, interquartile range; SD, standard deviation; ULN, upper limit of normal.
[a]n = 73 (LT-02 BID).
[b]n = 74 (LT-02 BID).
[c]At baseline of PCG-2.
[d]Left-sided includes proctosigmoiditis and left-sided colitis; extended includes subtotal colitis and pancolitis.

Discussion

This phase 3 clinical trial program for modified-release PC aimed to demonstrate the efficacy of LT-02 for induction and maintenance of remission in mild to moderate UC patients with inadequate response to mesalamine. During induction therapy, both LT-02 dose regimens failed to demonstrate benefit over placebo in the interim analysis and in the final analysis, justifying early termination of the trial. Because of this early termination, only 150 out of a planned 400 patients were enrolled in PCG-4. To exclude the possibility of important but subtle effects in the unexpectedly smaller data set obtained, analysis of the trial was extended longer than usual before publication.

The trial used a stringent primary end point (ie, deep remission) to reduce the placebo response because all patients received concomitant mesalamine. Across all treatment groups, the observed response rates for the primary end point were lower than anticipated in the initial protocol. The proportions of patients in the FAS achieving deep remission were 9.7% and 14.2% in the LT-02 groups (0.8 g QID/1.6 g BID, respectively) vs 13.5% in the placebo group, comparable with a study on

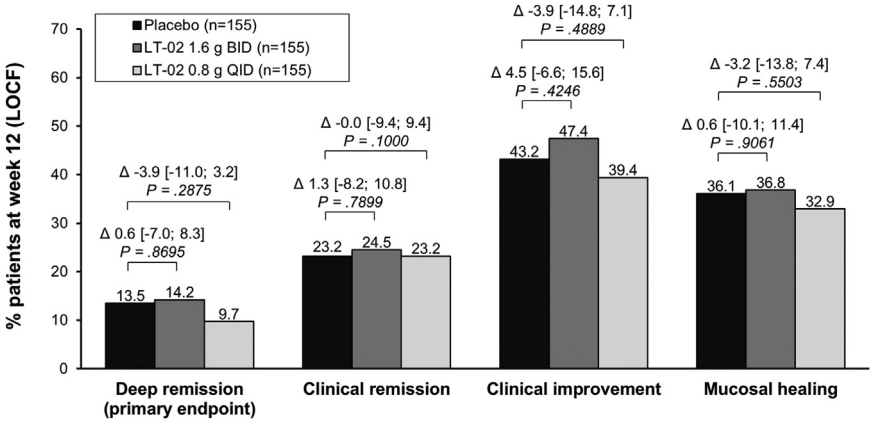

Figure 1. PCG-2: proportion of patients with deep remission (primary end point), clinical remission, clinical improvement, and mucosal healing. LOCF, last observation carried forward.

budesonide MMX 9 mg in a similar patient population that reported a 13% remission rate for the investigational drug versus 7.5% for placebo.[21]

The PCG-2 trial could not confirm the previous results from a phase 2 trial that reported superiority of LT-02 (0.8 g QID) over placebo. Although the patient populations were similar, the efficacy parameters were based on the Simple Clinical Colitis Activity Index (SCCAI).[15] In contrast, our current phase 3 trials with LT-02 used the mDAI, a broadly accepted, valid outcome measure that also includes an objective endoscopic mucosal assessment, which might explain the different outcome in these studies. The PCG-2 results also differ from findings using a previous PC formulation (LT-01)

Table 3. PCG-2 Key Secondary End Points at Week 12 (LOCF) (FAS; n = 465)

Parameter	Placebo (N = 155)	LT-02 1.6 g BID (N = 155)	LT-02 0.8 g QID (N = 155)
Total mDAI			
n	128	134	126
Change from baseline	–2.8 (3.04)	–2.8 (2.91)	–2.6 (3.08)
Difference vs placebo		–0.09 (–0.78, 0.60)	0.13 (–0.57, 0.83)
P value for difference		.791	.708
Total CAI			
N	152	149	147
Change from baseline	–2.53 (0.28)	–3.32 (0.28)	–3.01 (0.28)
Difference vs placebo		–0.79 (–1.56, – 0.02)	–0.49 (–1.26, 0.29)
P value for difference		.045	.215
EI			
N	128	134	127
Change from baseline	–2.48 (0.29)	–2.15 (0.29)	–2.03 (0.29)
Difference vs placebo		0.33 (–0.47, 1.13)	0.45 (–0.36, 1.27)
P value for difference		.421	.275
HI			
N	126	133	125
Change from baseline	–0.43 (0.08)	–0.62 (0.08)	–0.44 (0.08)
Difference vs placebo		–0.19 (–0.41, 0.03)	–0.01 (–0.23, 0.21)
P value for difference		.083	.953
Fecal calprotectin (μg/g)			
N	148	152	148
Change from baseline	–438 (113)	–622 (111)	–479 (113)
Difference vs placebo		–184 (–495, 127)	–41 (–354, 271)
P value for difference		.245	.795
CRP (mg/mL)			
N	153	154	153
Change from baseline	–1.09 (1.15)	–1.41 (1.14)	–0.57 (1.15)
Difference vs placebo		–0.32 (–3.50, 2.87)	0.52 (–2.67, 3.71)
P value for difference		.845	.748

NOTE. Least squares mean and standard error are reported for changes from baseline; least squares mean and 95% confidence interval are reported for differences in change from baseline.
LOCF, last observation carried forward; SD, standard deviation.

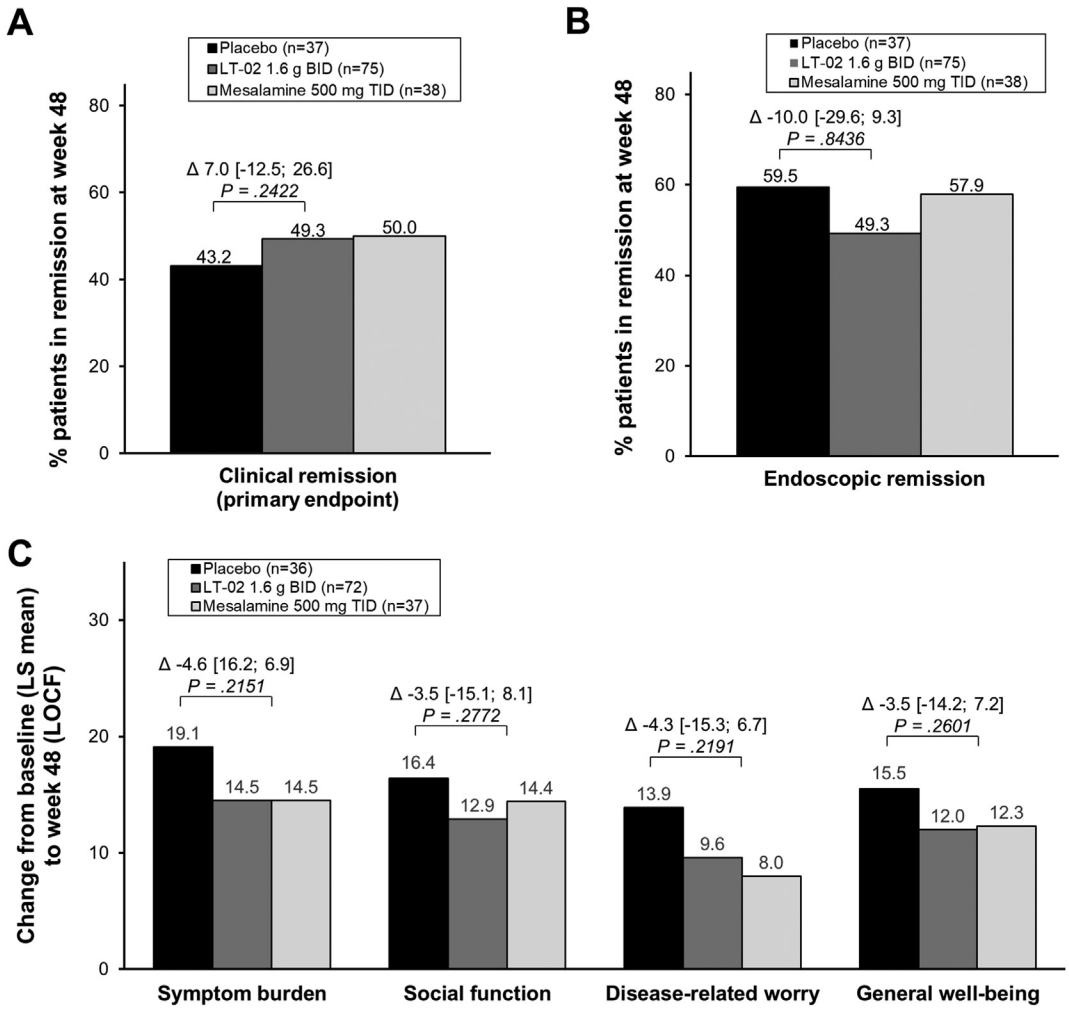

Figure 2. PCG-4: proportion of patients maintaining clinical remission (primary end point) (*A*) and endoscopic remission (*B*) through week 48 (95% CI in brackets) (*C*). Change in quality of life assessed using the Short Health Scale questionnaire. LS, least squares; TID, 3 times daily.

that showed higher response rates with PC versus placebo.[8,11,12] These trials had smaller sample sizes, were monocentric, and allowed other UC pre- and comedications (such as systemic steroids, azathioprine, or 6-mercaptopurine).

Furthermore, preliminary evidence has recently emerged that the amphiphilic properties of mesalamine may interfere with the mechanism of the phospholipid PC when administered concurrently. Biochemical and cell culture studies have revealed that mesalamine can inhibit binding of PC to mucin 2 and to the apical membrane of polarized human colonic cell lines in a dose-dependent manner.[22] Moreover, a post hoc analysis of the LT-02 phase 2B trial[15] revealed a dose-dependent effect of LT-02 alone, but no effects of LT-02 versus placebo in patients taking concomitant mesalamine.[22] However, this hypothesis must be considered speculative because of very small sample sizes and the limited applicability of in vitro studies to the in vivo human colon.

Because considerably fewer patients were enrolled in the maintenance trial PCG-4 than planned, this trial was no longer powered to demonstrate significant differences between LT-02 and placebo. Nonetheless, several numerical trends were observed that merit discussion. Outcomes were slightly improved for the primary and many secondary end points with LT-02 versus placebo, including total mDAI, CAI, and calprotectin levels. LT-02 may have a stronger impact on symptoms than endoscopy, because health-related quality of life data also revealed improvements in all 4 categories of the SHS questionnaire for LT-02 and mesalamine versus placebo. Nonetheless, endoscopic improvement and healing are crucial for long-term outcomes in ulcerative colitis, as reflected in the recent STRIDE-II consensus.[2] Hence, a mere tendency toward improvement in clinical outcomes may not be sufficient to recommend the use of LT-02 in UC. The beneficial effects of LT-02 over placebo in PCG-4 were observed to varying degrees. Noteworthy was the average time to clinical relapse, which was markedly longer with LT-02 than placebo (338 days vs 167 days). Response rates for mesalamine were generally similar to those observed with LT-02.

Response rates to LT-02 in the maintenance trial were slightly higher in patients with a shorter disease

Table 4. PCG-4 Key Secondary End Points at Week 48 (LOCF) (FAS; N = 150)

Parameter	Placebo (n = 37)	LT-02 1.6 g BID (n = 75)	Mesalamine (n = 38)
Total mDAI			
n	30	54	33
Change from baseline	2.45 (0.57)	2.17 (0.43)	2.64 (0.54)
Difference vs placebo		–0.28 (–1.69, 1.13)	
P value for difference		.346	
mDAI subscore: stool frequency			
n	36	73	38
Change from baseline[a]	0.7 (1.1)	0.7 (0.99)	0.7 (0.93)
mDAI subscore: rectal bleeding			
N	36	73	38
Change from baseline[a]	0.6 (0.80)	0.4 (0.76)	0.6 (0.83)
mDAI subscore: mucosal appearance			
N	30	55	33
Change from baseline[a]	0.5 (0.94)	0.5 (0.92)	0.8 (1.02)
Time to clinical relapse or discontinuation (days)			
N	37	75	38
Median (95% CI)	167.0 [52.0, -]	338.0 [172.0, -]	343.0 [101.0, -]
HR (95% CI) vs placebo		1.37 [0.80, 2.34]	
P value for HR		.122	
Total CAI			
N	36	71	38
Change from baseline	2.64 (0.59)	2.18 (0.42)	2.98 (0.57)
Difference vs placebo		–0.47 (–1.89, 0.95)	
P value for difference		.258	
HI			
N	30	53	34
Change from baseline	0.45 (0.15)	0.46 (0.11)	0.67 (0.14)
Difference vs placebo		0.01 (0.36, 0.38)	
P value for difference		.523	
Fecal calprotectin (μg/g)			
N	33	66	34
Change from baseline	244 (178)	–79 (126)	292 (175)
Difference vs placebo		–323 (–754, 107)	
P value for difference		.070	

NOTE. If not indicated otherwise, least squares mean and standard error are reported for changes from baseline; least squares mean and 95% confidence interval are reported for differences in change from baseline.
LOCF, last observation carried forward; SD, standard deviation.
[a]Mean and standard deviation.

duration ($\leq$5 years) and in patients pretreated with a combination of oral and rectal mesalamine 10–12 days before enrollment (versus oral-only treatment for 6 weeks). Response rates to LT-02 for maintaining remission exceeded those to mesalamine (64.0% vs 42.9%) in patients with a short UC history. Thus, LT-02 may work best when applied early after disease onset. However, data from these subgroup analyses should be interpreted with caution because of the small sample sizes.

LT-02 was safe and well-tolerated in both trials, and adverse events rates were similar across all treatment groups. No deaths occurred, and serious adverse events were infrequent and generally unrelated to LT-02 treatment. The incidence of adverse drug reactions was low and consistent with the previous phase 2 trial data.[15]

The main limitation of the PCG-4 trial was the small sample size as a result of the early termination of the preceding PCG-2 trial. Moreover, in the absence of a specific dose-finding trial for maintenance treatment, the 1.6 g LT-02 BID regimen in the PCG-4 trial was chosen for practical reasons, because a BID dosing regimen was thought to be more convenient for patients than a QID regimen. In addition, the local reading of endoscopies may have introduced variability to mDAI mucosal appearance subscores.

In conclusion, LT-02 in combination with mesalamine failed to show beneficial effects for the induction of remission in patients with mild to moderate UC and inadequate response to mesalamine treatment. Similarly, LT-02 was not found to be superior to placebo or

mesalamine for maintenance therapy, although there were some hints of efficacy in the unexpectedly small LT-02 population. LT-02 was safe and well-tolerated.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://doi.org/10.1016/j.cgh.2023.09.031.

References

1. Raine T, Bonovas S, Burisch J, et al. ECCO guidelines on therapeutics in ulcerative colitis: medical treatment. J Crohns Colitis 2022;2–17.
2. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) initiative of the International Organization for the Study of IBD (IOIBD)—determining therapeutic goals for treat-to-target strategies in IBD. Gastroenterology 2021;160:1570–1583.
3. Bernhard W, Postle AD, Linck M, et al. Composition of phospholipid classes and phosphatidylcholine molecular species of gastric mucosa and mucus. Biochim Biophys Acta 1995; 1255:99–104.
4. Butler BD, Lichtenberger LM, Hills BA. Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate. Am J Physiol 1983;244:G645–G651.
5. DeSchryver-Kecskemeti K, Eliakim R, Carroll S, et al. Intestinal surfactant-like material: a novel secretory product of the rat enterocyte. J Clin Investig 1989;84:1355–1361.
6. Ehehalt R, Wagenblast J, Erben G, et al. Phosphatidylcholine and lysophosphatidylcholine in intestinal mucus of ulcerative colitis patients: a quantitative approach by nanoElectrospray-tandem mass spectrometry. Scand J Gastroenterol 2004; 39:737–742.
7. Lichtenberger LM. The hydrophobic barrier properties of gastrointestinal mucus. Annu Rev Physiol 1995;57:565–583.
8. Braun A, Treede I, Gotthardt D, et al. Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: a clue to pathogenesis. Inflamm Bowel Dis 2009;15:1705–1720.
9. Stremmel W, Hanemann A, Ehehalt R, et al. Phosphatidylcholine (lecithin) and the mucus layer: evidence of therapeutic efficacy in ulcerative colitis? Dig Dis 2010;28:490–496.
10. Treede I, Braun A, Sparla R, et al. Anti-inflammatory effects of phosphatidylcholine. J Biol Chem 2007;282:27155–27164.
11. Stremmel W, Braun A, Hanemann A, et al. Delayed release phosphatidylcholine in chronic-active ulcerative colitis: a randomized, double-blinded, dose finding study. J Clin Gastroenterol 2010;44:e101–e107.
12. Stremmel W, Ehehalt R, Autschbach F, et al. Phosphatidylcholine for steroid-refractory chronic ulcerative colitis: a randomized trial. Ann Intern Med 2007;147:603–610.
13. Stremmel W, Hanemann A, Braun A, et al. Delayed release phosphatidylcholine as new therapeutic drug for ulcerative colitis: a review of three clinical trials. Expert Opinion on Investigational Drugs 2010;19:1623–1630.
14. Stremmel W, Merle U, Zahn A, et al. Retarded release phosphatidylcholine benefits patients with chronic active ulcerative colitis. Gut 2005;54:966–971.
15. Stremmel W, Vural H, Evliyaoglu O, et al. Delayed-release phosphatidylcholine is effective for treatment of ulcerative colitis: a meta-analysis. Dig Dis 2021;39:508–515.
16. Karner M, Kocjan A, Stein J, et al. First multicenter study of modified release phosphatidylcholine "LT-02" in ulcerative colitis: a randomized, placebo-controlled trial in mesalazine-refractory courses. Am J Gastroenterol 2014;109:1041–1051.
17. Riley SA, Mani V, Goodman MJ, et al. Microscopic activity in ulcerative colitis: what does it mean? Gut 1991;32:174–178.
18. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomized trial. BMJ 1989;298:82–86.
19. Hjortswang H, Jarnerot G, Curman B, et al. The Short Health Scale: a valid measure of subjective health in ulcerative colitis. Scand J Gastroenterol 2006;41:1196–1203.
20. Sandborn WJ, Kamm MA, Lichtenstein GR, et al. MMX Multi Matrix System mesalazine for the induction of remission in patients with mild-to-moderate ulcerative colitis: a combined analysis of two randomized, double-blind, placebo-controlled trials. Aliment Pharmacol Ther 2007;26:205–215.
21. Rubin DT, Cohen RD, Sandborn WJ, et al. Budesonide Multi-matrix is efficacious for mesalamine-refractory, mild to moderate ulcerative colitis: a randomised, placebo-controlled trial. J Crohns Colitis 2017;11:785–791.
22. Stremmel W, Staffer S, Gehrke S. The detergent effect of mesalazine interferes with phosphatidylcholine binding to mucin 2. Inflammatory Intestinal Diseases 2019;3:107–115.
23. Dignass A, Preiss JC, Aust DE, et al. Aktualisierte Leitlinie zur Diagnostik und Therapie der Colitis ulcerosa 2011 - Ergebnisse einer Evidenzbasierten Konsensuskonferenz [Updated German guideline on diagnosis and treatment of ulcerative colitis, 2011] [German]. Z Gastroenterol 2011;49:1276–1341.
24. Jennison C, Turnbull BW. Group sequential tests and repeated confidence intervals. In: Ghosh BK, Sen PK, eds. Handbook of sequential analysis. New York: Marcel Dekker, 1991:283–311.

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Conflicts of interest

These authors disclose the following: Axel Dignass reports receiving fees for participation in clinical trials, review activities such as data monitoring boards, statistical analysis and end point committees from Abivax, AbbVie, Arena Pharmaceuticals, Bristol Myers Squibb/Celgene, Dr. Falk Foundation, Galapagos, Gilead, Janssen, and Pfizer; receiving consultancy fees from AbbVie, Amgen, Arena Pharmaceuticals, Biogen, Boehringer Ingelheim, Bristol Myers Squibb/Celgene, Celltrion, Falk Foundation, Ferring Pharmaceuticals, Fresenius Kabi, Galapagos, Janssen, Lilly, MSD, Pfizer, Pharmacosmos, Roche/Genentech, Sandoz/Hexal, Takeda, Tillotts, and Vifor Pharma; receiving lecturing fees including service on speakers bureaus from AbbVie, Biogen, CED Service GmbH, Celltrion, Falk Foundation, Ferring, Galapagos, Gilead, High5MD, Janssen, Materia Prima, MedToday, MSD, Pfizer, Takeda, Tillotts, and Vifor Pharma; and receiving payment for manuscript preparation from Falk Foundation, Takeda, Thieme, and UniMed Verlag. 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Supplementary Methods

Patients

Patients were enrolled in the PCG-2 trial if they fulfilled the key inclusion criterion of a score of at least 1 in each subscore of the mDAI ("modified" meaning that patients showing any mucosal friability were given a mucosal appearance score of at least 2; range for each subscore 0–3, total 0–12, with higher scores indicating more severe disease).

Patients were required to exhibit inadequate response to mesalamine, defined as persisting symptoms despite (1) continued treatment with oral mesalamine $\geq$2.4 g/day (or therapeutic equivalent [either $\geq$2.4 g/day olsalalazine, $\geq$5.6 g/day balsalazide, or $\geq$6.2 g/day sulfasalazine]) for at least 6 weeks before baseline or (2) combination treatment with oral mesalamine $\geq$2.4 g/day (or therapeutic equivalent) and a rectal mesalamine product for at least 10–14 days (as recommended[1] and as escalation[23]) between screening and baseline for patients on mesalamine regimens before screening that did not meet the criteria in (1).

Subgroup Analyses

The primary end points were also evaluated by prespecified subgroup analyses (sex, mesalamine pretreatment, mDAI, disease localization, disease duration, smoking habits [only for the primary end point in the PCG-2 trial], calprotectin, and underlying mesalamine treatment).

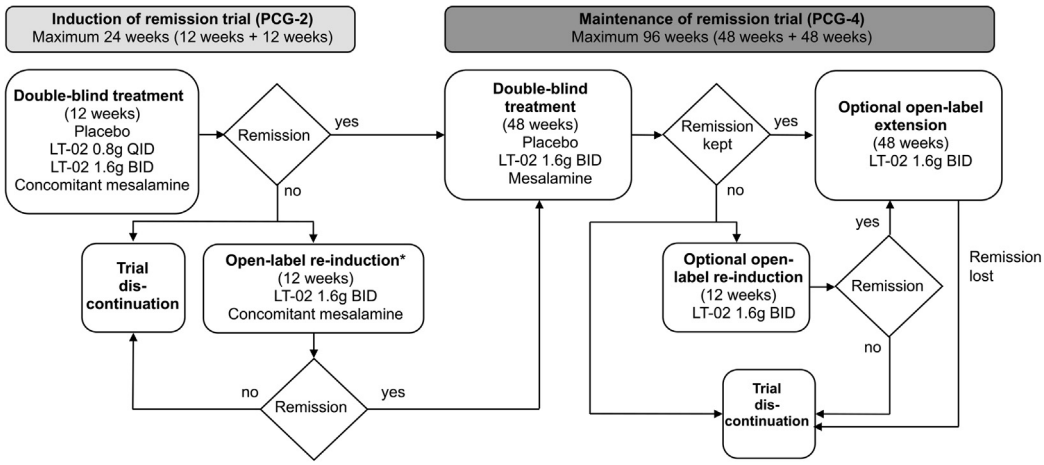
Sample Size and Statistical Analysis

The planned sample size of the PCG-2 trial was 720 (240 per group) on the basis of the primary end point. Assuming a response rate of 0.18 for the placebo group and 0.3 for both active treatment groups, this provided an overall power of at least 89.8% to detect a statistically significant difference between at least one LT-02 treatment group and placebo at the one-sided $\alpha = 0.025$. Accounting for 5% dropouts, 762 patients were to be enrolled in the PCG-2 trial. For PCG-4, 400 patients in total were planned to be randomized, 200 patients in the LT-02 and 100 patients each in the mesalamine and placebo groups, providing an overall power of at least 80%, assuming a difference in remission rates of 0.18 between the LT-02 and placebo groups at week 48 and placebo response rates between 5% and 60%.

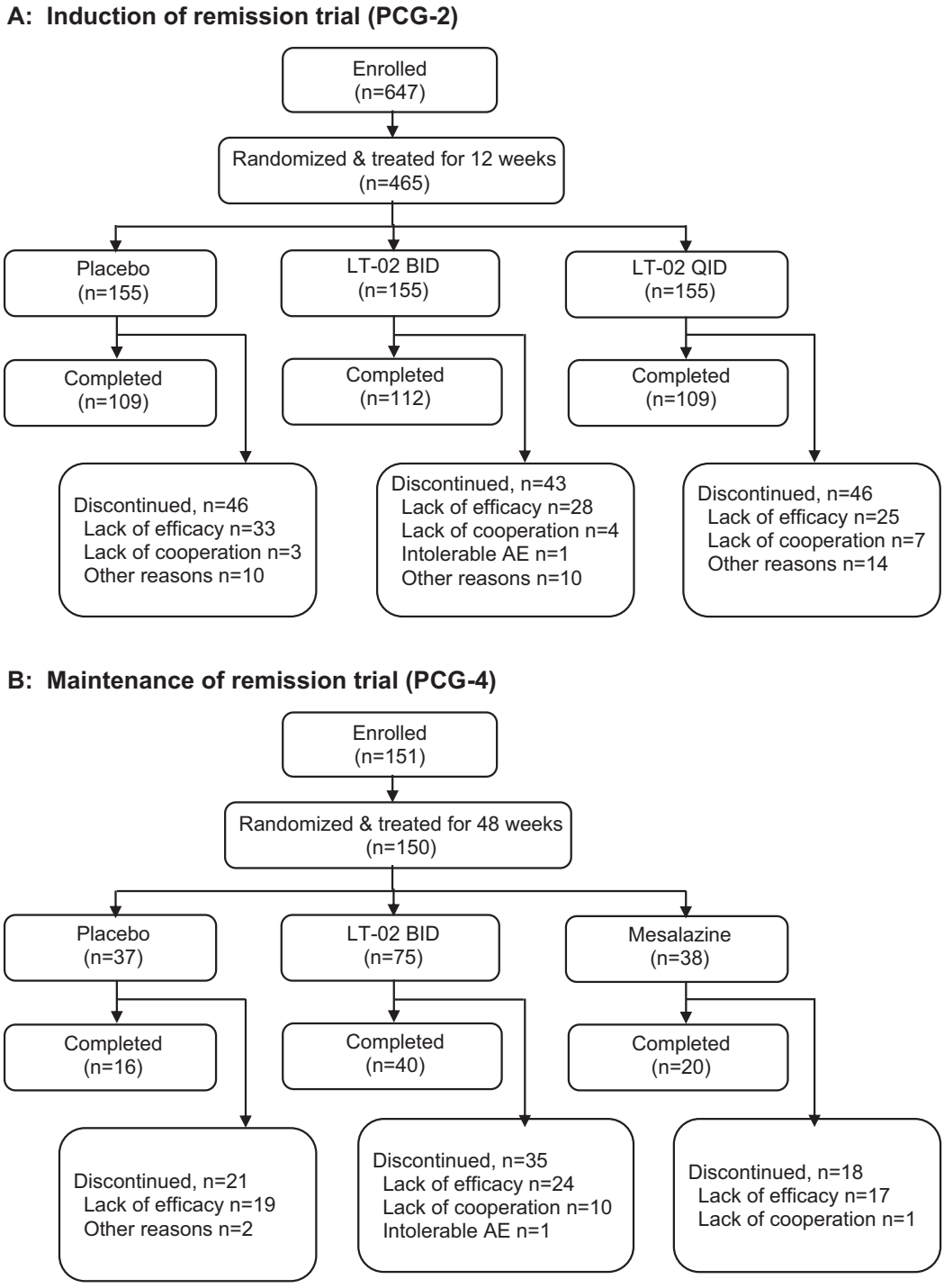
Both trials included a prespecified interim analysis, group-sequential adaptive design with the possibility to adjust sample size and treatments at the pre-planned interim analysis. For confirmatory hypothesis testing at the interim analysis as well as at the final analysis, the inverse normal method of combining the Simes adjusted *P* values of the normal approximation test for 2 rates was used. To estimate the treatment effect, the pairwise difference between the remission rates and the corresponding two-sided 95% multiplicity-adjusted repeated confidence interval (CI) for the pairwise differences were reported.[24]

For the primary end point in PCG-2, *P* values were calculated by using a χ^2 test for pairwise comparison against placebo. The 95% CIs were based on normal approximation. Patients who discontinued the trial because of lack of efficacy were categorized as nonresponders for the primary efficacy end point. Patients who discontinued for other reasons but without an endoscopy at discontinuation were also considered nonresponders. For the primary end point in PCG-4, the *P* value (one-sided) was based on a stratified (factor = stratum as per criterion of deep remission) Cochran-Mantel-Haenszel test comparing LT-02 BID against placebo. The 95% CI was based on a normal approximation.

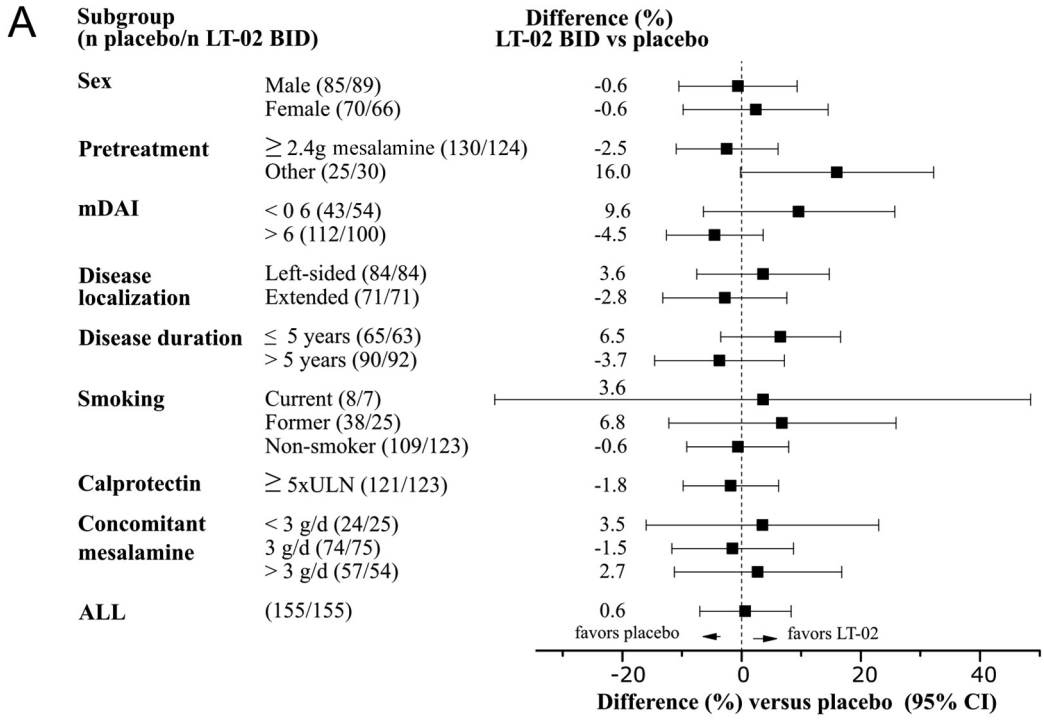
Secondary end points were analyzed descriptively by using an analysis of covariance, with treatment group as factor and baseline value of the respective parameter as covariate. For the PCG-4 trial, prespecified hierarchical testing was abandoned because of premature termination of patient enrollment and resulting low sample size. A last observation carried forward approach was used. For the time to clinical relapse or discontinuation, median and 95% CI were calculated by using Kaplan-Meier analysis. Hazard ratio and corresponding CI were based on a proportional hazards model with treatment group as factor. *P* value vs placebo was based on log-rank test.

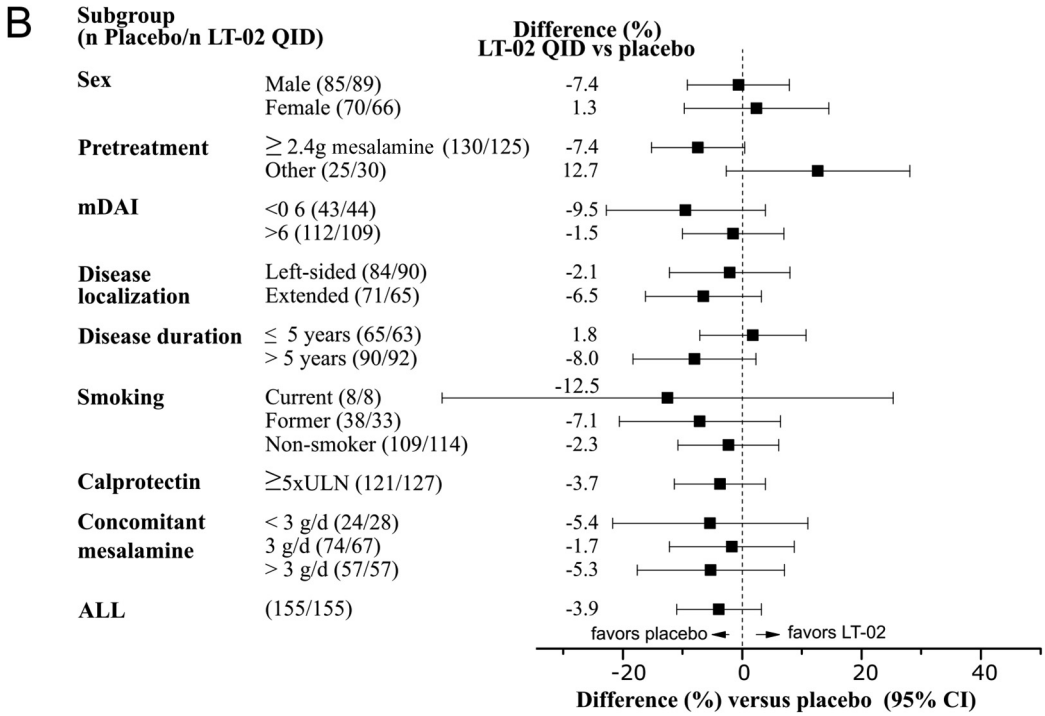


Supplementary Figure 1. Study design. *Patients who completed the double-blind treatment period as scheduled but did not achieve remission or discontinued the trial after at least 8 weeks of treatment because of lack of efficacy and with no improvement in mDAI (decrease by $\leq$1) had the option to continue in an open-label sub-trial.



Supplementary Figure 2. Patient disposition. (*A*) Induction of remission trial (PCG-2). (*B*) Maintenance of remission trial (PCG-4). AE, adverse event.





Supplementary Figure 3. PCG-2: Differences in proportions of patients with deep remission (primary end point) at week 12 (LOCF) between the BID regimen and placebo (*A*) or the QID regimen versus placebo (*B*) stratified by baseline characteristics. Deep remission was defined as mDAI score $\leq$1 with 0 points for rectal bleeding and stool frequency, and $\geq$1-point reduction from baseline in the mucosal appearance score, at week 12 (LOCF). *P* values (one-sided) were based on χ^2 test for comparison against placebo; 95% CIs were based on normal approximation. LOCF, last observation carried forward; ULN, upper limit of normal.

Supplementary Table 1. PCG-2 Proportion of Patients With Deep Remission by Baseline Characteristics (Efficacy Population, n = 465)

	Patients achieving deep remission, n/N (%)			Difference LT-02 BID or QID vs placebo			
	Placebo	LT-02 BID	LT-02 QID		%	95% CI	*P* value
Sex							
Male	11/85 (12.9)	11/89 (12.4)	5/91 (5.5)	BID	–0.6	–10.5, 9.3	.9081
				QID	–7.4	–16.0, 1.1	.0859
Female	10/70 (14.3)	11/66 (16.7)	10/64 (15.6)	BID	2.4	–9.8, 14.5	.7009
				QID	1.3	–10.8, 13.4	.8280
Pretreatment							
≥2.4 g mesalamine ≥6 weeks	20/130 (15.4)	16/124 (12.9)	10/125 (8.0)	BID	–2.5	–11.0, 6.1	.5709
				QID	–7.4	–15.2, 0.4	.0673
Other[a]	1/25 (4.0)	6/30 (20.0)	5/30 (16.7)	BID	16.0	–0.2, 32.2	.0763
				QID	12.7	–2.7, 28.1	.1335
mDAI							
≤6	7/43 (16.3)	14/54 (25.9)	3/44 (6.8)	BID	9.6	–6.4, 25.7	.2518
				QID	–9.5	–22.8, 3.9	.1666
>6	14/112 (12.5)	8/100 (8.0)	12/109 (11.0)	BID	–4.5	–12.6, 3.6	.2835
				QID	–1.5	–10.0, 7.0	.7309
Disease localization							
Left-sided	12/84 (14.3)	15/84 (17.9)	11/90 (12.2)	BID	3.6	–7.5, 14.7	.5286
				QID	–2.1	–12.2, 8.0	.6880
Extended	9/71 (12.7)	7/71 (9.9)	4/65 (6.2)	BID	–2.8	–13.2, 7.6	.5956
				QID	–6.5	–16.2, 3.2	.1963
Disease duration							
≤5 y	4/65 (6.2)	8/63 (12.7)	5/63 (7.9)	BID	6.5	–3.5, 16.6	.2041
				QID	1.8	–7.1, 10.7	.6933
>5 y	17/90 (18.9)	14/92 (15.2)	10/92 (10.9)	BID	–3.7	–14.6, 7.2	.5101
				QID	–8.0	–18.3, 2.3	.1281
Smoking status							
Current	2/8 (25.0)	2/7 (28.0)	8/1 (12.5)	BID	3.6	–41.4, 48.5	.8760
				QID	–12.5	–50.3, 25.3	.5218
Former	5/38 (16.7)	5/25 (20.0)	12/33 (6.1)	BID	6.8	–12.2, 25.9	.4672
				QID	–7.1	–20.6, 6.4	.3171
Non-smokers	14/109 (12.8)	15/123 (12.2)	12/114 (10.5)	BID	–0.6	–9.2, 7.9	.8814
				QID	–2.3	–10.8, 6.1	.5898
Calprotectin							
≥5 × ULN	15/121 (12.4)	13/123 (10.6)	11/127 (8.7)	BID	–1.8	–9.8, 6.2	.6543
				QID	–3.7	–11.4, 3.9	.3372
Concomitant mesalamine							
<3 g/day	3/24 (12.5)	4/25 (16.0)	2/28 (7.1)	BID	3.5	–16.0, 23.0	.7263
				QID	–5.4	–21.7, 11.0	.5136
3 g/day	9/74 (12.2)	8/75 (10.7)	7/67 (10.4)	BID	–1.5	–11.7, 8.7	.7740
				QID	–1.7	–12.2, 8.7	.7486
>3 g/day	9/57 (15.8)	10/54 (18.5)	6/57 (10.5)	BID	2.7	–11.3, 16.8	.7028
				QID	–5.3	–17.6, 7.1	.4059

NOTE. Deep remission was defined as mDAI score ≤1 with 0 points for rectal bleeding and stool frequency, and ≥1-point reduction from baseline in the mucosal appearance score at week 12 (LOCF). *P* values (one-sided) were based on χ^2 test for comparison against placebo. The 95% confidence intervals were based on normal approximation.

LOCF, last observation carried forward; ULN, upper limit of normal.

[a]Other pretreatments included combination treatment with oral mesalamine ≥2.4 g/day (or therapeutic equivalent) and a rectal mesalamine preparation for at least 10–14 days before baseline.

Supplementary Table 2. PCG-4 Proportion of Patients Maintaining Remission Through Week 48 by Baseline Characteristics (Efficacy Population, N = 150)

	Patients maintaining remission, n/N (%)			Difference LT-02 BID vs placebo		
	Placebo	LT-02 BID	Mesalamine	%	95% CI	*P* value
Sex						
Male	9/24 (37.5)	22/43 (51.2)	8/20 (40.0)	13.7	–10.8, 38.1	.1411
Female	7/13 (53.8)	15/32 (46.9)	11/18 (61.1)	–7.0	–39.1, 25.2	.6642
Pretreatment (PCG-2)[a]						
≥2.4 g mesalamine >6 wk	16/34 (47.1)	29/64 (45.3)	14/30 (46.7)	–1.7	–22.5, 19.0	.5656
Other[b]	0/3 (0.0)	8/11 (72.7)	5/8 (62.5)	72.7	46.4, 99.0	.0120
mDAI (PCG-2)[a]						
≤6	4/9 (44.4)	15/26 (57.7)	8/16 (50.0)	13.2	–24.4, 50.9	.2458
>6	12/28 (42.9)	21/48 (43.8)	11/22 (50.0)	0.9	–22.2, 24.0	.4698
Disease localization						
Left-sided	9/20 (45.0)	22/43 (51.2)	14/25 (56.0)	6.2	–20.3, 32.6	.3244
Extended	7/17 (41.2)	15/32 (46.9)	5/13 (38.5)	5.7	–23.4, 34.8	.3513
Disease duration (PCG-2)[a]						
≤5 y	6/15 (40.0)	16/25 (64.0)	6/14 (42.9)	24.0	–7.1, 55.1	.0698
>5 y	0/22 (45.5)	21/50 (42.0)	13/24 (54.2)	–3.5	–28.4, 21.4	.6075
Calprotectin (PCG-2)[a]						
≥5 × ULN	10/27 (37.0)	30/58 (51.7)	13/27 (48.1)	14.7	–7.6, 37.0	.1033

NOTE. Percentage of patients maintaining remission through week 48, ie, patients without any relapse (defined as mDAI rectal bleeding score ≥1 and mDAI mucosal appearance score ≥2) and not being a treatment failure (defined as premature withdrawal for whatever reason) were stratified by baseline characteristics. *P* values (one-sided) were based on χ^2 test for comparison against placebo. The 95% confidence intervals were based on normal approximation.
N = number of patients in the analysis set; n = number of responders.
[a]At baseline of preceding PCG-2.
[b]Other pretreatment included combination treatment with oral mesalamine ≥2.4 g/day (or therapeutic equivalent) and a rectal mesalamine preparation for at least 10–14 days before baseline of PCG-2.

Supplementary Table 3. Treatment-emergent Adverse Events (Safety Population: PCG-2: N = 465, PCG-4: N = 150)

	No. (%) of patients experiencing at least one of the following adverse events					
	PCG-2 (N = 465)			PCG-4 (N = 150)		
	Placebo (n = 155)	LT-02 1.6 g BID (n = 155)	LT-02 0.8 g QID (n = 155)	Placebo (n = 37)	LT-02 (n = 75)	Mesalamine (n = 38)
Any TEAE	82 (52.9)	83 (53.5)	80 (51.6)	25 (67.6)	46 (61.3)	25 (65.8)
Serious TEAE	4 (2.6)	8 (5.2)	5 (3.2)	0	4 (5.3)	1 (2.6)
ADRs	20 (12.9)	23 (14.8)	11 (7.1)	0	7 (9.3)	1 (2.6)
TEAEs leading to discontinuation	13 (8.4)	13 (8.4)	14 (9.0)	2 (5.4)	9 (12.0)	3 (7.9)
Common TEAEs						
Colitis ulcerative	21 (13.5)	18 (11.6)	21 (13.5)	20 (54.1)	27 (36.0)	19 (50.0)
Headache	22 (14.2)	12 (7.7)	13 (8.4)	1 (2.7)	3 (4.0)	0
Nasopharyngitis	14 (9.0)	10 (6.5)	12 (7.7)	2 (5.4)	7 (9.3)	4 (10.5)
Nausea	4 (2.6)	3 (1.9)	8 (5.2)	1 (2.7)	2 (2.7)	0
Abdominal pain	7 (4.5)	5 (3.2)	4 (2.6)	0	2 (2.7)	1 (2.6)
Flatulence	5 (3.2)	3 (1.9)	4 (2.6)	1 (1.3)	0	0
Vomiting	2 (1.3)	2 (1.3)	5 (3.2)	1 (1.3)	0	0
Blood CK increased	5 (3.2)	2 (1.3)	2 (1.3)	1 (1.3)	0	0
Abdominal distention	1 (0.6)	4 (2.6)	3 (1.9)	0	0	1 (2.6)
Influenza-like illness	4 (2.6)	2 (1.3)	0	0	0	0
Abdominal pain upper	3 (1.9)	0	1 (0.6)	2 (5.4)	4 (5.3)	0

NOTE. Results are provided as the number (%) of patients with at least one TEAE. TEAEs reported by at least 3% of patients in any treatment group are listed.
ADR, adverse drug reaction; CK, creatine kinase; SD, standard deviation.