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First Multicenter Study of Modified Release Phosphatidylcholine “LT-02” in Ulcerative Colitis: A Randomized, Placebo-Controlled Trial in Mesalazine-Refractory Courses

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- OBJECTIVES:** Phosphatidylcholine is a key component of the mucosal barrier. Treatment with modified release phosphatidylcholine aims to improve the impaired barrier function. The primary objective is to evaluate the efficacy of LT-02, a newly designed modified release phosphatidylcholine formula, in a multicenter setting.
- METHODS:** This is a double-blinded, randomized, placebo-controlled, superiority study conducted in 24 ambulatory referral centers in Germany, Lithuania, and Romania. A total of 156 patients with an inadequate response to mesalazine, a disease activity score (Simple Clinical Colitis Activity Index (SCCAI)) of ≥ 5 , and bloody diarrhea underwent treatment with 0, 0.8, 1.6, or 3.2 g LT-02. The primary end point was defined *a priori* as changes in SCCAI from baseline to the end of treatment. The primary statistical model was a general linear least-squares model. The study was funded by the sponsor Lipid Therapeutics, Heidelberg, Germany, and registered at <http://clinicaltrials.gov/show/NCT01011322>.
- RESULTS:** Baseline characteristics and dropouts were well balanced between all groups. The primary analyses revealed an SCCAI drop of 33.3% in the placebo group (from 9.0 to 6.0 points) compared with 44.3% in the 0.8 g LT-02 (from 8.8 to 4.9, $P > 0.05$) and 40.7% in the 1.6 g groups (from 8.6 to 5.1, $P > 0.05$). The 3.2 g group improved 51.7% from 8.5 to 4.1 ($P = 0.030$ in comparison with placebo). The remission rate was 15% (6/40) in the placebo group compared with 31.4% (11/35) in the highest LT-02 dose group ($P = 0.089$). Mucosal healing was achieved in 32.5% of placebo patients compared with 47.4% of LT-02 patients ($P = 0.098$); the rates for histologic remission were 20% compared with 40.5%, respectively ($P = 0.016$). There were 17 (48.6%) treatment-emergent adverse events in the highest dose group (and 0 serious adverse events (SAEs)) compared with 22 (55%) in the placebo group (4 SAEs).
- CONCLUSIONS:** The primary end point analysis showed a statistically significant improvement in disease activity during LT-02 treatment in comparison with placebo. The drug was found to be very safe.

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INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease that affects the distal colon, potentially spreading over the entire colon. The incidence is 5–20/100,000 in western countries, with a prevalence of 0.02–0.23% (1). First-line pharmacotherapy consists of 5-aminosalicylic acids and/or steroids for acute episodes. Aminosaliculates and thiopurines, but not steroids, should be used for maintenance therapy. Calcineurin and tumor necrosis factor- α antagonists may work in refractory cases, but the effects wear off over time and adverse events can be limiting (2–5); see also FDA block warning on tumor necrosis factor blockers). Moreover, tumor necrosis factor antibodies are highly cost intensive. Current therapeutic regimens therefore are not always successful. The 10-year cumulative risk of colectomy is ~9% (6). An unmet medical need for a safe and effective therapy remains.

Phosphatidylcholine (PC) was found to be an essential protective component of colonic mucus (7–9). The novel treatment of modified release PC was based on the observation that specifically patients with UC had a low intrinsic mucus PC content that reduces the hydrophobic barrier function of the intestinal mucus (10,11). Colonic bacteria may then permeate the intestinal mucus barrier, and the consecutive unspecific but aggressive immune responses lead to inflammation and ulceration (12). Moreover, the intrinsic anti-inflammatory property of PC is lacking which, in turn, perpetuates the mucosal damage (13,14). The purpose of modified release PC is to reconstitute the low mucus PC reservoir and to re-establish the mucosal barrier (9,15–17). Three previous studies have shown efficacy using a modified release preparation of soy lecithin containing 30% PC (18–20). The altered bioavailability of modified release PC aims to release PC in the distal ileum, thereby avoiding early intestinal absorption. The formula was optimized to LT-02, which contains >94% PC concentrated soy lecithin, to allow for regulatory approval.

The goal of the present trial was to evaluate the clinical efficacy, optimal dose, and safety of LT-02.

METHODS

We calculated 160 patients with mesalazine-refractory UC for the screening phase in order to randomize 144 patients. A planned interim analysis included the possibility of increasing the sample size if necessary. The main inclusion criteria were as follows: an active disease with the Simple Clinical Colitis Activity Index (SCCAI) of ≥ 5 and a subscore for “blood in stool” of ≥ 2 at baseline; a history of bloody diarrhea for at least 6 weeks before inclusion despite mesalazine treatment at a dose of ≥ 3 g/day; or a documented intolerance to mesalazine (for details of criteria, see legend of **Figure 1**). Patients were required to maintain a stable comedication throughout the study; steroid tapering was not allowed. UC was defined in accordance with the European consensus conference (21). Recruitment took place in 24 referral centers in Germany, Lithuania, and Romania. The Contract Research Organization (CRO) produced computer-generated

randomization lists for every study center with the allocation of 1:1:1:1 in blocks of 4. The study interventions consisting of three different doses of LT-02 (0.8, 1.6, and 3.2 g) were tested against placebo and were provided in sequentially numbered containers. Doses were selected based on the results of the previous studies (18–20). The study medication was provided in sachets with pellets taken orally four times daily. The study medication was produced, packed, and labeled according to Good Manufacturing Practice and stored at 2–8°C.

Patients were interviewed, examined, and screened for eligibility at the screening visit (V1, for inclusion and exclusion criteria see legend of **Figure 1**). Patients to be included received detailed study information, gave written informed consent, and were instructed in completing the study diary (comprising SCCAI and other interview parameters). Stool samples were taken to exclude for infectious enterocolitis (including *Clostridium difficile* and *Escherichia coli* 0157:H7). If the patient was still eligible after 1 week of screening (V2 = baseline), a safety lab was taken and a sigmoidoscopy/colonoscopy was performed. At the interim visits 2 and 6 weeks after baseline (V3 and V4), possible disease exacerbation and changes in medication or adverse events (AEs) were assessed. The treatment period ended 12 weeks after baseline at V5 and involved an interview, a physical examination, a sigmoidoscopy, a safety lab, and the SCCAI assessment. The maximal duration of the study was 21 weeks per patient: a 1-week screening period plus 12 weeks of treatment period; patients reaching partial or complete remission (SCCAI <5 at end of treatment) underwent an additional 8-week follow-up without study medication (see **Appendix Figure A1**).

Patients could discontinue the study at any time without reason. The investigator could withdraw a patient in case of AEs or disease exacerbation or if therapeutic intervention was required. Discontinuation criteria were the development of complications such as pseudomembranous colitis, an SCCAI increase of ≥ 7 over baseline, or fever >39°C. Discontinuation because of disease exacerbation was defined as an AE. Patients who discontinued the study early qualified as “premature discontinuation” which resulted in a final study visit. Dropouts were not replaced.

Patients’ compliance was monitored by returned sachets, diary entries, and interviews. The central ethics committees approved the study protocol in all participating countries. No changes to the study methods were made after study initiation.

Precautions against bias

To avoid selection bias, all patients who fulfilled all criteria were included into the study in the predefined, randomized order. Pellets, sachets, and containers were completely indistinguishable between treatment arms. Both patients and study personnel remained blinded and unaware of the allocation method throughout the study until database closure. It was not possible for patients or investigators to guess the next allocated medication in order to avoid selection or allocation biases. To avoid attrition bias, we handled incomplete data in a conservative manner: all patients with premature treatment termination were included in all final analyses with their last available data (last observation

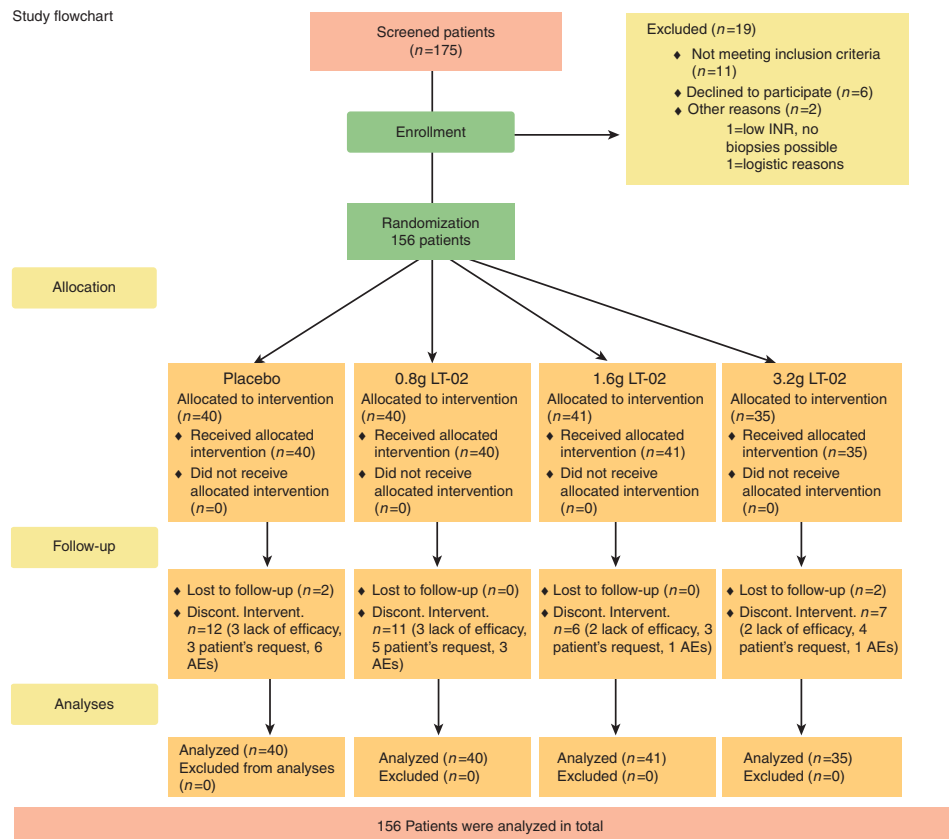


Figure 1. Study flowchart. Inclusion criteria were as follows: proven mesalazine-refractory ulcerative colitis (European consensus definition (16)) with an inadequate response to mesalazine for 6 weeks at a dose of ≥ 3 g/day for over 4 weeks or documented intolerance to mesalazine (a documented intolerance required previous doctors' letters or medical notes that stated that an adverse event possibly related to mesalazine led to a discontinuation of its therapy); active disease with blood in stool for at least 6 weeks; SCCAI ≥ 5 and SCCAI subscore for "blood in stool" ≥ 2 at baseline visit (V2); comedication was allowed if on a stable dose for 4 weeks (e.g., 5-ASA, systemic acting steroids (if taken for ≥ 8 weeks before the start of the study), azathioprine (2–2.5 mg/kg), 6-mercaptopurine (1–1.5 mg/kg), both if taken for ≥ 3 months); and a negative pregnancy test at V1 and V2 plus the use of adequate contraception, if applicable. Exclusion criteria were as follows: toxic megacolon or fulminant courses; therapy with cyclosporine, tacrolimus, methotrexate, or TNF- α -antagonists within 3 months before study entry; current treatment with opiates or loperamide; current antibiotic treatment; rectal applications of aminosalicylates, steroids, or budesonide; oral application of topically acting steroids; ulcerative proctitis with a disease extent < 10 cm; inflammatory or bleeding disorders of the gastrointestinal tract other than UC, or diseases that may cause diarrhea or gastrointestinal bleeding; condition after surgery of the colon; any other uncontrolled systemic diseases (e.g., cardiac, renal, pulmonary, hepatic) or severe chronic diseases (e.g., malignancies, HIV infection); and pregnant or nursing women. 5-ASA, 5-aminosalicylic acid; AE, adverse event; Discont. Intervent., discontinued intervention; INR, international normalized ratio; SCCAI, Simple Clinical Colitis Activity Index; TNF- α , tumor necrosis factor- α ; UC, ulcerative colitis.

carried forward (LOCF)), so that a possible LT-02 treatment effect was rather reduced than increased. More placebo patients may have stopped treatment owing to a lack of efficacy or for other reasons; it is unlikely that the results would have improved if treatment had been continued, and carrying forward the last value appeared to be conservative. All dropouts and losses to follow-up are described in **Figure 1**. Individuals with possible conflict of interest were neither allowed to recruit patients nor were they involved in the conduct or analyses of the study. There were no changes in the study methods or outcome parameters after study initiation. All authors had access to the study data and have reviewed and approved this manuscript.

Outcomes

The *disease activity* was assessed using the SCCAI (22) that is validated and has been proven to correlate well with other indices

comprising invasive methods (23–26). The primary end point was changes in SCCAI from baseline (V2) to V5. The SCCAI assesses stool frequency during the day and at night, defecation urgency, blood in stool, general well-being, abdominal pain, and extraintestinal manifestations. The score ranges from 0 to 19 points—the lower the score, the lower the disease activity.

We assessed the following secondary and *a priori* defined end points in an exploratory sense: complete remission (< 3 mean SCCAI (26) and "blood in stool" subscore of 0 (see European Crohn's and Colitis Organization (ECCO) definition of remission (21), LOCF); partial remission (SCCAI < 5 , LOCF); clinical response (SCCAI decrease ≥ 2 (26)); mucosal healing (endoscopic Mayo Score (EMS) ≤ 1); achievement of mucosal healing (EMS ≤ 1 at V5 and improvement of EMS ≥ 1 from V2 to V5); patients with complete remission; and a "bowel frequency" subscore of 0. All SCCAI-based end points refer to the patient's mean values of

1 week rounded to integer numbers. Time to symptom resolution was defined as the first 3 days with ≤ 3 stools per day without visible blood (21).

The EMS is categorized as follows: 0, inactive; 1, mild disease (erythema, decreased vascular pattern, minimal granularity); 2, moderate disease (marked erythema, friability, granularity, absent vascular pattern, bleeding on minimal trauma, no ulcerations); and 3, severe disease (ulceration, spontaneous bleeding). The histologic index (by Riley) ranges from 1 representing remission to 4 representing severe colitis; a central pathologist, who was blinded to the groups, assessed the score.

Statistics

Data from a dose-finding study (19) showed that a relative effect size for the SCCAI of ~ 0.8 could be expected from the 3.2 g dose group compared with placebo. Under the parametric assumptions of the *t*-test, 30 patients per group were needed to detect a difference of a relative effect size of 0.8 between placebo and an active group with a power of 85% ($\alpha=0.025$, one sided). The sample size was increased from 30 to 36 patients per group to accommodate for nonresponse, loss to follow-up, and other deviations from planned study conditions. We also included dose groups of 0.8 and 1.6 g LT-02 to allow for dose-finding and dose-response analyses, as the drug LT-02 was new and had not been tested in studies.

Primary analysis was based on the full analysis set (intention to treat) that included all randomized patients. If a patient had a missing value at the final visit (V5), the latest available value was carried forward. Analysis of covariance was used to model the primary end point, including the following baseline covariates: (i) mean SCCAI in the past 7 days of the screening period; and (ii) disease extent. The model including these two covariates and the treatment group effect was considered to be the core model. It was used to compare the effects of placebo and the individual dose groups in fixed sequence, starting with the highest dose group of 3.2 g LT-02 daily, followed by the lower dose groups in descending order. The confirmatory testing procedure stopped at the first nonsignificant result; superiority was given if the one-sided *P* value was < 0.025 , equivalent to a two-sided α level of 0.05.

Survival methods were used to analyze time-to-event variables; the log-rank test compared time with event curves between treatment groups. likelihood ratio χ^2 tests based on nominal logistic regression were planned to compare the results of qualitative variables between treatment groups. The analysis plan did not specify how the groups should be compared: in order to increase the statistical power, we focused on the comparisons between placebo and the three active groups pooled.

The statistical power to detect treatment effects in categorical parameters in this small sample size is far below the usual 80–90%. This is why we defined categorical variables such as rate of remission or mucosal healing *a priori* as secondary and exploratory, in order to discover trends in treatment effects to gather information for the sample-size calculation for future phase III studies.

Linearity was checked for important continuous variables compared with log transformation. Normality for continuous

parameters was assumed if the absolute value of skewness was < 1 . In addition, the Shapiro–Wilk goodness-of-fit tests were applied.

A planned interim analysis was conducted by an independent data monitoring committee after half of the patients had terminated the treatment period to adjust the sample size or to stop the trial for futility, if indicated.

Software for sample-size estimation included nQuery Advisor V5.0 (Statistical Solutions Ltd, Cork, Ireland) and StudySize V2.0 (CREOSTAT HB, V.Frolunda, Sweden). Statistical analyses were performed using SAS JMP V9 and SAS JMP V8 and higher (JMP, SAS Institute Inc., Cary, NC).

RESULTS

The study was conducted between November 2009 and December 2010. A total of 175 patients were screened, of which 156 UC patients (119 in Germany, 22 in Lithuania, and 15 in Romania) were randomized and treated (see **Figure 1**). When we received the recommendation of the independent data monitoring committee to continue the study as planned, we had already randomized 12 patients more than the planned 144 patients; the independent data monitoring committee then recommended including these additional patients in the analyses.

In total, 14, 12, 6, and 7 patients of the placebo, 0.8, 1.6, and 3.2 g LT-02 groups, respectively, terminated the study prematurely after randomization, most frequently at their own request owing to the lack of efficacy or AEs. In addition, 18 patients did not return all empty sachets of study medication and were therefore classified as noncompliant (7 placebo, 6 0.8 g LT-02, 2 1.6 g LT-02, and 3 3.2 g LT-02 patients). One placebo patient suffered a serious adverse event (SAE; atrial fibrillation) and was unblinded prematurely; one patient in the 3.2 g LT-02 group was accidentally unblinded by a peripheral study nurse, but remained single-blinded.

Overall, disease-specific baseline characteristics indicated no major differences across the four patient groups (**Table 1**). There were no relevant country effects in the interaction analyses. Concomitant medication was comparable across all study groups, but azathioprine intake was higher in the 0.8 g LT-02 group. The mean SCCAI varied from 8.5 to 9.0, which represents a moderately active UC population. There were no colectomies reported during the study or during follow-up.

Efficacy results

We found a higher absolute SCCAI reduction in all LT-02 groups compared with placebo. In the primary analysis, the disease activity score (SCCAI) in the highest dose group (3.2 g LT-02) dropped from 8.5 to 4.1 (51.7%) compared with 9.0 to 6.0 (33.3%) in the placebo group (two-sided *P* value = 0.030, see **Figure 2**; the corresponding one-sided *P* value is $P=0.015$ which met the goal of the study.)

The secondary analyses found a remission rate of 31.4% (11/35) in the highest LT-02 dose group compared with 15% (6/40) under placebo ($P=0.09$; two-sided likelihood ratio χ^2 test; **Appendix Figure A2**). The response rates increased from 24/40 (60%) under placebo to 29/35 (83%) in the highest LT-02 dose group ($P=0.030$;

Table 1. Key demographic and baseline characteristics

		Placebo (n=40)	0.8 g LT-02 (n=40)	1.6 g LT-02 (n=41)	3.2 g LT-02 (n=35)
Female	n (%)	17 (42.5%)	20 (50.0%)	14 (34.1%)	12 (34.3%)
Age (years)	Mean (s.d.)	45.2 (11.6)	40.7 (12.5)	42.5 (15.2)	41.1 (12.0)
BMI (kg/m ²)	Mean (s.d.)	26.3 (4.8)	24.6 (3.8)	25.2 (5.5)	24.1 (4.2)
Duration of disease (years)	Median (range)	9.65 (0.4–25.2)	6.3 (0.7–32.9)	7.4 (0.4–27.4)	7.2 (1.2–25.5)
Number of previous episodes	Mean (s.d.)	8.6 (7.09)	8.1 (7.53)	9.3 (10.64)	5.2 (4.99)
Duration of present acute episode (days)	Median (range)	114.0 (22–2,688)	96.0 (15–1,260)	87.5 (30–1,107)	134.0 (7–2,626)
Localization					
Proctosigmoiditis	n (%)	1 (2.5%)	4 (10.0%)	2 (4.9%)	2 (5.7%)
Left-sided colitis	n (%)	23 (57.5%)	23 (57.5%)	30 (73.2%)	20 (57.1%)
Extensive colitis	n (%)	16 (40.0%)	13 (32.5%)	9 (22.0%)	13 (37.1%)
SCCAI	Mean (s.d.)	9.0 (2.1)	8.8 (1.7)	8.6 (2.5)	8.5 (2.0)
Endoscopic Mayo score	Mean (s.d.)	2.2 (0.6)	2.2 (0.7)	2.0 (0.6)	2.1 (0.6)
Histologic Riley index (HI)	Mean (s.d.)	2.7 (0.7)	2.2 (0.9)	2.6 (0.9)	2.6 (0.9)
Concomitant IBD treatment					
(i) 5-ASA/sulfasalazine	n (%)	30 (75%)	34 (85%)	31 (75.6%)	27 (77.1%)
(ii) Steroids	n (%)	14 (35.0%)	13 (32.5%)	12 (29.3%)	13 (37.1%)
(ii) Azathioprine	n (%)	6 (15.0%)	12 (30.0%)	6 (14.6%)	3 (8.6%)
No comedication (i–iii)	n (%)	7 (17.5%)	4 (10.0%)	8 (19.5%)	4 (11.4%)

5-ASA, 5-aminosalicylic acid; BMI, body mass index; IBD, inflammatory bowel disease; SCCAI, Simple Clinical Colitis Activity Index.

The longer duration of disease in the placebo group was not significant ($P>0.4$, Dunnett test, Wilcoxon test). In addition, there was no statistically significant effect on SCCAI ($P=0.22$) and no effect modification in the sense of an interaction with treatment ($P=0.29$).

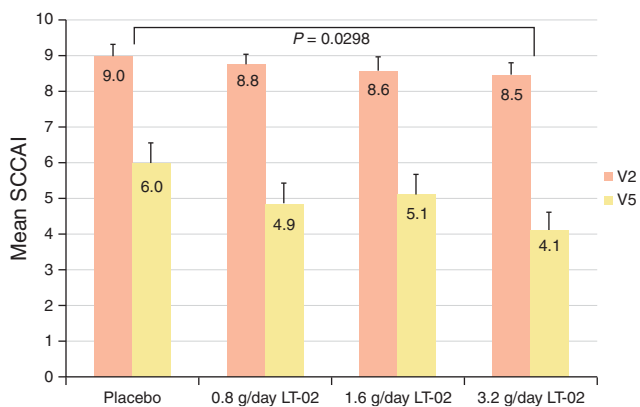


Figure 2. Primary end-point analysis.

Comparison	Estimate	Two-sided p-value	95% confidence limits	
0.8 g LT-02 vs. Placebo	-0.944	0.1725	-2.305	0.417
1.6 g LT-02 vs. Placebo	-0.445	0.5189	-1.803	0.914
3.2 g LT-02 vs. Placebo	-1.559	0.0298	-2.963	-0.155

Note: Treatment comparisons based on ANCOVA with class variable "treatment" and covariates "SCCAI at baseline" and "extent of disease".

The comparison between placebo and the highest dose group revealed an estimate of -1.56 and a two-sided P value of 0.03 with a 95% confidence interval of -2.96 to -0.16 . SCCAI, Simple Clinical Colitis Activity Index.

two-sided likelihood ratio χ^2 test; **Table 2**). The number needed to treat for LT-02 in mesalazine-refractory UC was 6.1 to reach complete remission and 4.3 to reach clinical response. The rate for achievement of mucosal healing was 32.5% in the placebo group compared with 47.4% in the pooled LT-02 groups ($P=0.098$, **Table 2**); the rate for histologic healing (histologic index = 1) was 20.0% in the placebo compared with 35.3% in the LT-02 groups ($P=0.016$). The time to first symptom resolution was 33 days in the median among LT-02 responders. Symptom resolution occurred ~ 2 weeks earlier and was almost twice as often under LT-02 treatment than under placebo treatment ($P=0.02$; **Figure 3**).

Safety results

Safety evaluations of lab results, vital signs, and physical examinations did not show any treatment-related changes between the study groups. The frequency of possibly drug-related AEs was low in all four study arms. Mainly mild or moderate treatment-related AEs occurred. There were 17 treatment-emergent AEs (48.6%) in the highest dose group (0 SAEs) compared with 22 (55%) in the placebo group (4 SAEs). There were no relevant or significant differences of adverse drug reactions between the treatment groups (see **Table 3**; **Appendix Table A2**). In all, 12 patients experienced

Table 2. Further secondary end-point analyses

		Placebo (n=40)	0.8 g LT-02 (n=40)	1.6 g LT-02 (n=41)	3.2 g LT-02 (n=35)	All LT-02 (n=116)	P value ^a
Complete remission ^b	n (%)	6 (15.0%) ^c	11 (27.5%)	9 (22.0%)	11 (31.4%) ^c	31 (26.7%) ^c	P=0.120 ^c
		5 (12.5%) ^d	11 (27.5%)	9 (22.0%)	10 (28.6%) ^d	30 (25.9%) ^d	P=0.067 ^d
Clinical response ^{c,e}	n (%)	24 (60%)	31 (77.5%)	30 (73.2%)	29 (82.9%)	90 (77.6%)	P=0.035 ^c
Mucosal healing (EMS ≤1)	n (%)	16 (40.0%) ^c	23 (57.5%) ^c	23 (56.1%) ^c	18 (51.4%) ^c	64 (55.2%) ^c	P=0.097 ^c
		12 (30.0%) ^d	21 (52.5%) ^d	22 (53.7%) ^d	17 (48.6%) ^d	60 (51.7%) ^d	P=0.016 ^d
Achievement of mucosal healing (EMS ≤1 plus EMS improvement ≥1)	n (%)	13 (32.5%) ^c	19 (47.5%) ^c	20 (48.8%) ^c	16 (45.7%) ^c	55 (47.4%) ^c	P=0.098 ^c
		11 (27.5%) ^d	19 (47.5%) ^d	19 (46.3%) ^d	15 (42.9%) ^d	53 (45.7%) ^d	P=0.040 ^d
Histologic remission (HI=1)	n (%)	8 (20.0%) ^c	16 (40%) ^c	17 (41.5%) ^c	14 (40%) ^c	47 (40.5%)	P=0.016 ^c

EMS, Endoscopic Mayo Score; HI, Histologic Index (varies from 1 to 4, with 1 showing remission and 4 being the worst disease activity).

^aAnalysis of placebo vs. pooled LT-02 patients; two-sided *P* values of likelihood ratio (LR) χ^2 testing.

^bComplete remission was defined by a mean Simple Clinical Colitis Activity Index (SCCAI) of <3 without blood in stool.

^cLast observation carried forward (LOCF).

^dData with dropouts considered as failures—sensitivity analyses upon request of reviewers to adjust for possible underestimations of treatment effects (28).

^eClinical response was a decrease from baseline by at least 2.

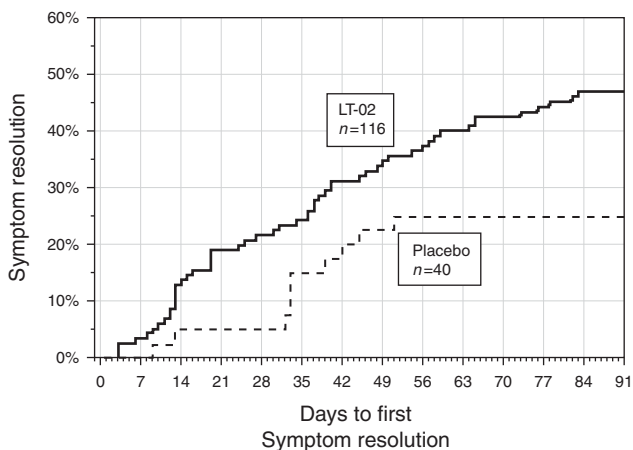


Figure 3. Time to first symptom resolution: all active LT-02 groups pooled vs. placebo. LT-02 patients reached the end point of first symptom resolution more than 2 weeks earlier than placebo ($P=0.02$, preplanned, two-sided log-rank test). In total, almost twice as many LT-02 patients reached complete symptom resolution compared with placebo.

17 SAEs; these consisted of 5, 2, and 1 patient in the 0.8, 1.6, and 3.2 g LT-02 treatment groups, respectively, and 4 patients in the placebo group. Only one SAE, atrial fibrillation, was assessed as being possibly related to study treatment; it occurred in the placebo group.

Follow-up

Responders of all study arms entered an 8-week follow-up period without study medication. Patients in the LT-02 group were able to avoid relapses over a longer period and in a higher percentage of patients ($P=0.02$, log-rank test; **Appendix Figure A3**).

DISCUSSION

The aim of the current trial was to evaluate the efficacy, safety, and optimal dose of a newly designed, modified release formulation of highly purified PC (LT-02). This drug is a first-in-class therapy for UC and the first treatment with a mucoprotective substance to reach study phase II or phase III.

The primary analysis revealed a statistically significant treatment effect for LT-02 in mesalazine-refractory UC: whereas the two lower doses of LT-02 showed improvement that was statistically not significant, the highest LT-02 dose group (3.2 g) showed a significantly higher drop of the index compared with placebo ($P=0.030$, two sided). Mixed modeling was performed as sensitivity analyses for the SCCAI primary model to confirm the primary analysis: all data of visits under treatment were used as the dependent variable (without applying LOCF), with repeated measures on the same patient taken into account with three different versions of simple covariance structures of residuals. Fixed effects included into the mixed model were the same as in the primary model, namely dose group, SCCAI at baseline, and extent of disease. The resulting two-sided *P* values for the primary comparison of 3.2 g LT-02 vs. placebo were between 0.008 and 0.036 depending on the type of the covariance structure used. These analyses confirmed the statistical significance of the originally planned primary analysis.

SCCAI subcategory analyses revealed that “Extraintestinal Manifestations” showed no significant differences between LT-02 and placebo, and this is not surprising for a topical and not systemic agent. “Bowel Movements at Night” indicated a statistical trend with a two-sided *P* value of 0.127, whereas “Stool Urgency” resulted in a *P* value of 0.006. (Both categories represent major patient complaints: nightly defecation is highly disruptive and affects patients’ recreation; stool urgency requires immediate access to bathrooms, which may result in pain and

Table 3. Drug-related adverse events as defined by the site investigator, *n* (%)

	Placebo (<i>n</i> =40)	0.8 g LT-02 (<i>n</i> =40)	1.6 g LT-02 (<i>n</i> =41)	3.2 g LT-02 (<i>n</i> =35)
ADRs	6 (15.0%) ^a	5 (12.5%) ^a	3 (7.3%) ^a	4 (11.4%) ^a
Tachyarrhythmia	1 (2.5%)	0	0	0
Abd. distension/flatulence	5 (12.5%)	2 (5.0%)	2 (4.9%)	1 (2.9%)
Constipation	0	0	0	1 (2.9%)
Nausea	0	1 (2.5%)	0	0
Vomiting	0	1 (2.5%)	0	1 (2.9%)
Asthenia	0	0	0	1 (2.9%)
Chills	0	0	0	1 (2.9%)
Edema	0	0	1 (2.4%)	0
Pain	0	0	0	1 (2.9%)
Arthralgia	0	0	1 (2.4%)	0
Headache	0	1 (2.5%)	1 (2.4%)	1 (2.9%)
Proteinuria	0	1 (2.5%)	0	0
Pruritus	0	0	1 (2.4%)	0

Abd., abdominal; ADR, adverse drug reaction.

Coded according to MedDRA, Version 13.0.

^aNo. of patients with at least one ADR; some patients had multiple ADRs.

There was no evidence for any treatment-related difference of adverse events (AEs). As may be expected from the patient population and the disease under treatment in this study, gastrointestinal AEs as well as infections were the most frequent AEs. Neither these nor other AEs showed any treatment-related differences.

humiliating stool incontinence.) “Bowel Frequency,” “General Wellbeing,” and “Blood In Stool” show borderline significant *P* values that are not much higher compared with those of the total SCCAI.

In the main secondary outcome analyses, we found that the clinical and histologic remission rates doubled between placebo and the highest LT-02 dose groups. Approximately 50% more LT-02 than placebo patients achieved mucosal healing ($P < 0.1$, **Table 2**). As the applied LOCF analyses bear the risk of underestimating treatment effects (27), we conducted a sensitivity analysis upon request of reviewers with dropouts treated as failures: the effects that we observed magnified and indicated statistical significance (**Table 2**). The preplanned analyses of clinical response and time to first symptom resolution also revealed statistically significant results (**Figure 3**). Follow-up analyses revealed that placebo patients relapsed earlier and more frequently than LT-02 patients ($P = 0.02$; **Appendix Figure A3**). This underlines the LT-02 efficacy as the placebo effect in the placebo group became more apparent. The treatment effect of LT-02 seems to last longer than the period of actual drug intake. We believe that an interruption of the vicious cycle of barrier defect and mucosal damage might be responsible for this effect.

The treatment effects in mesalazine-refractory UC were good (number needed to treat was 6.1 for complete remission and 4.3 for clinical response), and the safety profile was excellent: AEs occurred equally among placebo and LT-02 groups and no LT-02-related SAE occurred.

Limitations

The primary end point “changes of disease activity index” instead of remission rates is unusual for a larger study. The clinical effect and the practical impact of treatment may be overestimated with the use of numerical changes, but they detect clinical effects in smaller study populations. Information is lost by qualitative variables such as remission rates. This is why quantitative target variables are important in dose-finding studies, as they have a higher sensitivity concerning dose-response effects of a new drug such as LT-02.

On the basis of this primary, quantitative end point, we had planned to include 36 evaluable patients per group. Remission end points require much higher sample sizes of ~200 patients per group (equals $N = 800$ for this study), and these are targeted for pivotal studies. The small sample size of the current trial resulted in a reduced statistical power for secondary end points, making statistical significance unlikely. With our small sample size, success rate estimates of qualitative variables have a high variability; statistically significant results or strict dose-response patterns cannot be expected for qualitative parameters such as remission or mucosal healing.

Most pivotal studies in UC use the Mayo score instead of the SCCAI. The European Medicines Agency, EMA, however, states that it does not favor any score, but recommends the use of indices using signs and symptoms rather than endoscopy, as this correlates well with the former but varies strongly between observers (www.tga.gov.au/pdf/euguide/ewp1846306en.pdf). The SCCAI is the most comprehensive clinical score; it requires no diagnostic

interventions and allows for continuous evaluation throughout the study. Unlike the Mayo or CAI scores, it allows for time-to-event (remission/response) analyses (see **Figure 3**, **Appendix Table A1** and **Appendix Figure A3**) that are essential for a new drug with an unknown time to treatment response. The SCCAI is a relatively new, but well-established, score that has been used frequently in clinical studies recently. Cutoff points for remission (<2.5), relapse (>4.5), and response (index drop >1.5) have been validated (24–26). As the SCCAI only allows for natural numbers, our end points of remission <3 , partial remission ≤ 5 , and response (SCCAI drop ≥ 2) are equivalent to these cutoffs.

Of the 24 study centers, 9 had <4 patients and an additional 7 centers had <8 patients, and this explains a certain deviation from a better balance between study arms (N varies between 35 and 41). This, however, is not an issue because statistical tests are fitted more to simple randomization than to restricted randomization.

The randomization ratio was 1:1:1 with blocks of four per center. This could mean a potential limited bias in case of the premature unblinding of a patient, as the investigators might draw conclusions concerning the treatment of the remaining patients at their centers. In an isolated occurrence, a patient was accidentally unblinded by a study nurse. As the investigators were completely uninformed regarding the above-described packaging strategy, it is highly unlikely that this could influence the study outcome. A randomization ratio with blocks of eight would have been preferable, but it was financially not feasible.

Some authors expect a two-sided P value for the primary analysis. A one-sided P value, however, is often used in regulatory superiority studies with a stricter significance level of $P=0.025$ that equals a two-sided P value of 0.05. Inferiority to placebo was not assumed, as previous studies had shown superiority. All presented P values are two sided; only the P value for the primary analysis is presented as both one and two sided to facilitate the comparison with the stricter limit of 0.025.

Fecal calprotectin is an essential marker in UC at present. During the planning phase of the study, however, it was not established and was included into the running study. As the study progressed so fast, the resulting data are too little to be analyzed.

Conclusion

There is a pressing need for treatment alternatives in refractory UC. The goal of the study with the *a priori* defined primary end point of changes in disease activity was reached: the disease activity score dropped significantly under 3.2 g LT-02 compared with placebo. Moreover, the drug was found to be very safe. Although the improvement of the disease activity in the lower doses (0.8 and 1.6 g LT-02) was not statistically significant compared with placebo, the highest dose was effective and is intended for the planned pivotal studies.

The treatment effects of modified release PC appear to correlate with the shift of paradigm from immunological disorder to mucosal barrier dysfunction that has taken place in our understanding of inflammatory bowel disease in recent years. Our findings could possibly further the treatment and understanding of ulcerative colitis.

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CONFLICT OF INTEREST

Guarantor of the article: Max Karner, MD.

Specific author contributions: M.K. was the principal investigator of the study; he helped in the planning and conduct of the trial and prepared the manuscript; he had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. A.K., J.S., S.S., G.v.B., P.U., C.S., L.K., and I.D. were the site investigators of the most active study centers. F.Z. and G.K. are representatives of Lipid Therapeutics (sponsor), Heidelberg, Germany, and helped in the planning and organization of the study. W.S. is the inventor of mrPC; he helped in the study design and prepared the manuscript together with M.K.

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Potential competing interests: M.K. was reimbursed for his costs to present the study data at a national and an international congress. A.K., J.S., S.S., G.B., P.U., C.S., L.K., and I.D. had no potential conflict of interest. F.Z. and G.K. are representatives of the sponsor. The adult and independent children of W.S. hold the patent for modified release phosphatidylcholine. W.S., F.Z., and G.K. were not involved in patient recruitment, conduct, or analyses of the study. Moreover, F.Z. and G.K., as representatives of the sponsor, were not involved in the preparation, review, or approval of the manuscript, nor in the decision to submit the manuscript for publication.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ There is a medical need for treatment alternatives in refractory ulcerative colitis.
- ✓ Modified release phosphatidylcholine was found to be effective and safe in three previous single-center studies. Critics request multicenter confirmation of efficacy and safety.
- ✓ A new modified release formula of a highly purified (>94%) phosphatidylcholine “LT-02” is required to allow for regulatory approval (all former studies used a phosphatidylcholine concentration of ~30%).

WHAT IS NEW HERE

- ✓ The study shows the first multicenter data for the efficacy of modified release phosphatidylcholine in ulcerative colitis.
- ✓ The drug was found to be effective, even in refractory disease, and has an excellent safety profile.
- ✓ It is a first-in-class treatment and the first mucoprotective substance in ulcerative colitis to reach a phase III level.

REFERENCES

1. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology* 2004;126:1504–17.
2. Baumgart DC, Macdonald JK, Feagan B. Tacrolimus (FK506) for induction of remission in refractory ulcerative colitis. *Cochrane Database Syst Rev* 2008;3: CD007216.
3. Shibolet O, Regushevskaya E, Brezis M *et al*. Cyclosporine A for induction of remission in severe ulcerative colitis. *Cochrane Database Syst Rev* 2005;1: CD004277.
4. De Vries HS, van Oijen MG, de Jong DJ. Serious events with infliximab in patients with inflammatory bowel disease: a 9-year cohort study in the Netherlands. *Drug Saf* 2008;31:1135–44.
5. Fidler H, Schnitzler F, Ferrante M *et al*. Long-term safety of infliximab for the treatment of inflammatory bowel disease: a single-center cohort study. *Gut* 2009;58:501–8.
6. Hoie O, Wolters FL, Riis L *et al*. Low colectomy rates in ulcerative colitis in an unselected European cohort followed for 10 years. *Gastroenterology* 2007;132:507–15.
7. DeSchryver-Kecsckemeti K, Eliakim R, Carroll S *et al*. Intestinal surfactant-like material. A novel secretory product of the rat enterocyte. *J Clin Invest* 1989;84:1355–61.
8. Lichtenberger LM. The hydrophobic barrier properties of gastrointestinal mucus. *Annu Rev Physiol* 1995;57:565–83.
9. 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 Opin Investig Drugs* 2010;Dec:19:1623–30.
10. 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;39:737–42.
11. 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;15:1705–20.
12. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417–29.
13. Treede I, Braun A, Jeliaskova P *et al*. TNF-alpha-induced up-regulation of pro-inflammatory cytokines is reduced by phosphatidylcholine in intestinal epithelial cells. *BMC Gastroenterol* 2009;9:53.
14. Treede I, Braun A, Sparla R *et al*. Anti-inflammatory effects of phosphatidylcholine. *J Biol Chem* 2007;282:27155–64.
15. Braun A, Schönfeld U, Welsch T *et al*. Reduced hydrophobicity of the colonic mucosal surface in ulcerative colitis as a hint at a physicochemical barrier defect. *Int J Colorectal Dis* 2011;26:989–98.
16. Gibson PR, Muir JG. Reinforcing the mucus: a new therapeutic approach for ulcerative colitis?. *2005;54:900–3*.
17. Croft NM. Phospholipid in UC: novel, safe and works—is it too good to be true? *Gastroenterology* 2006;130:1003–4; discussion 1004–5.
18. Stremmel W, Merle U, Zahn A *et al*. Retarded release phosphatidylcholine benefits patients with chronic active ulcerative colitis. *Gut* 2005;54:966–71.
19. 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:e101–7.
20. Stremmel W, Ehehalt R, Autschbach F *et al*. Efficacy of retarded release phosphatidylcholine for treatment of chronic, steroid refractory ulcerative colitis. *Ann Intern Med* 2007;147:603–10.
21. Stange EF, Travis SP, Vermeire S *et al*. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis of the European Crohn's and Colitis Organisation (ECCO). *J Crohns Colitis* 2008;2:1–23.
22. Walmsley RS, Ayres RC, Pounder RE *et al*. A simple clinical colitis activity index. *Gut* 1998;43:29–32.
23. Higgins PD, Leung J, Schwartz M *et al*. The quantitative validation of non-endoscopic disease activity indices in ulcerative colitis. *Aliment Pharmacol Ther* 2007;25:333–42.
24. Higgins PD, Schwartz M, Mapili J *et al*. Is endoscopy necessary for the measurement of disease activity in ulcerative colitis? *Am J Gastroenterol* 2005;100:355–61.
25. Jowett SL, Seal CJ, Phillips E *et al*. Defining relapse of ulcerative colitis using a symptom-based activity index. *Scand J Gastroenterol* 2003;38:164–71.
26. Higgins PD, Schwartz M, Mapili J *et al*. Patient defined dichotomous end points for remission and clinical improvement in ulcerative colitis. *Gut* 2005;54:782–8.
27. Lane P. Handling drop-out in longitudinal clinical trials: a comparison of the LOCF and MMRM approaches. *Pharm Stat* 2008;7:93–106.



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APPENDIX

Table A1. Remission rates over time

			Placebo (n=40)	0.8 g LT-02 (n=40)	1.6 g LT-02 (n=41)	3.2 g LT-02 (n=35)
V3	Complete remission	n (%)	3/38 (7.9%)	3/38 (7.9%)	2/40 (5.0%)	5/33 (15.2%)
V4	Complete remission	n (%)	6/36 (16.7%)	7/35 (20.0%)	5/38 (13.2%)	8/32 (25.0%)
V5	Complete remission	n (%)	6/28 (21.4%)	11/30 (36.7%)	9/36 (25.0%)	11/29 (37.9%)
V5 (LOCF)	Complete remission	n (%)	6/40 (15.0%)	11/40 (27.5%)	9/41 (22.0%)	11/35 (31.4%)

LOCF, last observation carried forward.

Complete remission was defined as the mean Simple Clinical Colitis Activity Index (SCCAI) of <3 without blood in stool.

Table A2a. Categories of adverse events (AEs)

		Placebo (n=40)	0.8g LT-02 (n=40)	1.6g LT-02 (n=41)	3.2g LT-02 (n=35)
Any pretreatment AE	n (%)	5 (12.5%)	6 (15.0%)	8 (19.5%)	3 (8.6%)
Any treatment-emergent AE	n (%)	22 (55.0%)	25 (62.5%)	20 (48.8%)	17 (48.6%)
Any posttreatment AE	n (%)	7 (17.5%)	8 (20.0%)	7 (17.1%)	6 (17.1%)
Any adverse drug reaction (ADR)	n (%)	6 (15.0%)	5 (12.5%)	3 (7.3%)	4 (11.4%)
Any serious adverse event (SAE)	n (%)	4 (10.0%)	5 (12.5%)	2 (4.9%)	1 (2.9%)
Any treatment-emergent SAE	n (%)	4 (10.0%)	4 (10.0%)	2 (4.9%)	0
Any potentially study medication-induced SAE	n (%)	1 (2.5%)	0	0	0

Table A2b. Detailed listing of adverse events

		Placebo (n=40)	0.8g LT-02 (n=40)	1.6g LT-02 (n=41)	3.2g LT-02 (n=35)
Blood and lymphatic system disorders	n (%)	3 (7.5%)	1 (2.5%)	0	1 (2.9%)
Cardiac disorders	n (%)	2 (5.0%)	0	0	0
Gastrointestinal disorders	n (%)	13 (32.5%)	13 (32.5%)	13 (31.7%)	9 (25.7%)
Infections and infestations	n (%)	8 (20.0%)	10 (25.0%)	5 (12.2%)	5 (4.3%)
Musculoskeletal and connective tissue disorders	n (%)	5 (12.5%)	3 (7.5%)	3 (7.3%)	1 (2.9%)
Nervous system disorders	n (%)	4 (10.0%)	5 (12.5%)	9 (22.0%)	1 (2.9%)
Psychiatric disorders	n (%)	1 (2.5%)	2 (5.0%)	0	0
Renal and urinary disorders	n (%)	2 (5.0%)	3 (7.5%)	2 (4.9%)	1 (2.9%)
Respiratory, thoracic and mediastinal disorders	n (%)	0	2 (5.0%)	0	0
Skin and subcutaneous tissue disorders	n (%)	0	2 (5.0%)	2 (4.9%)	1 (2.9%)
Vascular disorders	n (%)	2 (5.0%)	0	2 (4.9%)	1 (2.9%)

Table A2c. Serious adverse events (SAEs)

Treatment	Terminology	SAE	Causality
Placebo	Anemia (progression)/cytomegalovirus infection	No SUSAR	Not related/unlikely
Placebo	Gallstones and ERCP-induced pancreatitis	No SUSAR	Not related
Placebo	Rectal carcinoma	No SUSAR	Not related
Placebo	Atrial fibrillation with thromboembolic event	SUSAR	Possibly
0.8g LT-02	Deep vein thrombosis	No SUSAR	Unlikely
0.8g LT-02	Disease exacerbation of UC	No SUSAR	Not related
0.8g LT-02	Disease exacerbation of UC	No SUSAR	Not related
0.8g LT-02	Disease exacerbation of UC	No SUSAR	Not related
0.8g LT-02	Acute appendicitis	No SUSAR	Not related
0.8g LT-02	Abscess of Bartholin's gland	No SUSAR	Unlikely
1.6g LT-02	Atrial flutter	No SUSAR	Not related
1.6g LT-02	Disease exacerbation of UC	No SUSAR	Not related
1.6g LT-02	Rectal bleeding after Colonoscopy with biopsies	No SUSAR	Not related
3.2g LT-02	Primary sclerosing cholangitis	No SUSAR	Not related

ERCP, endoscopic retrograde cholangiopancreatography; SUSAR, suspected unexpected serious adverse reaction; UC, ulcerative colitis.
The only serious adverse event that was categorized as possibly drug related occurred in the placebo group.

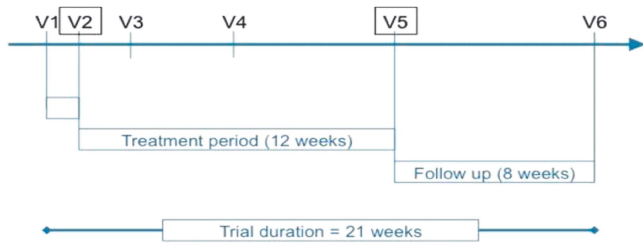


Figure A1. The course of the trial. Patients were screened for inclusion and exclusion criteria (see legend of **Figure 1**) at V1. If patients still fulfilled the study criteria at baseline (V2, 1 week after V1), they were then randomized into the study and received their first study medication at the study center after baseline investigations (interview, physical examination, sigmoidoscopy/colonoscopy, lab tests). At the interim visits 2 and 6 weeks after baseline (V3 and V4w), the Simple Clinical Colitis Activity Index (SCCAI), possible disease exacerbations, changes in medication, and adverse events (AEs) were assessed. The treatment period ended 12 weeks after baseline at V5 that involved the final study assessment (interview, physical examination, lab test, sigmoidoscopy). Responders of all study arms entered a 8-week follow-up period without study medication; those patients were asked to continue their comedication as taken before, unless they relapsed.

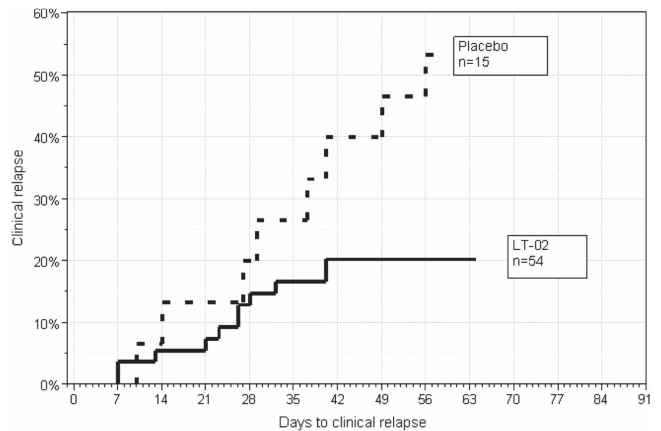


Figure A3. Time to relapse (Simple Clinical Colitis Activity Index (SCCAI) ≥ 5) in the responder group after discontinuation of the study medication. A total of 69 patients who had a time to clinical relapse or information on censoring related to clinical relapse were included in the analysis. Patients treated with LT-02 relapsed later and less frequently than placebo patients (preplanned, two-sided log-rank test, $P=0.016$).

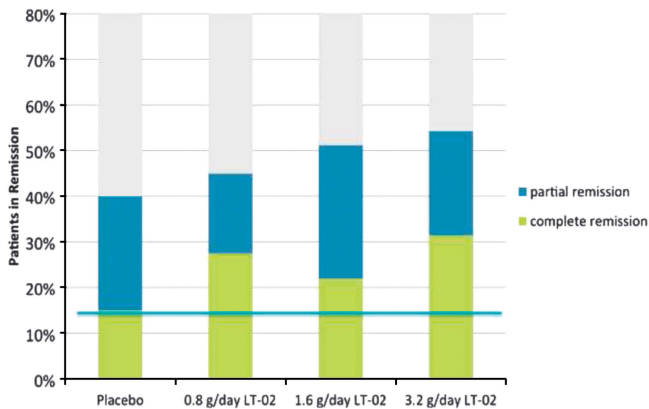


Figure A2. Complete remission rates by dose groups. The rates of complete remission were 15.0% under placebo compared with 31.4% in the 3.2 g LT-02 group ($P=0.089$); the other rates were 27.5% (0.8g LT-02) and 22.0% (1.6g LT-02). Complete remission was defined by a mean Simple Clinical Colitis Activity Index (SCCAI) of <3 without blood in stool. When adding a normal stool frequency to this definition, the remission rate then increased by factor 2.3, from 12.5% in the placebo group to 28.6% in the highest LT-02 dose group ($P=0.105$). The blue columns show the rates for partial remission defined by an SCCAI <5 .