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# The Probiotic VSL#3 Has Anti-inflammatory Effects and Could Reduce Endoscopic Recurrence After Surgery for Crohn's Disease

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**BACKGROUND & AIMS:** **Probiotic formulations of single species of bacteria have not been effective in preventing the recurrence of Crohn's disease after surgery. We investigated the ability of VSL#3, a mixture of 8 different bacterial probiotic species, to prevent Crohn's disease recurrence after surgery in a multicenter, randomized, double-blind, placebo-controlled trial.**

**METHODS:** **Within 30 days of ileocolonic resection and re-anastomosis, patients with Crohn's disease were randomly assigned to groups given 1 sachet of VSL#3 (900 billion viable bacteria, comprising 4 strains of *Lactobacillus*, 3 strains of *Bifidobacterium*, and 1 strain of *Streptococcus salivarius* subspecies thermophilus) (n = 59) or matching placebo (n = 60). Colonoscopy was performed at days 90 and 365 to evaluate the neoterminal ileum for disease recurrence and obtain mucosal biopsies for cytokine analysis. Patients from both groups with either no or mild endoscopic recurrence at day 90 received VSL#3 until day 365. The primary outcome was the proportion of patients with severe endoscopic recurrence at day 90.**

**RESULTS:** **At day 90, the proportion of patients with severe endoscopic lesions did not differ significantly between VSL#3 (9.3%) and placebo (15.7%, $P = .19$). The proportions of patients with non-severe lesions at day 90 who had severe endoscopic recurrence at day 365 were 10.0% in the early VSL#3 group (given VSL#3 for the entire 365 days) and 26.7% in the late VSL#3 group (given VSL#3 from days 90 through 365) ($P = .09$). Aggregate rates of severe recurrence (on days 90 and 365) were not statistically different, 20.5% of subjects in the early VSL#3 group and 42.1% in the late VSL#3 group. Patients receiving VSL#3 had reduced mucosal inflammatory cytokine levels compared with placebo at day 90 ($P < .05$). Crohn's disease activity index and inflammatory bowel disease quality of life scores were similar in the 2 groups.**

**CONCLUSIONS:** **There were no statistical differences in endoscopic recurrence rates at day 90 between patients who received VSL#3 and patients who received placebo. Lower mucosal levels of inflammatory cytokines and a lower rate of recurrence among patients who received early VSL#3 (for the entire 365 days) indicate that this probiotic should be further investigated for prevention of Crohn's disease recurrence. Clinical trials.gov number: NCT00175292.**

*Keywords:* Inflammatory Bowel Disease; IBDQ; Microbiota; Bifidobacteria; Streptococcus; Treatment.

*Abbreviations used in this paper:* CDAI, Crohn's Disease Activity Index; IBDQ, Inflammatory Bowel Disease Questionnaire.


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See editorial on page 936.

Recurrence after intestinal resection in patients with Crohn's disease is common. Sequential colonoscopy in a cohort of Belgian patients showed that 1 year after surgery, 73% of patients developed endoscopic recurrence, and 44% had severe (grade 3 or 4) lesions.[1] A meta-analysis confirmed a pooled severe endoscopic recurrence rate of approximately 50%.[2]

Studies to limit postoperative recurrence with conventional medication have recently been reviewed; however, the results have not been consistent or were of limited efficacy.[3] Open-label studies with anti–tumor necrosis factor suggest this may be a much more effective prevention strategy in the future.[4,5]

Crohn's disease is characterized by a dysregulated intestinal immune response to normal and/or abnormal intestinal microbial antigens. The intestinal microbiota of patients with active Crohn's disease is altered compared with that of patients with quiescent disease or their unaffected siblings, showing a global decrease in microbial diversity with marked reductions in Firmicutes, *Clostridium*, lactobacilli, and bifidobacteria groups.[6,7] Patients with rapid postoperative Crohn's recurrence were found to be deficient in the Firmicutes species *Faecalibacterium prausnitzii*.[8,9] Ex vivo experiments demonstrated supplementation with *F prausnitzii* attenuated intestinal inflammation.[9] These observations raise the possibility that probiotic manipulation of endogenous intestinal microbiota might be an effective approach to preventative therapy.

Five clinical trials have examined the efficacy of probiotics in the prevention of Crohn's disease recurrence after ileal resection. *Lactobacillus johnsonii* LA1[10,11] was no more effective than placebo in preventing endoscopic recurrence. *Lactobacillus* GG had no effect on endoscopic postoperative recurrence after 1 year.[12] A mixture of 4 lactobacilli and prebiotics was ineffective in preventing endoscopic recurrence at 2 years.[13] In contrast, a small randomized trial found the probiotic mixture VSL#3 was superior to mesalamine in preventing endoscopic recurrence at 1 year.[14]

VSL#3 has 2 innovative characteristics, a high number of viable bacteria (900 billion/sachet) and a mixture of 8 different bacteria.[15] This mixture could confer protective effects where single-strain or lactobacillus-only formulations had failed. VSL#3 is effective in the interleukin 10 gene-deficient murine model of inflammatory bowel disease,[16] in maintenance and prevention of pouchitis,[17] and in induction[18–20] and maintenance[21] of remission in ulcerative colitis. The safety of the VSL#3 preparation is well-established.[22]

We conducted a 1-year study in which patients with an ileal resection and ileocolonic re-anastomosis for Crohn's disease were randomly assigned to oral VSL#3 or placebo for 3 months, and then both study groups received open-label VSL#3.

## Methods

### Overall Study Design

This randomized, double-blind, placebo-controlled, multicenter study compared the efficacy of VSL#3 with placebo for the prevention of endoscopic recurrence of Crohn's disease in patients who had recently undergone ileocolonic surgical resection with a small-intestine-to-colon anastomosis. The study design comprised 3 phases: a screening phase (day 0), a double-blind treatment phase (days 1–90, in which patients received placebo or VSL#3, 1 sachet twice daily), and an open-label treatment phase (days 91–365, in which all patients without severe endoscopic recurrence received VSL#3 one sachet twice daily). Only patients who had demonstrated either no (Rutgeerts grade 0) or mild endoscopic evidence of recurrence (Rutgeerts grade 1 or 2) at day 90 were offered continuation in the study, receiving open-label VSL#3, one sachet twice daily.

The protocol was approved by the institutional ethics committee at each participating center. All patients gave written informed consent.

### Study Participants

The trial was conducted at 17 tertiary inflammatory bowel disease university-associated centers in Canada between December 2003 and March 2007. Participants were 16 years of age or older with a radiologic, endoscopic, or surgical diagnosis of Crohn's disease of at least 3-month duration. Patients underwent resection of ileocolonic Crohn's disease at the physician's discretion, with margins macroscopically free of disease, and small-bowel-to-colon anastomosis no more than 30 days before randomization. Patients with residual luminal disease were not eligible.

### Concomitant Therapy

Patients receiving a tumor necrosis factor antagonist within 8 weeks of resection were not eligible. After resection, treatment of Crohn's disease was not permitted. Codeine, loperamide, diphenoxylate, and cholestyramine were allowed for diarrhea.

### Study Medication

Commercial VSL#3 (VSL Pharmaceuticals Inc, Towson, MD) was provided in packets that contained 900 billion viable lyophilized bacteria consisting of 4 strains of *Lactobacillus (L pracasei DSM24733, L plantarum*

*DSM24730, L acidophilus DSM24735,* and *L delbrueckii* subsp bulgaricus *DSM24734*), 3 strains of *Bifidobacterium (B longum DSM24736, B breve DSM24732,* and *B infantis DSM24737),* and 1 strain of *Streptococcus salivarius* subsp thermophilus *DSM24731*. Placebo was provided in identical sachets containing 3 g corn starch. VSL#3 and placebo were administered twice daily. The study drug and the placebo were identical in taste, smell, color, texture, and consistency.

## Study Interventions

**Screening phase**

Patients were screened for eligibility at study visit 1 (day 0) at the time of discharge from hospital or immediately thereafter. Eligible patients were randomly assigned in a 1 to 1 ratio to VSL#3 or placebo for a period of 90 days. The computer-generated randomization was performed in permutated blocks and stratified by center. Investigators and patients were unaware of the treatment assignment.

**Double-blind treatment phase A**

This phase of the study comprised days 1–90. One sachet of VSL#3 or placebo was taken in the morning and at night. Patients were reviewed at days 30 and 90. Telephone contacts occurred on days 14 and 60. At each visit a physical exam and medication adherence check were performed, and the Crohn's Disease Activity Index (CDAI) and Inflammatory Bowel Disease Questionnaire (IBDQ) were calculated. At day 90, participants underwent a colonoscopy to evaluate endoscopic recurrence according to the Rutgeerts score (Table 1).[1,23] Each endoscopist and research coordinator reviewed a videotape prepared by Dr Rutgeerts demonstrating endoscopic scoring. At the time of each colonoscopy, a color photographic example of each endoscopic grade was available to ensure accurate assessment of the endoscopic score.

**Open-label treatment phase B**

This phase of the study comprised days 91–365. Only patients with either no or mild endoscopic recurrence (Rutgeerts grade 0, 1, or 2) at day 90 were allowed to continue in the open-label treatment phase for an additional 9 months. Those with severe endoscopic recurrence (Rutgeerts grade 3 or 4) were considered treatment failures and withdrawn from the study. All patients received 1 sachet open-label VSL#3 twice daily. At each visit a physical exam and medication adherence check were performed, and CDAI and IBDQ were calculated. Clinic visits were scheduled for days 180, 270 and 365, with telephone contacts on days 135, 225, and 315. Endoscopic recurrence was assessed by colonoscopy on day 365.

With the above design, we were also able to assess outcome relative to those patients who received VSL#3 early (ie, immediately within the first 30 days after surgery, early VSL#3 treatment group) compared with those who received VSL#3 late (ie, from days 91 to 365, 90 after surgery, late VSL#3 treatment group) (Figure 1).

**Table 1.** Rutgeerts Endoscopic Assessment Criteria[1,25]

| | |
|---|---|
| Grade 0 | No lesions |
| Grade 1 | Fewer than 5 aphthous ulcerations |
| Grade 2 | More than 5 aphthous ulcerations with normal mucosa between the ulcerations or skip areas of larger lesions, or lesions confined to the ileocolonic anastomotic ring (less than 1 cm) |
| Grade 3 | Diffuse aphthous ileitis with diffusely inflamed mucosa |
| Grade 4 | Diffuse ileal inflammation with larger ulcers, nodules, or narrowing |

## Outcomes

The primary outcome measure was severe endoscopic recurrence (Rutgeerts grade 3 or 4) within 90 days of study treatments. The secondary outcomes were (1) any endoscopic recurrence of Crohn's disease (Rutgeerts grades 1–4) within 90 days of study treatment, (2) mucosal cytokine levels at day 90, (3) quality of life as determined by the IBDQ scores at days 90 and 365, (4) Crohn's disease activity as measured by the CDAI at days 90 and 365, and (5) severe endoscopic recurrence of Crohn's disease (Rutgeerts grade 3 or 4) at day 365 in subjects who received early (within 30 days after resection) versus late (>90 days after resection) VSL#3 (Figure 1).

## Mucosal Cytokine Measurements

Total RNA was isolated from colonic mucosal biopsies. The mRNA (1 $\mu$g) was reverse transcribed by using the High Capacity cDNA reverse transcription kit (Applied Biosystems, Branchburg, NJ) with random primers. Real-time polymerase chain reaction analysis was performed with ABI 7900HT real-time polymerase chain reaction system (Applied Biosystems). All reactions were completed in triplicate, and a GAPDH primer set was used as the endogenous control primers for normalization.

## Randomization and Blinding, Sample Size Calculations, Statistical Analysis, and Role of Funding Source

Randomization and blinding, sample size calculations, statistical analysis, and role of funding source are included in the Supplementary Material. Representatives from VSL Pharmaceuticals, Inc had the opportunity to review and comment on the study design and on the manuscript; however, the principal investigators made the final decisions regarding the design of the trial, and all of the authors had access to the study data and reviewed and approved the content of the manuscript.

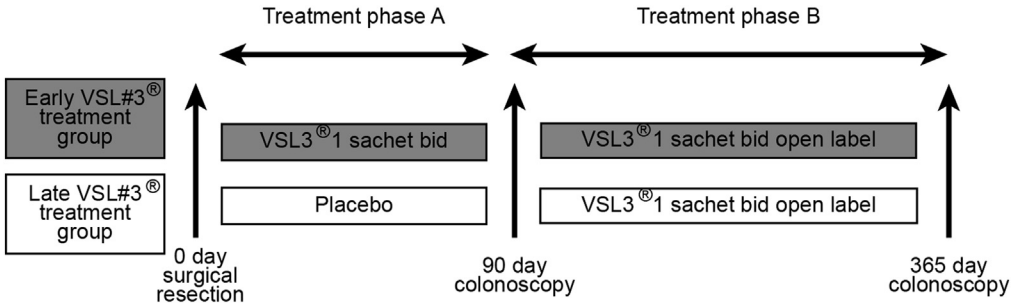


**Figure 1.** Schematic diagram outlining the group of patients who received VSL#3 early after surgery (within the first 30 days after surgery, early VSL#3 treatment group, *shaded bar*) compared with those who received VSL#3 late (from days 91 to 365 after surgery, late VSL#3 treatment group, *open hatched bar*). The late VSL#3 treatment group included patients with no or mild endoscopic recurrence (Rutgeerts grade 0, 1, 2).

## Results

### *Participant Flow*

Of 120 patients enrolled in the trial, 58 received VSL#3, and 62 received placebo. The details of participant flow are shown in Supplementary Figure 1. Study drug was started on discharge from hospital in all patients, with a range between 2 to 3 weeks after resection. Nonevaluable patients at 90 days totaled 22 of 120 (18.3%). Ninety-four patients had endoscopic evaluation at day 90 (n = 92 patients) or at the time of withdrawal (n = 2 patients). Of the VSL#3-treated patients at day 90, 14 of 58 (24.1%) were nonevaluable (withdrew consent, n = 5; noncompliance, n = 1; non-serious adverse events, n = 4; lost to follow-up, n = 4).

Among patients who continued into the open-label treatment phase (n = 81), 57 completed the study to day 365, and 56 of 57 had endoscopic evaluation at day 365 or at the time of withdrawal (n = 4 patients).

### *Subject Recruitment*

Subjects were recruited and attended study visits between December 2003 and June 2006. Follow-up and study visits continued until March 2007.

### *Baseline Subject Characteristics*

The baseline characteristics were similar in the 2 treatment groups (Table 2). No important differences were observed in age, gender, duration or characteristics of Crohn's disease, medication use immediately before surgery, number of previous surgical resections, CDAI, or IBDQ scores.

### *Endoscopic Recurrence*

At day 90, the proportion of patients with severe endoscopic recurrence (grades 3 and 4) by intent-to-treat analysis was not significantly different for those taking VSL#3 (4 of 43, 9.3%) relative to placebo (8 of 51, 15.7%) ($P = .19$) (Figure 2). It is noteworthy that the day 90 recurrence rate in the placebo arm (15.7%) was markedly lower than anticipated in the sample size calculation (45%). The rates of all endoscopic recurrences (grades 1–4) at day 90 were similar for those taking VSL#3 (32 of 43, 74.4%) compared with placebo (39 of 51, 76.5%) ($P = .82$). The proportion of patients free of severe endoscopic recurrence (grades 0, 1, and 2) was also similar for those taking VSL#3 (43 of 58, 74.1%) relative to placebo (39 of 62, 62.9%). Prior resection did not affect recurrence rates in either group (data not shown).

At day 365, the proportion of patients who at day 90 were free of severe endoscopic recurrence and then developed severe endoscopic recurrence (grades 3 and 4) was 10.0% (3 of 30) in patients who had received early treatment with VSL#3 within 30 days after surgery compared with 26.7% (8 of 30) for those who received

**Table 2.** Baseline Subject Characteristics

| Characteristic | VSL#3 (n = 58) | Placebo (n = 62) | *P* value |
|---|---|---|---|
| Age, *y*, mean (± SD) | 37.6 ± 12.4 | 35.9 ± 11.8 | .45 |
| Male, n (%) | 30 (51.7) | 32 (51.6) | .99 |
| Months since diagnosis, mean (±SD) | 103.9 ± 85.6 | 96.2 ± 94.7 | .64 |
| Smoking status, n (%) | | | .14 |
| Current smokers | 13 (22.4) | 19 (30.7) | |
| Former smokers | 16 (27.6) | 23 (37.1) | |
| Never smokers | 29 (50.0) | 20 (32.3) | |
| Prior mesalamine use, n (%) | 47 (81.0) | 49 (79.0) | .78 |
| Prior corticosteroid use, n (%) | 50 (86.2) | 51 (82.3) | .55 |
| Prior immune modifier agents, n (%) | 29 (50.0) | 35 (56.5) | .48 |
| Prior infliximab use, n (%) | 7 (12.1) | 9 (14.5) | .69 |
| Number of prior surgeries, n (%) | | | .44 |
| 0 | 34 (58.6) | 43 (69.4) | |
| 1 | 20 (34.5) | 15 (24.2) | |
| 2 | 4 (6.9) | 4 (6.5) | |
| CDAI, mean (± SD) | 169.7 ± 83.1 | 164.8 ± 81.4 | .74 |
| IBDQ score, mean (± SD) | 155.0 ± 29.1 | 157.1 ± 30.7 | .69 |

SD, standard deviation.

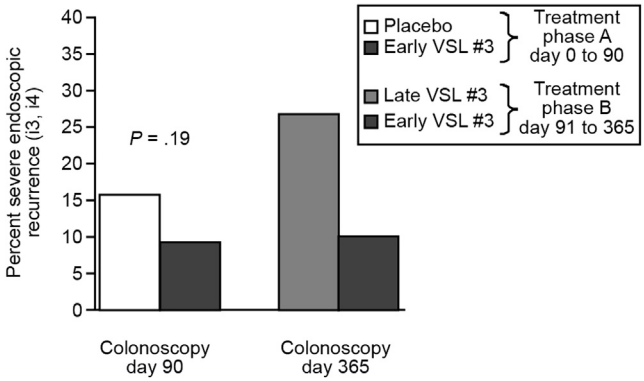


**Figure 2.** Percent severe endoscopic recurrence (Rutgeerts score i3, i4) at day 90 and day 365. Between day 0 and day 90, patients received either placebo (*open bar*) or early VSL#3 treatment (*black bar*). Between day 91 and day 365, all patients received VSL#3, defining a group who received early VSL#3 (early VSL#3 treatment group, *black bar*) and one who received VSL#3 late (ie, from days 91 to 365, late VSL#3 treatment group, *open hatched bar*). At day 90, the proportions of patients with severe endoscopic lesions did not differ significantly between VSL#3 and placebo. Similarly, the proportions of patients with non-severe lesions at day 90 who had severe endoscopic recurrence at day 365 were numerically but not statistically different in the early VSL#3 group (given VSL#3 for the entire 365 days) compared with the late VSL#3 group (given VSL#3 from days 90 through 365) ($P = .09$).

placebo for 90 days and then late treatment with VSL#3 from day 91 to day 365 (Figure 2) ($P = .09$). For those patients who finished both double-blind phase and open phase, the proportion of patients free of endoscopic severe recurrence (grades 3 and 4) at day 365 was 89.6% (26 of 29) in the early VSL#3-treated group compared with 71.4% (20 of 28) for the group who received placebo and then VSL#3. Furthermore, 5 patients from the placebo group withdrew from the study because of treatment failure, whereas none of the VSL#3-treated patients were withdrawn for treatment failure. Individual endoscopic scores for each time point are shown in Table 3.

Similarly, aggregate (from both day 90 and day 365) severe recurrence rates were numerically but not statistically different, 20.5% (7 of 34) in the group treated with early VSL#3 from study start to day 365 and 42.1% (16 of 38) in the group treated with placebo from study start to day 90 and then late VSL#3 from day 90 to day 365.

These results from day 365 suggest that early VSL#3 treatment in the immediate postoperative period may be of more benefit in reducing Crohn's disease endoscopic recurrence after resection and re-anastomosis compared with starting VSL#3 late, 90 days after surgery (Figure 2). This likely implies a critical time interval for probiotic exposure relative to its therapeutic effects.

### *Crohn's Disease Activity Index and Inflammatory Bowel Disease Questionnaire*

The CDAI and IBDQ scores were similar in the 2 treatment groups (data not shown).

### *Colonic Mucosal Cytokine Expression*

At day 90 and day 365, patients assigned to VSL#3 starting from day 0 had reduced pattern of mucosal inflammatory cytokine expression compared with those who received placebo (Figure 3). Individual cytokine expression in each patient at day 365 was compared with their own baseline cytokine expression at day 90. There was no significant difference in expression of any cytokine in the placebo or the VSL#3 group between day 90 and day 365 (data not shown). This would suggest that although starting patients late after surgery on VSL#3 was not effective in reducing cytokine expression, those patients receiving VSL#3 immediately after re-anastomosis were able to maintain their reduced cytokine expression during this time period. These findings further support the concept of a critical time interval for successful probiotic therapy.

### *Safety and Tolerability*

The proportions of patients with adverse events and side effects in each group are shown in Supplementary Table 1. There were 6 serious adverse events in the placebo-treated group (traumatic stabbing, 1; small bowel obstruction due to adhesions, 2; worsening of disease, 1; postoperative wound infection, 1; ventral hernia repair, 1). There was 1 serious adverse event in the VSL#3-treated group (postoperative wound infection).

## Discussion

This study is a prospective, placebo-controlled double-blind study to assess the efficacy of VSL#3 in

**Table 3.** Endoscopic Recurrence Scores

| | | | | | |
|---|---|---|---|---|---|
| Day 90 | 0 | 1 | 2 | 3 | 4 |
| VSL#3 (n = 43) | 11 (25.6%) | 15 (34.9%) | 13 (30.2%) | 3 (7.0%) | 1 (2.3%) |
| Placebo (n = 51) | 12 (23.5%) | 15 (29.4%) | 16 (31.4%) | 7 (13.7%) | 1 (2.0%) |
| Day 365 | 0 | 1 | 2 | 3 | 4 |
| Early VSL#3 (n = 30) | 6 (20.0%) | 11 (36.7%) | 10 (33.3%) | 2 (6.7%) | 1 (3.3%) |
| Late VSL#3 (n = 30) | 6 (20.0%) | 8 (26.7%) | 8 (26.7%) | 6 (20.0%) | 2 (6.7%) |

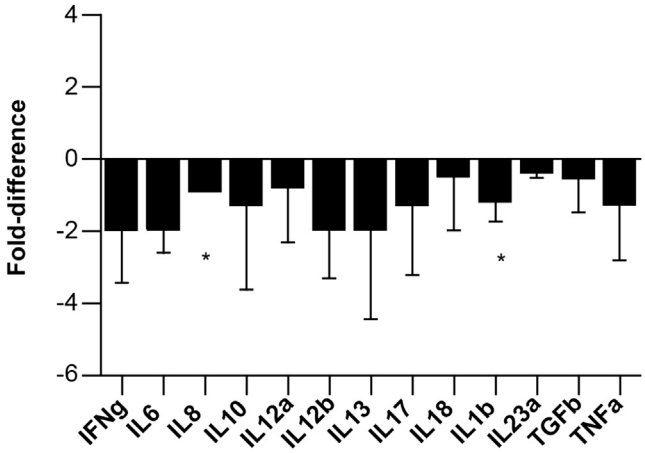


**Figure 3.** Mucosal expression of cytokines at day 90 as expressed as relative-fold change in early VSL#3 group compared with placebo group. For comparisons of relative expression, the housekeeping gene GAPDH was used to normalize each cytokine before calculating fold change. To show cytokine expression differences between the early VSL#3 and placebo groups at day 90, each patient was calibrated against the group average of the placebo group for each cytokine before averaging. Patients receiving VSL#3 from day 0 had overall reduced cytokine expression compared with the placebo group. *$P < .05$ compared with placebo group at day 90. IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

maintaining postoperative remission in patients with Crohn's disease. Overall, treatment with VSL#3 within 30 days after ileal resection and re-anastomosis resulted in a trend toward reduction of severe endoscopic recurrence (grades 3 and 4) and proinflammatory colonic mucosal cytokine expression at day 90.

The rate of recurrence in the placebo group was markedly less than anticipated, rendering the study underpowered to detect a statistical difference of its primary objective. Our sample size calculation was based on 45% recurrence rate at day 90 and reduction of 25% in the placebo group; however, a considerably lower than expected 90-day recurrence rate of 15.7% was observed. This placebo recurrence rate is similar to that of Van Gossum et al.[11]

Rutgeerts et al[1] observed that by 1 year, 73% of nontreated patients had developed endoscopic recurrence, and 44% had severe lesions (grade 3 or 4). In our study, only 26.7% of patients developed severe (grade 3 or 4) endoscopic recurrence when they received placebo for 90 days followed by VSL#3 from day 91 to day 365 (ie, late treatment with VSL#3), compared with only 10% of patients who received early treatment with VSL#3 within 30 days of surgery and then continued VSL#3 to day 365. This suggests that when the VSL#3 probiotic treatment is administered late (ie, more than 90 days after resection and re-anastomosis), it does not confer the same protection as when VSL#3 is administered within 30 days of surgery.

Because the incidence of severe recurrence at 90 days was substantially lower in the control group (15.7%) than anticipated (45%), the study had limited statistical power to detect a clinically meaningful effect. Several European studies have reported the rate of severe endoscopic recurrence to range from 43% to 53% at 3 months after surgery.[24,25,9] The reason for the lower endoscopic recurrence rate at 90 days in this Canadian study remains unexplained, although it may relate to differences in the genetic and/or phenotypic inflammatory bowel disease variants, diet, patient selection for surgery, or the course of treatment before operation. Future studies of postoperative recurrence will need to assess this issue.

It is intriguing to consider that the luminal microbiota drives initiation of a Crohn's disease inflammatory response and that the presence of probiotic bacteria early in the process can abrogate disease, whereas the presence of probiotics administered 3 months postoperatively does not have the same degree of benefit. This is in keeping with both animal[9] and human studies.[9]

In a review of probiotics, Hedin et al[26] concluded that there was insufficient evidence of benefit for probiotics in Crohn's disease, and recently, *Saccharomyces boulardi*, a probiotic yeast, was shown not to be effective in preventing Crohn's disease relapse after medical induction of remission.[27] Furthermore, previous studies addressing maintenance of postoperative remission in Crohn's disease used single probiotic strains (*L rhamnosus GG*[12] and *L johnsonii*[10,11]) or were small pilot studies. The current study is the largest probiotic postoperative prevention trial and the only one to show a trend toward benefit with the timing of the administration of a multiple species probiotic.

The importance of innate immunity in the pathogenesis of Crohn's disease through genome-wide association studies has highlighted the importance of barrier function and innate immunity as determinants of the dysregulated host response to microbes.[28,29] In particular, subsets of patients with Crohn's disease exhibit an impaired innate immunity and are unable to clear bacteria that breach the epithelial barrier.[30] The present study demonstrates that oral administration of VSL#3 alters mucosal cytokine balance. Patients receiving VSL#3 from day 0 (ie, early VSL#3 group) exhibited a reduction in expression of proinflammatory cytokines as compared with patients receiving placebo. In contrast, patients who began VSL#3 at day 90 (late VSL#3 group) showed no significant changes in their cytokine profile, supporting the concept that early probiotic treatment is necessary to confer optimal benefit. It is yet to be determined whether doses of VSL#3 higher than the 1800 billion colony-forming units used in this study would have additional preventative effects. Indeed, many of the active treatment studies with VSL#3 have used daily doses $\geq$3600 billion colony-forming units.[18–20,31]

In summary, patients receiving VSL#3 within 30 days after ileal resection and ileocolonic re-anastomosis demonstrated a numeric, but not statistically significant, reduction in endoscopic recurrence and lower colonic mucosal proinflammatory cytokine levels

compared with placebo-treated controls after 90 and 365 days. CDAI and IBDQ end points were similar in both groups. Early treatment with VSL#3 had a larger effect than late treatment. Future larger studies will be needed to confirm the effect of VSL#3 in prevention of postoperative recurrence.

## 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://dx.doi.org/10.1016/j.cgh.2014.10.031.

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**Conflicts of interest**
This author discloses the following: Dr Fedorak has served on a VSL3 Pharmaceuticals Inc speakers bureau. The remaining authors disclose no conflicts.

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## Supplementary Material

## Randomization and Blinding

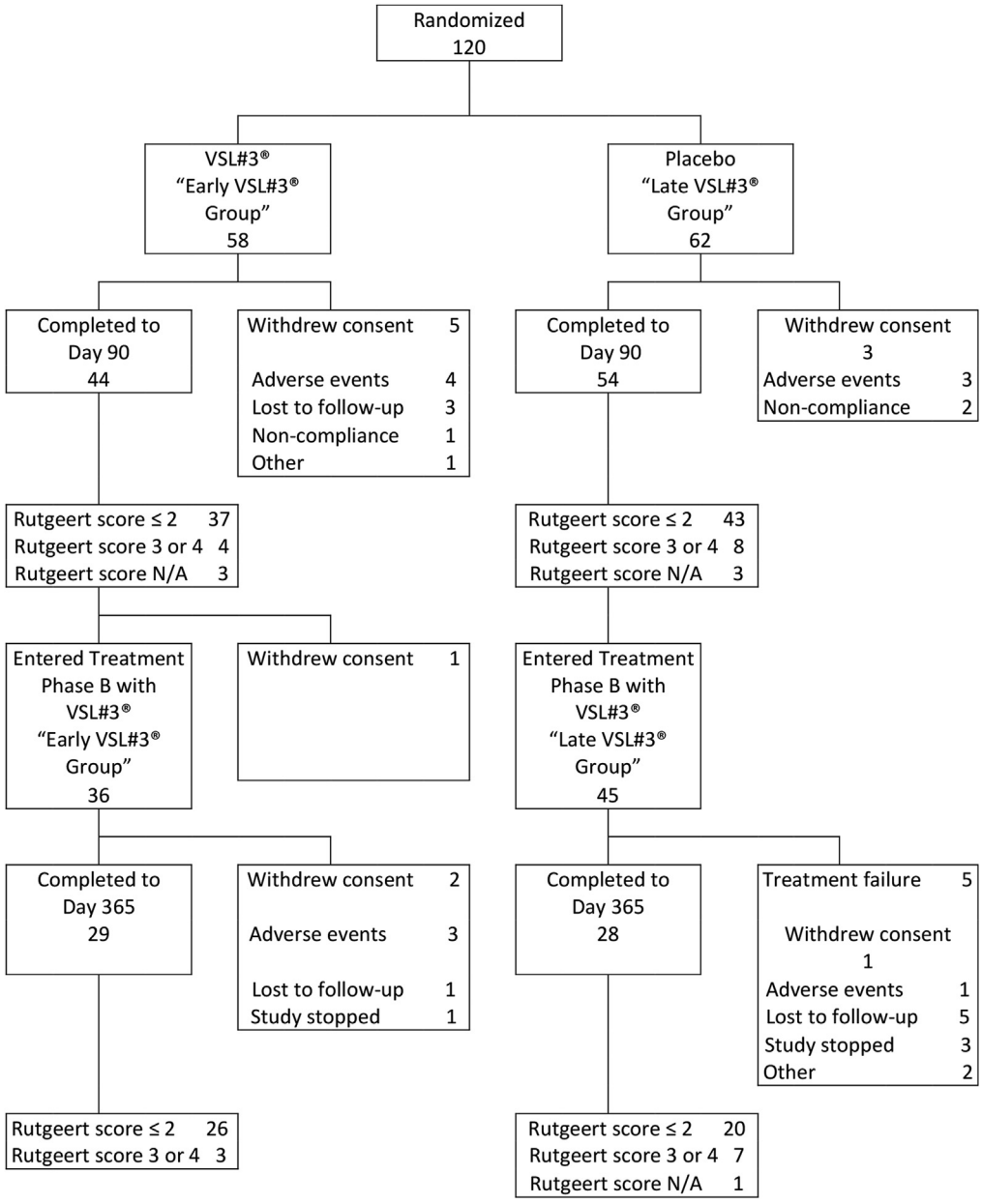
Eligible subjects were assigned to 1 of 2 treatment groups in a 1:1 ratio by random allocation that was based on a computer-generated randomization schedule prepared before the study by Robarts Inc. Randomization was stratified by study center. The site investigator, study coordinator, and patient were blinded to the treatment allocation during double-blind treatment days 1–90.

## Sample Size Determination

The primary efficacy parameter used to determine sample size was the proportion of patients with severe endoscopic recurrence of Crohn's disease after 90 days of study drug treatment. Rutgeerts et al[25] reported the rate of severe endoscopic recurrence to be 43% at 3 months after surgery. Under the assumption that the rate of severe endoscopic recurrence in placebo-treated patients would be 45%, 52 evaluable patients per group were required to detect an absolute difference of 25% (ie, 20% rate of severe endoscopic recurrence in patients treated with VSL#3) at the .05 level of significance with 80% power. Consequently, a total of 120 patients were enrolled, allowing for a nonevaluable rate of up to 13%.

## Statistical Analysis

Demographic and baseline characteristics of the patients were analyzed by using descriptive methods. The primary efficacy analysis compared the proportions of patients with severe endoscopic recurrence within 90 days after surgery between the VSL#3 and placebo groups by means of the $\chi^2$ test among patients who had endoscopic evaluation performed. A sensitivity analysis was also performed to compare the proportion of patients who were free of severe endoscopic recurrence at day 90, that is, patients who did not have an endoscopic evaluation done were considered treatment failures. The primary efficacy analyses were performed according to the intent-to-treat principle. For secondary efficacy parameters, the following analyses were applied: the proportion of patients with any endoscopic recurrence also was analyzed by the $\chi^2$ test for double-blind phase (90 day). The proportion of patients experiencing drug-related adverse events during the placebo-controlled phase of the trial was assessed by using Fisher exact test. Descriptive analysis was used for open-phase outcomes such as severe endoscopic recurrence of Crohn's disease (Rutgeerts grade 3 or 4) during the 1-year follow-up period in subjects who received early (within 30 days after resection) versus late (>90 days after resection) VSL#3. All statistical tests were 2-sided and were performed at the .05 level of significance.




**Supplementary Figure 1.** Study participant flow.

**Supplementary Table 1.** Adverse Events

| | VSL#3 n (%) | Placebo n (%) |
|---|---|---|
| Double-blind treatment phase A (days 0–90) | | |
| All | 31 (53.4) | 40 (64.5) |
| Related (probable) | 4 (6.9) | 5 (8.1) |
| Serious | 1 (1.7) | 5 (8.1) |
| Deaths | 0 | 0 |
| Withdrawals | 5 (8.6) | 5 (8.1) |
| Open-label treatment phase B (days 91–365) | | |
| All | 28 (48.3) | 27 (43.5) |
| Related (probable) | 0 | 1 (1.6) |
| Serious | 0 | 1 (1.6) |
| Deaths | 0 | 0 |
| Withdrawals | 2 (3.4) | 6 (9.7) |