# SYN-010, a Proprietary Modified-Release Formulation of Lovastatin Lactone, Lowered Breath Methane and Improved Stool Frequency in Patients with IBS-C: Results of a Multi-Center Randomized Double-Blind Placebo-Controlled Phase 2a Trial

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## ABSTRACT

**BACKGROUND:** Observational studies show a strong association between delayed intestinal transit and intestinal methane production. Experimental data suggest a direct inhibitory activity of methane on the colonic and ileal smooth muscle. The archaeon *Methanobrevibacter smithii* is the predominant methanogen in the human intestine. Certain statins can inhibit archaeal methane production without affecting other gut organisms as demonstrated in livestock and humans. The aim of this trial (NCT02495623) is to assess the efficacy of a proprietary modified-release lovastatin lactone in lowering intestinal methane production.
**METHODS:** Patients with IBS-C and a breath methane value > 10 parts per million (ppm) at screening were randomly assigned to receive placebo, SYN-010 dose 21 mg or SYN-010 dose 42 mg orally once daily for 28 days. The primary endpoint was the change from baseline in the area under the curve (AUC) of breath methane production at Day 7, based on a 180-minute lactulose breath test (LBT). Secondary efficacy assessments included change in methane AUC at Day 28, stool frequency and consistency, abdominal pain, bloating, and safety data. A necessary normalization of the severely left-skewed breath test data, not prespecified in the statistical analysis plan, was accomplished by square root transformation and paired t-tests were performed allowing each patient to serve as their own control. The analyses of the clinical outcomes were performed with untransformed raw data.
**RESULTS:** 63 Patients were enrolled in the trial. After 7 days statistically significant (SS) reductions in breath methane levels were seen in the 42 mg dose group (p = 0.02 but not the 21 mg dose group (p=0.64). In contrast, after 28 days both dose groups, 21 mg and 42 mg, showed SS reductions (Δ) of breath methane levels, except for placebo (Table in published abstract)). Placebo Δ = – 31.0, p= 0.15, SD = 74.3; 21 mg dose Δ = – 22.6, p = 0.03, SD = 55.0; 42 mg dose Δ = -34.3, p=0.01, SD = 59.0. Unit: ppm*hours. Paired t-test. Percentage of weekly abdominal pain intensity and stool frequency responses are shown in the body of the poster. The definition of these outcomes are consistent with the FDA IBS guidance. A SS improvement in the stool frequency response for the 21 mg dose group was apparent. The 42 mg group was numerically better (Figure 4). No serious adverse events were observed.
**CONCLUSION:** This is one of the first trials to target a specific component of the intestinal microbiome. It shows that SYN-010 can reduce intestinal methane production as measured by breath test in patients with IBS-C. This study was not powered to show improvement in clinical parameters. The unexpected finding of statistically significant improvement in stool frequency response for the 21 mg dose group and encouraging trends in other clinical parameters are therefore particularly noteworthy. Further development of SYN-010 appears warranted.

## BACKGROUND

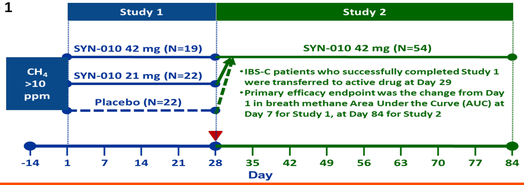
There is an association between irritable bowel syndrome with constipation (IBS-C) and high breath methane levels, and there is experimental evidence suggesting that methane may slow down intestinal transit. Statins, specifically lovastatin lactone, have been shown to lower methanogenesis in human stool samples. In addition, recent research examining the effects of lovastatin in a rat model of diet-induced constipation and *M. smithii* proliferation showed that gavage with lovastatin significantly reduced the ratio of *M. smithii* to total bacteria in the ileum without altering the architecture and composition of overall colonic flora. Most importantly, there was an increase in stool wet-weight (a proxy for soft stools in rats) noted in rats receiving lovastatin lactone gavage. We developed SYN-010, which is a proprietary modified-release formulation of lovastatin lactone that achieves high concentrations in the intestinal tract with low systemic exposure.

## CLINICAL TRIAL DESIGN

This phase 2 trial consisted of two studies, Study 1 and 2. Study 1 was a 4-week long double-blind, parallel-group, placebo-controlled study in patients with IBS-C at doses of 21 mg and 42 mg of SYN-010. The primary objective of this study was to evaluate the change from baseline in breath methane ($CH_4$) in patients with IBS-C as determined by a full lactulose breath test (LBT), after 7 days of treatment with SYN-010 compared with placebo. In order to qualify for the study, patients needed to have a spot (single-sample) methane breath test > 10 ppm upon screening.

One of the secondary objectives of Study 1 was to evaluate the changes from baseline in the number and consistency of bowel movements (BMs) and abdominal pain and bloating at 1, 2, and 4 weeks of study treatment with SYN-010 compared with placebo. Study 2 was an open-label extension of Study 1, where all patients received 42 mg SYN-010 for 8 weeks. Studies 1 and 2 were exploratory in nature and p-values, if mentioned, are nominal.

**Figure 1**

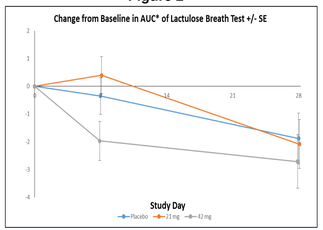



## RESULTS

Demographics and baseline characteristics were well balanced among the groups.

| Table 1 | Treatment Group | | |
|---|---|---|---|
| | Placebo (N=22) | 21 mg (N=22) | 42 mg (N=19) |
| **Age, mean (range)** | 46.4 (32-62) | 42.6 (34-57) | 44.7 (25-62) |
| **Female, n (%)** | 17 (77.3%) | 19 (86.4%) | 14 (73.7%) |
| **Race, n (%)** | | | |
| White | 16 (72.7%) | 21 (95.5%) | 15 (78.9%) |
| Black or African American | 4 (18.2%) | 1 (4.5%) | 4 (21.1%) |
| Other | 2 (9.1%) | 0 | 0 |

**Figure 2**



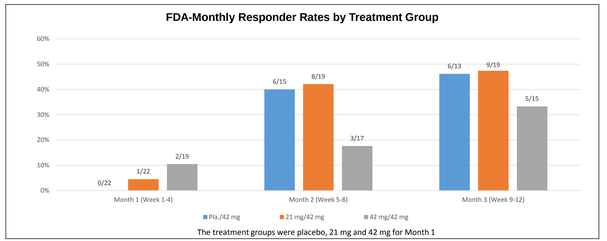

**Study 1**: **Methane AUC in response to intervention (placebo, SYN-010 21 mg and 42 mg).** At Day 7, there was a significant reduction in the AUC in the 42 mg group with relatively small nominal p-values, and no reduction was observed in the 21 mg group. At Day 28, there was a trend in the reduction of the AUC in the breath methane for both active treatment groups (Figure 2).

The Least Square mean and Standard Error are based on an ANCOVA model compared with the placebo group, AUC (methane) is transformed by square root due to skewed distribution of the data. For an analysis based on paired-t tests, see abstract of this poster.

| Table 2 | AUC Methane per Treatment Group | | |
|---|---|---|---|
| **Study Day** | **Pla./42mg (N=17)** | **21 mg/42mg (N=20)** | **42mg/42mg (N=17)** |
| **Baseline** Mean (SD) | 58.4 (53.7) | 88.1 (77.3) | 88.8 (59.9) |
| **Day 28** | N = 16 | N = 20 | N = 17 |
| Mean (SD) | 50.4 (44.2) | 65.4 (83.1) | 54.5 (57.1) |
| **Day 84 (on 42 mg all groups)** | N = 14 | N = 18 | N = 16 |
| Mean (SD) | 33.3 (34.2) | 63.9 (75.7) | 73.8 (70.6) |
| Change from Baseline(SD) | -20.7 (40.5) | -29.9 (60.2) | -4.69 (62.7) |
| p-value* | 0.078 | 0.050 | 0.769 |
| p-value** | 0.084 | 0.018 | 0.359 |
| Change from Day 28(SD) | -23.2 (42.7) | -7.9 (51.4) | 23.3 (51.9) |
| p-value* | 0.063 | 0.523 | 0.093 |
| p-value** | 0.070 | 0.777 | 0.040 |

**Table 2 - Studies 1 and 2:** In the placebo/42 mg group (started in Study 1 on placebo, switched to 42 mg in study 2) at Day 84, there was significant reduction in the methane AUC from both baseline and Day 28 with small nominal p-values. In the 21 mg/42 mg group, at Day 84, there was a significant reduction in the methane AUC from baseline, but no obvious reduction from Day 28. In the 42 mg/42 mg group, at Day 84, no obvious reduction was observed from baseline, and there was an increase from Day 28. P-values were from a paired t-test assessing the within-treatment group change from baseline or Day 28. The rows with '*' were based on the raw data, and rows with '**' were based on the transformed data with the square root transformation.

**Figure 3**




**Monthly Responder** is an FDA-defined composite measure incorporating improvements in CSBMs and abdominal pain. A Monthly Responder is defined as a patient who had a weekly response in at least 50% of the weeks of treatment during the month. A Weekly Responder is defined as a patient who experienced a decrease in weekly average score for worst abdominal pain in the past 24 hours of at least 30% compared with baseline and a stool frequency increase of 1 or more Complete Spontaneous Bowel Movements (CSBMs) per week compared with baseline.
In Study 1, there was an increase in the percentage of patients identified as Monthly Responders in the 21 mg and 42 mg groups, when compared with the placebo group. In Study 2, the percentages of monthly responders further increased when patients were on 42 mg SYN-010 with some exceptions (Figure 3).

**Figure 4**

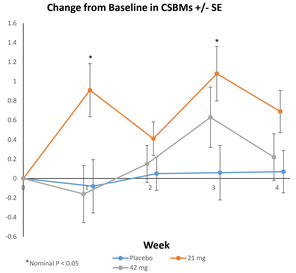
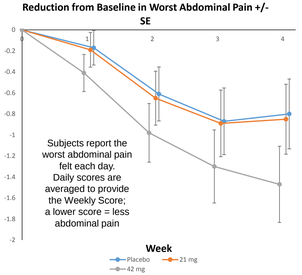



**Figure 5**




**Weekly Changes in Study 1**: Figures 4 and 5 show change from baseline in weekly average CSBM and abdominal pain score over time, respectively. There appeared to be an improvement (increase) in the weekly average CSBMs for the two active treatment groups. There was also a trend for the reduction of the abdominal pain score over time for the two active treatment groups. Least-squares mean changes are based on an ANCOVA at each week.

| Table 3 | Percent of (n/N) Patients With Response | | |
|---|---|---|---|
| **Study Week** | **Placebo/ 42 mg** | **21 mg / 42 mg** | **42 mg / 42 mg** |
| | **Increase of ≥1 CSBM per Week vs Baseline** | | |
| Week 4 | 24% (4/17) | 40% (8/20) | 29% (5/17) |
| Week 8 | 47% (7/15) | 58% (11/19) | 31% (5/16) |
| Week 12 | 57% (8/14) | 78% (14/18) | 31% (5/16) |
| | **≥30% Decrease in Abdominal Pain Score vs Baseline** | | |
| Week 4 | 25% (4/16) | 25% (5/20) | 44% (7/16) |
| Week 8 | 50% (6/12) | 53% (10/19) | 60% (9/15) |
| Week 12 | 83% (10/12 ) | 41% (7/17) | 64% (9/14) |
| | **≥30% Decrease in Bloating Score vs Baseline** | | |
| Week 4 | 25% (4/16) | 35% (7/20) | 50% (8/16) |
| Week 8 | 50% (6/12) | 63% (12/19) | 67% (10/15) |
| Week 12 | 83% (10/12) | 76% (13/17) | 71% (10/14) |

**Response Rates:** At the conclusion of Study 1, patients over the next 8 weeks were on open-label 42 mg SYN-010. Response rates appeared to increase over time for all the 3 endpoints. The response rates further increased when the patients were transferred from placebo or 21 mg group to 42 mg group (Table 3).

**Safety**: No serious adverse events were observed. The few treatment emergent adverse events observed were typically mild and comparable among the treatment groups. Particularly, diarrhea was rare, and, if present, mild.

## CONCLUSIONS

- No SAEs were oberved in Study 1 and Study 2. The few treatment emergent AEs were balanced between groups, and diarrhea was rare, and, if present, mild.
- A numerical reduction of breath methane values was seen in Study 1 and confirmed in Study 2 when placebo patients were switched to SYN-010 at 42 mg.
- Study 1, while not powered to show differences in clinical parameters, nevertheless showed reductions in abdominal pain and improvements in Monthly Responders and CSBMs with a noticeable dose and/or duration effect.
- These favorable trends continued in Study 2 (open-label).
- Further development of SYN-010 appears warranted, and a Phase 3 study is planned.

## DISCLOSURES, REFS. & ACKGTS.

KG, JS, OC, and HM are employees of Synthetic Biologics, Inc. VW is a consultant to Synthetic Biologics. MP is a member of the Synthetic Biologics' IBS Advisory Board. The authors thank Dr. Chenxiong (Charles) Le for statistical advice.
References:
1. Gottlieb K, Wacher V, Sliman J, Pimentel M. Review article: inhibition of methanogenic archaea by statins as a targeted management strategy for constipation and related disorders. Alimentary pharmacology & therapeutics. 2016 Jan 1;43(2):197-212.
2. Morales W, Marsh E, Yu A, Marsh Z, Weitsman S, Barlow GM, Rezaie A, Chang C, Wacher V, Pimentel M. Lovastatin improves stool form in *Methanobrevibacter smithii* colonized rats with constipation. Gastroenterology 2015 Apr 1;148(4):S779-S780.