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, 4-week acute Phase 2a and an 8-week follow-on extension Phase 2 study

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A growing body of clinical and preclinical evidence has demonstrated that elevated intestinal methane production reduces motility and is an underlying cause of symptoms in irritable bowel syndrome with constipation (IBS-C)

Methane in humans is produced almost entirely by Methanobrevibacter smithii (M. smithii), an archeon that predominantly resides in the colon but is also found in the small intestine of some patients; intestinal methane production can be estimated using a non-invasive methane breath test

Methane production by M. smithii in human stool is inhibited by the lactone form of lovastatin, but not by its cholesterol-lowering β-hydroxyacid metabolite, indicating a distinct mode of action for lovastatin lactone as an IBS-C therapy

Lovastatin lactone and its β-hydroxyacid metabolite were not microbicidal in rats, suggesting that lovastatin can exert its antimethanogenic effect while avoiding significant perturbations to the intestinal microbiome

SYN-010 is a proprietary, modified-release formulation of lovastatin lactone intended to act in the intestine to reduce methane production and alleviate symptoms in patients with IBS-C.

The SYN-010 modified-release capsule is designed to avoid drug release in the stomach (reducing conversion of methane-inhibiting lovastatin lactone to the non-inhibiting β-hydroxyacid) then deliver different pulses of lovastatin lactone to the small and large intestine in proportion with the anticipated levels of *M. smithii* in each location (Figure 1).

Systemic absorption of lovastatin is not required for the treatment of IBS-C: release of lovastatin lactone lower in the intestinal tract, and reduced conversion of the more poorly-absorbed lovastatin lactone to the more readily-absorbed β-hydroxyacid, are expected to decrease systemic exposure to lovastatin species.

**Figure 1:** Dual pulse release profile of SYN-010 modified-release lovastatin lactone capsules. The release profile was evident in a pharmacokinetic study in healthy volunteers, where continued drug release over 24 h (well into the colon) was also observed.
Sixty-three (63) IBS-C patients with high breath methane (CH$_4$ >10 ppm) at Screening were enrolled in a multicenter, randomized, controlled, double-blinded clinical trial (RCT) in which they received SYN-010 21 mg, SYN-010 42 mg or Placebo once daily for 4 weeks.

Fifty-four (54) subjects who completed Study 1 continued into an open-label extension (EXT) in which all subjects received SYN-010 42 mg once daily for an additional 8 weeks.

Breath methane production was measured using a lactulose breath test at baseline (day 1), then days 7, 28, 35 and 84 (Figure 2).

**Figure 2**
Phase 2a clinical trials of SYN-010.
- ClinicalTrials.gov identifiers: NCT02495623 (RCT) and NCT02493036 (EXT).
- Days where a lactulose breath test was conducted are indicated with a triangle (▼).
Objectives

• Primary objective
  • **RCT:** Determine the effects of SYN-010 on intestinal methane production by measuring changes in breath methane AUC from baseline to day 7 using a lactulose breath test (Figure 3).
  • **EXT:** Evaluate the sustainability of SYN-010 42 mg effects on breath methane production.

• Secondary objectives
  • **RCT and EXT:** evaluate potential changes from baseline in IBS-C clinical symptoms¹:
    • Weekly number of complete spontaneous bowel movements (CSBM), and
    • Weekly average worst abdominal pain score and weekly average bloating score.

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**Figure 3**
Exemplary breath methane and hydrogen concentration vs time profiles during a lactulose breath test.

- Subjects followed a restricted diet in the days preceding the test, then fasted for at least 12 h prior to providing the first breath sample.
- Breath was collected by exhaling into a coated test tube and samples analyzed by gas chromatography; time 0 samples were taken prior to ingestion of a lactulose solution.
- Area under the concentration vs time curve (AUC) was calculated using the linear trapezoidal method.
- Breath hydrogen levels increase as the lactulose is metabolized by intestinal bacteria; however, breath methane levels tend to remain stable during the test.

¹Studies were not prospectively powered for formal statistical evaluation of clinical endpoints.
RESULTS

• Study cohorts were well-matched demographically, although Cohort 2 had a lower average weekly number of CSBMs at baseline than both Cohorts 1 and 3 (Table 1).

• SYN-010 was well-tolerated over 12 weeks of treatment and the few reported adverse events were all of mild or moderate intensity (Table 2).
  • No serious adverse events were reported and no drug-related diarrhea was observed.

• Breath methane AUC was highly variable, but was reduced from baseline in SYN-010 treatment groups, with greatest effects observed for SYN-010 42 mg (Figure 4)¹.

• Weekly number of CSBMs was increased from baseline in the SYN-010 21 mg treatment group during the RCT (P<0.05 vs Placebo) and in subjects transferred from Placebo to SYN-010 42 mg in the EXT (Figure 5)¹.
  • Regression analysis for all subjects who completed the EXT demonstrated an inverse correlation between breath methane AUC and weekly number of CSBMs (Figure 6).
  • Less rescue medication was used in SYN-010 treatment groups than the Placebo group during the RCT (Figure 7).

• Abdominal Pain scores (Figure 8) and Bloating scores (Figure 9) were reduced from baseline in SYN-010 treatment groups, with greatest reductions observed for SYN-010 42 mg during the RCT and subjects transferred from Placebo to SYN-010 42 mg in the EXT¹.

¹Statistical P values, where reported, are nominal
# Subject Demographics

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>COHORT 1</th>
<th>COHORT 2</th>
<th>COHORT 3</th>
</tr>
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<tbody>
<tr>
<td><strong>Study</strong></td>
<td>RCT</td>
<td>EXT</td>
<td>RCT</td>
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<tr>
<td>SYN-010 dose</td>
<td>Placebo</td>
<td>42 mg</td>
<td>21 mg</td>
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<tr>
<td>Dosing period, weeks</td>
<td>4</td>
<td>8</td>
<td>4</td>
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<tr>
<td>No. Subjects (Female)¹</td>
<td>22 (17)</td>
<td>17 (13)</td>
<td>22 (19)</td>
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<tr>
<td>Study Drug Compliance</td>
<td>99.2%</td>
<td>98.6%</td>
<td>97.9%</td>
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<td><strong>Baseline Parameters (Day 1)</strong></td>
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<tr>
<td>Age (years)</td>
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<td>42.6±6.0</td>
<td>44.7±9.5</td>
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<tr>
<td>White/Black-African Amer./Other²</td>
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<td>BMI (kg/m²)</td>
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<td>Breath methane (ppm)</td>
<td>25.3±18.7</td>
<td>24.9±26.2</td>
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<td>No. CSBMs per week</td>
<td>0.41±0.73</td>
<td>0.27±0.63</td>
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<td>Bristol Stool Form Scale³</td>
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<td>1.63±0.99</td>
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<td>Abdominal Pain Score (0-10)³</td>
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<td>Bloating Score (0-4)³</td>
<td>2.44±0.65</td>
<td>2.52±0.76</td>
<td>2.46±0.56</td>
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</table>

¹RCT completers were eligible to continue into the EXT, no new subjects were enrolled in the EXT.  
²Over 90% of subjects identified as Hispanic. ³Weekly average. Data are mean±SD unless indicated.
## Table 2: Treatment emergent adverse events (TEAEs), serious adverse events (SAEs) and withdrawals

<table>
<thead>
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<tr>
<td>Study</td>
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<td>EXT</td>
<td>RCT</td>
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<tr>
<td>SYN-010 Dose</td>
<td>Placebo</td>
<td>42 mg</td>
<td>21 mg</td>
</tr>
<tr>
<td>Enrolled (n)</td>
<td>22</td>
<td>17</td>
<td>22</td>
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<tr>
<td>Withdrew (n)</td>
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<tr>
<td>Reported TEAE (n)</td>
<td>1</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Reported SAE (n)</td>
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</table>

### Description of TEAE (Relationship to Treatment)

- **RCT (4 weeks)**
  - 01 Gastroenteritis (unlikely)
  - 04 Headache (probable)
  - 05 Intermittent rectal bleeding (unrelated)
  - 07 Elevated GGT (probable)
  - 08 Elevated AST creatine kinase (possible)

- **EXT (8 weeks)**
  - 02 Diarrhea (unrelated)²
  - 03 Elevated ALT AST ALP LDH GGT (unlikely)³
  - 05 Proctitis (unrelated)
  - 06 First degree AV block (unrelated)
  - 07 Elevated creatine kinase (unrelated)

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¹Numbers are Subject ID: TEAEs were all of mild or moderate intensity.
²Commenced after last dose of study drug. ³Resulted in withdrawal from the study.
Figure 4: Change in breath methane AUC (ppm*h) from day 1 baseline (mean±SEM; mITT population). Open bars represent the RCT and closed bars represent the EXT. Statistical tests were performed on square root transformed values to account for a strong left skew of the data\textsuperscript{1}.

\textsuperscript{1}Nominal P values for within-group change from day 1 (paired t-test): \textsuperscript{†}P<0.05, \textsuperscript{‡}P<0.005
**SYN-010 Increased Weekly No. CSBMs**

**Figure 5:** Change in weekly number of CSBMs from day 1 baseline (mean±SEM; mITT population). Open bars represent the RCT and closed bars represent the EXT. BMs (if any) were reported by subjects each day in an electronic diary¹.

¹Nominal P value for SYN-010 vs Placebo (mixed effect model): **P<0.05

¹Nominal P values for within-group change from day 1 (paired t-test): †P<0.05, ‡P<0.005, §P<0.0005
Lower Methane AUC Correlated with More BMs

**Figure 6:** Inverse correlation between breath methane AUC and the weekly number of CSBM and SBMs (inset) for all subjects who completed the EXT (at least 8 weeks of SYN-010 42 mg; mITT population). Lower breath methane correlated with more bowel movements.

¹Line represents least-squares linear regression modeling
Less Rescue Medication Use in SYN-010 Groups

Figure 7: Subjects in SYN-010 treatment groups used less rescue medication (bisacodyl) than the Placebo group (RCT study; mITT population). Rescue medication use was reported by subjects each day in an electronic diary.¹

¹Nominal P values for Fisher’s exact test vs Placebo
SYN-010 Reduced Abdominal Pain Score

Figure 8: Percentage change in weekly average worst abdominal pain score (mean±SEM; mITT population). Open bars represent the RCT and closed bars represent the EXT. Pain was reported by subjects each day in an electronic diary using an 11 point scale (0-10)¹.

¹Nominal P values for within-group change from day 1 (paired t-test): †P<0.05, ‡P<0.005, §P<0.0005
SYN-010 Reduced Bloating Severity

Figure 9: Percentage change in weekly average bloating score from day 1 baseline (mean±SEM; mITT population). Open bars represent the RCT and closed bars represent the EXT. Bloating was reported by subjects each day in an electronic diary using a 5 point scale (0-4).¹

¹Nominal P values for within-group change from day 1 (paired t-test): †P<0.05, ‡P<0.005, §P<0.0005
CONCLUSIONS

• The SYN-010 modified-release formulation of lovastatin lactone was designed to reduce intestinal methane production, thereby treating an underlying cause of constipation in IBS-C.

• Daily doses of SYN-010 were well-tolerated by IBS-C patients over the 12 week treatment period (at least 8 weeks of SYN-010 42 mg). No SAEs were reported and there were no incidences of drug-related diarrhea, which is an important potential benefit of SYN-010 as an IBS-C therapy.

• Breath methane was reduced relative to baseline in SYN-010 treatment groups and lower breath methane levels correlated with increased number of CSBMs, consistent with the proposed methane-inhibiting action of lovastatin lactone.

• Although these studies were not prospectively powered for formal statistical evaluation of clinical endpoints, compelling improvements in CSBMs, abdominal pain, and bloating were observed in SYN-010 treatment groups.

• These results validate the need to evaluate optimal dosing of SYN-010 in a larger patient population and a Phase 2b/3 clinical trial of SYN-010 is in development.
SYN-010 Seeking Normalization of Bowel Habits

- CONSTIPATION 😞: Laxatives Lubiprostone Linaclotide
- NORMAL 😊: SYN-010
- DIARRHEA 😥: Laxatives Lubiprostone Linaclotide

☑ Treating the Cause