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Development of a Modified-Release Formulation of Lovastatin Targeted to Intestinal Methanogens Implicated in Irritable Bowel Syndrome With Constipation

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ABSTRACT

There is growing evidence that methane production, predominantly by *Methanobrevibacter smithii*, in the intestines is a cause of constipation, pain, and bloating in irritable bowel syndrome with constipation (IBS-C). *M smithii* resides primarily in the large intestine but can also colonize the small intestine. *In vitro* studies found that the prodrug lactone form of lovastatin, found in cholesterol-lowering drugs, inhibited methane production in stool samples from patients with IBS-C. However, the cholesterol-lowering lovastatin β-hydroxyacid was ineffective at inhibiting methane production in this system. A considerable amount of lovastatin is converted to hydroxyacid in the stomach and is absorbed. It was hypothesized that galenic innovations could protect lovastatin from the stomach and allow release in 2 strategic locations, the duodenum and the ileocecal region, to reach *M smithii*. The desired release profile was achieved by developing an oral dosage form containing lovastatin and coated with 2 different enteric polymers that enabled a pH-dependent "dual pulse" drug release. Combinations of the 2 coated tablets were encapsulated together to deliver the desired amount of lovastatin to the targeted intestinal locations. The capsules have been tested *in vitro* and *in vivo* and show promise in treating IBS-C.

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder characterized by recurring abdominal pain or discomfort. The onset is associated with a change in frequency and/or a change in form (appearance) of stool, and relief is associated with defecation.[1] IBS with constipation (IBS-C) is a subtype associated with predominantly hard or lumpy stool and a low frequency of loose or watery stool bowel movements.[2,3] The prevalence of IBS is estimated at 3% to 20% of the United States adult population; it affects about twice as many women as men and is most common in people younger than 45 years of age.[4-7] The distribution of IBS subtypes is highly influenced by the nature of patient reporting; however, a systematic evaluation found that IBS-C comprises approximately one-third of the reported IBS cases.[6,8] IBS-C has a negative impact on health-related quality of life and contributes to high health care costs.[9] Many theories have been put forward but the exact causes and pathophysiology of IBS are still uncertain.[10]

There are few desirable therapies for IBS-C. A combination of rare adverse effects, which is often diarrhea, and a very low tolerance of adverse effects have led to the withdrawal or restriction of some effective therapies.[10,11] Many new potential therapeutics do not have a defined mechanism of action (e.g., prebiotics and probiotics) and are often poorly characterized and highly variable in composition, making it a particular challenge to measure any consistent or reproducible benefit in clinical practice. Recent interest has therefore focused on drugs whose actions are confined to

Conflict of interest: Steve Hubert is the Senior Director of Manufacturing at Synthetic Biologics, Inc. Andrew Bristol is Vice President Development at Synthetic Biologics, Inc. Olivia Coughlin is Vice President, Product Development at Synthetic Biologics, Inc. Alan Chadwick was Formulation Technical Manager at Aesica during this study and is now Formulation Technical Manager at Boots UK. John Kokai-Kun is Vice President of Non-Clinical Affairs at Synthetic Biologics, Inc. Vince Wacher is Synthetic Biologics' Product Development Consultant.

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the intestinal lumen with a perceived low likelihood of unwanted systemic effects and have a compelling mechanism of action.

Lovastatin, a fungal metabolite, was originally approved for human use as a cholesterol-lowering agent by the U.S. Food and Drug Administration in 1987 (Mevacor®; Merck). The natural product, lovastatin, is not itself a cholesterol-lowering agent; rather, it is converted *in vivo* to its hydroxyacid (HA) form, which inhibits 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and blocks cholesterol biosynthesis.[12] It has long been understood that intestinal microorganisms produce gases including methane.[13] Methane production *in vivo* is due to methanogenic archaea, with the predominant methanogen in humans being *Methanobrevibacter smithii*.[14] There has been rapid development in our understanding of the relationship between the gut microbiome and health, including the influence of *in vivo* production of methane.[15] A growing body of preclinical and clinical evidence has found that methane, which can be measured in the breath,[16] slows intestinal transit and elevated intestinal methane production is associated with symptoms of IBS-C.[15,17] Lovastatin has been shown to inhibit methane production in methanogenic microorganisms *in vivo*[18] and *in silico* docking simulations are consistent with lovastatin having its antimethanogenic effect through direct inhibition of enzymes in the archaeal methanogenesis pathway.[19] In a comparative study of the effects of statins on methane production by *M smithii* in stool from IBS-C patients, lovastatin reduced methane production, whereas the HA form of lovastatin and all other statins tested were ineffective.[20]

The evidence of a causative relationship of methane to IBS-C symptoms and the ability of lovastatin to inhibit methane production provided compelling scientific justification for developing a modified-release lovastatin as a therapeutic agent for IBS-C. This drug reformulation and repurposing strategy was further supported by the possibility to draw on previous lovastatin's safety data available in: (1) Mevacor® product information, (2) nonclinical, clinical, and postmarketing data generated for Mevacor® tablets, and (3) the published literature.[21] The investigational drug formulation to treat IBS-C has been designated SYN-010. Considering that patients suffering from IBS-C may not desire the systemic cholesterol-lowering effect of HA and knowing that HA is ineffective as an inhibitor of methane production, development of SYN-010 modified-release lovastatin was thus based on 3 key guiding principles: (1) minimizing presystemic conversion of lovastatin to HA, (2) delivery of lovastatin to the sites in the intestine where the methanogens reside, and (3) reducing systemic exposure to lovastatin species.

Mevacor® is an immediate-release tablet formulation of lovastatin. The prodrug form is released in the stomach where the soluble form undergoes a reversible reaction between the lactone and HA forms. Conversion and absorption of both forms occurs throughout the GI tract. Both forms reach the portal circulation and are extracted by the liver. The HA form and the lactone form, after enzymatic biotransformation to the HA form, inhibit cholesterolgenesis.[22] Bioavailability data reported by Aura Laboratories (Hackensack, NJ) indicated that following administration of a single dose of Mevacor® (40 mg) to fasted healthy volunteers, plasma concentrations of lovastatin peaked at 2 h with highest plasma levels of lovastatin HA observed around 4 h.[23] Detectable plasma levels are observed very quickly after Mevacor® administration,[24] and detailed preclinical studies have confirmed that absorption of lovastatin from the stomach can be rapid and extensive.[22] After administration of oral [^{14}C] lovastatin, 83% of the total administered radioactivity is recovered in the feces of human patients.[22] This suggests that protecting lovastatin from the stomach would help minimize presystemic hydrolysis of lovastatin, thereby reducing plasma concentrations of HA. Preclinical studies have indicated that

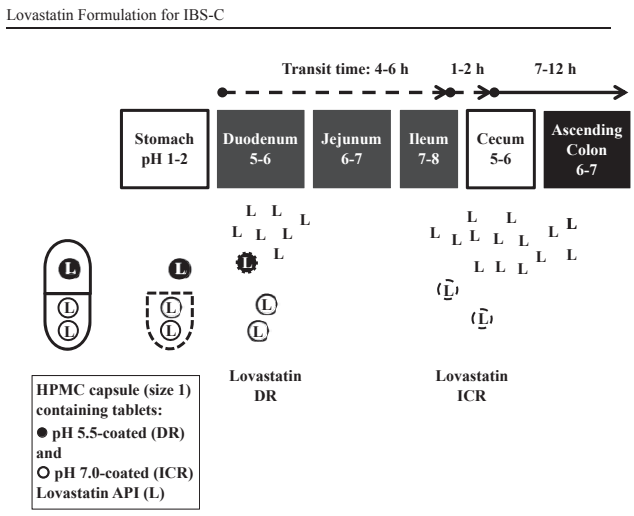

Figure 1. Intestinal delivery strategy using SYN-010 for IBS-C. Upon oral administration, the capsule shell was designed to dissolve in the stomach, and thus allow the tablets to pass into the small intestine. The duodenal release (DR) tablets were designed to dissolve in the small intestine at pH > 5.5. The ileocecal release (ICR) tablets were designed to transit down the small intestine and dissolve at the ileocecal region of the gastrointestinal tract at pH > 7.0.

lovastatin is more poorly absorbed than the HA,[22] so preservation of lovastatin should limit overall lovastatin absorption in addition to delivering more of the antimethanogenic lovastatin species to the intestinal lumen.

M smithii predominantly resides in the colon; however, low levels of *M smithii* have been observed in small intestinal aspirates from some patients.[25] Considering that the small intestine is a significant site of lovastatin absorption[26] and the objective is to limit the systemic absorption of lovastatin, it is was decided to release only a limited quantity of lovastatin in the small intestine with the bulk being released near the ileocecal junction. In order to address potential methane production in both regions of the intestine, while limiting lovastatin absorption, a “dual pulse” lovastatin release strategy was developed. It has been reported that the fasted small intestine contained a total volume of 43 ± 14 mL of resting water. Patients take capsules with approximately 240 mL (8 fluid ounces) of water. Twelve min after ingestion of water, small intestine water content rose to a maximum value of 94 ± 24 mL of water.[27] Lovastatin is effective in inhibiting the production of methane in human stool homogenates from 0.04 to 5.0 mg/g stool.[20] This would suggest a tablet of approximately 4 mg designed to release immediately after leaving the stomach would be effective in inhibiting the production of methane in the human small intestine. The tablet size of 7 mg was selected to provide some overage, while not supplying the amount of lovastatin expected to significantly modify the patient's lipid profile. A dose of 35 mg for the colon was selected based on the significantly larger quantity of resident methanogens.

In order to achieve the desired “dual pulse” release profile, it was decided to design a drug product format to begin releasing the 7 mg dose in the duodenum and the larger dose in the ileocecal region. The drug product strategy would also allow for dose escalation in either location of the GI tract. Commercial manufacturing considerations indicated that it would be desirable to have a direct compression (DC) tablet process for lovastatin tablets.[28] Since the target amount of the duodenal release formulation is a 7 mg dose per tablet, it would be operationally efficient to have a corresponding 7 mg tablet for release in the ileocecal region. This would allow a single common core tablet containing 7 mg. The tablets