

2018

# Protective Actions of Luminally Restricted 5-HT<sub>4</sub> Receptor Agonist in Dextran Sodium Sulfate Induced Colitis

ALISHA Anne LINTON  
*University of Vermont*

Follow this and additional works at: <https://scholarworks.uvm.edu/graddis>



Part of the [Neuroscience and Neurobiology Commons](#)

---

## Recommended Citation

LINTON, ALISHA Anne, "Protective Actions of Luminally Restricted 5-HT<sub>4</sub> Receptor Agonist in Dextran Sodium Sulfate Induced Colitis" (2018). *Graduate College Dissertations and Theses*. 894.  
<https://scholarworks.uvm.edu/graddis/894>

This Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks @ UVM. It has been accepted for inclusion in Graduate College Dissertations and Theses by an authorized administrator of ScholarWorks @ UVM. For more information, please contact [donna.omalley@uvm.edu](mailto:donna.omalley@uvm.edu).

PROTECTIVE ACTIONS OF LUMINALLY RESTRICTED 5-HT<sub>4</sub> RECEPTOR  
AGONIST IN DEXTRAN SODIUM SULFATE INDUCED COLITIS

A Thesis Presented

by

Alisha Anne Linton

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
Specializing in Neuroscience

May, 2018

Defense Date: March 15, 2018  
Thesis Examination Committee:

Gary M. Mawe, Ph.D., Advisor  
Sayamwong Hammack, Ph.D., Chairperson  
Margaret Vizzard, Ph.D.  
Rebecca Wilcox, M.D.  
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

## ABSTRACT

The 5-hydroxytryptamine receptor 4 (5-HT<sub>4</sub> receptor) is heavily expressed on colonic epithelial cells and has been targeted as a therapeutic for functional bowel symptoms and pain; however, adverse cardiac events related to 5-HT<sub>4</sub> agonist treatment limited their therapeutic use. Previous studies in the Mawe laboratory have demonstrated that intraluminal application of a 5-HT<sub>4</sub> agonist exerts protective epithelial actions in animal models of colitis, and accelerates recovery from colitis. The aim of this study was to test the effects of a lumenally restricted 5-HT<sub>4</sub> agonist in a mouse model of experimental colitis.

The lumenally restricted 5-HT<sub>4</sub> agonist (Takeda Pharmaceuticals; 10 mg/kg) was administered to mice during active dextran sodium sulfate (DSS) induced colitis. Colitis activity was evaluated using disease activity index, a fecal lipocalin-2 assay, and histological damage scoring. Epithelial proliferation and colonic motility were also measured as readouts of the potential protective actions and colonic function, respectively.

Oral gavage and intracolonic delivery of this lumenally restricted 5-HT<sub>4</sub> agonist had no detectable effect on recovery from colitis or colonic motility as compared to vehicle. Additionally, in positive control experiments, we failed to see an effect of the 5-HT<sub>4</sub> agonist, tegaserod, on colitis severity or colonic motility in any of the measures tested.

In conclusion, it is unclear if the lumenally restricted 5-HT<sub>4</sub> agonist has any effect on recovery from DSS colitis. Given inconsistencies with the model and lack of an effect of tegaserod, additional studies will be required, possibly involving different doses and time points, to fully assess the actions of this lumenally restricted compound in colitis recovery.

## **ACKNOWLEDGEMENTS**

Graduate school is hard. It is far more challenging than it is possible to convey, and I am beyond grateful that I had an amazing support system to help me through. First, my advisor, Gary Mawe. His continued support, guidance, and (at times) brutal advice, were crucial to my success. I would also like to thank all current and former members of the Mawe Lab – specifically Brigitte Lavoie, Stephanie Spohn, Estelle Spear, and Melody Haag. These four women are not only top notch role models for women in science, but also the kindest and most helpful lab members I could have asked for. The Mawe Lab cultivates a unique atmosphere, prioritizing the strength of the lab over individual accomplishments. It is a place that fosters collaboration and friendship, and where everyone gets a cake on their birthday.

Additionally, I would like to thank the Neuroscience Graduate Program for their support and guidance. Especially Carrie Perkins, for always listening to my ideas and for giving me advice whenever I asked. Without her wisdom and insight, I would not be where I am today. I would also like to thank Tony Morielli for boldly steering the NGP forward and providing mentorship to all who ask.

It should go without saying that I would like to thank my family. Their constant love and support from afar has been crucial to my success, and I am forever grateful for their understanding my need to continuously move farther north. I would also like to thank my partner, Jason, for his love, support, hugs and acceptance of my late-night rants about the trials and tribulations of science and graduate school. I would be amiss if I did not mention our dog Mira, for her unconditional love and constant reminder to not take

life too seriously. Lastly, I would like to thank all the friends I have made in Vermont that have made this place feel like home.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF FIGURES .....	v
LIST OF TABLES.....	vi
CHAPTER 1: LITERATURE REVIEW .....	1
I. Structure and Organization of the Gastrointestinal Tract.....	1
A. Organization of the Gastrointestinal Tract.....	1
B. Tissues of the Gastrointestinal System .....	4
II. Serotonin .....	7
A. History.....	7
B. Gastrointestinal Mucosal Serotonin.....	7
C. 5-HT <sub>4</sub> Receptor .....	8
D. Mucosal 5-HT <sub>4</sub> Receptor Actions.....	10
E. Epithelial 5-HT <sub>4</sub> receptors as a therapeutic target .....	12
III. Inflammatory Bowel Disease.....	14
A. Crohn's Disease .....	15
B. Ulcerative Colitis .....	16
C. Current Therapies.....	16
IV. Animal Models of Colitis.....	18
A. Dextran Sodium Sulfate.....	18
B. Trinitrobenzine Sulfonic Acid .....	19
C. Other Models of IBD: IL-10 knockout and Oxazolone colitis .....	20
V. Specific Aims.....	21
CHAPTER II: PROTECTIVE ACTIONS OF LUMINALLY RESTRICTED 5-HT <sub>4</sub> RECEPTOR AGONISTS IN DEXTRAN SODIUM SULFATE INDUCED COLITIS ..	30
I. Abstract.....	30
II. Introduction .....	31
IV. Results.....	38
V. Discussion .....	42
VI. Works Cited.....	47
CHAPTER III: FINAL CONCLUSIONS AND FUTURE DIRECTIONS .....	62
I. Summary and Conclusions .....	62
II. Future Directions.....	62
COMPREHENSIVE BIBLIOGRAPHY .....	67

## LIST OF FIGURES

Figure 1 .....	50
Supplemental Figure 1 .....	52
Supplemental Figure 2 .....	54
Supplemental Figure 3 .....	56
Supplemental Figure 4 .....	58
Supplemental Figure 5 .....	60

## LIST OF TABLES

Table 1. Drug and vehicle formulations for 15 day recovery paradigm. ....	34
Table 2. Details of disease activity index rubric. ....	35
Table 3. Scoring system for disease activity index from Wirtz et al. (2017).....	63

## **CHAPTER 1: LITERATURE REVIEW**

### **I. Structure and Organization of the Gastrointestinal Tract<sup>1</sup>**

#### **A. Organization of the Gastrointestinal Tract**

The gastrointestinal (GI) tract is a long tube that begins in the mouth and ends at the anus. Each part of the GI tract is specialized to serve the overall function of the organ system, which is to breakdown ingested food into absorbable molecules, and to absorb those molecules for use as nutrients. While the GI tract has specialized parts, the basic structure of the gut wall is maintained throughout the length of the organ system. The four tissue layers of the gut are the mucosa, submucosa, muscularis externa and serosa. Additionally, the gut contains its own nervous system, the enteric nervous system, that is organized as several sets of ganglionated plexi, and that serve to coordinate the movements and functions of the gut. The myenteric (Auerbach's) plexus is located within the muscularis externa and serves to control motor patterns, and the submucosal (Meissner's) plexus is located within the submucosa and helps to coordinate secretion and vasodilation. The enteric nervous system is highly specialized and autonomous, receiving only moderate autonomic input from the central nervous system via the vagus nerve, sacral spinal roots S2-4, and prevertebral ganglia (celiac, superior and inferior mesenteric ganglia) within the abdominal cavity.

Starting from the mouth, food first enters the oral cavity and is mechanically broken down by mastication, and enzymatically by salivary amylase. From here, food is

---

<sup>1</sup> Information within this section is textbook material. See Silverthorn DJ, BR; Ober, WC; Garrison, CW; Silverthorn, AC (2013) Human Physiology: and Integrated Approach. Pearson.

swallowed and descends the esophagus via peristalsis, passes through the lower esophageal sphincter and enters the stomach. The stomach serves to temporarily store food, further digest food using acids, lipases and peptidases, and control the rate at which food enters the duodenum. Specialized parietal cells are responsible for maintaining the acidic environment of the stomach by secreting hydrochloric acid. The acidic environment of the stomach is important not only for helping to break down food particles, but also to kill any potential bacterial hazards. This acid, along with the other enzymes and mechanical movement, begin the process of digestion, primarily breaking down large food particles to create a soupy substance known as chyme. Chyme exits the stomach via the powerful pyloric sphincter and enters the duodenum.

The duodenum is the first part of the small intestine and secretes alkaline mucus from Brunner's glands to neutralize the acidic pH of the semi-solid chyme exiting the stomach. Two accessory GI organs, the pancreas and the gallbladder, secrete their contents into the duodenum at the ampulla of Vater. The entrance of these substances is controlled by the small circular muscle that surrounds the ampulla, called the sphincter of Oddi. The pancreas synthesizes many digestive enzymes, and the gallbladder stores and concentrates bile which contains enzymes to help break down fats. The distal two regions of the small intestine are the jejunum and ileum, respectively. Their border is ill-defined, but differences can be noted in the thickness of the mucosa. The jejunum has a thicker, more folded mucosa, while the ileum has a thinner mucosa and the presence of Peyer's patches, seen as mounds under the epithelium. Peyer's patches are nodes of lymphatic tissue contained within the submucosal layer of the gut, and play an important role in

maintaining gut homeostasis. The main function of the small intestine is to further break down food particles into nutrients and then readily absorb those nutrients into the bloodstream. The mucosa of the small intestine has specialized folds to increase surface area, including mucosal folds (plica circulares in the jejunum), villi (finger-like projections of mucosa), and microvilli (on the luminal side of individual epithelial cells). These specialized folds result in a 20-30 fold increase in surface area, resulting in more efficient nutrient absorption. Additionally, peristalsis and segmentation help to mix and mechanically break down food particles. Peristalsis is a coordinated motor pattern that helps push contents forward through the gut. Segmentation occurs to help mix intestinal contents with the digestive enzymes, and to ensure that all chyme contacts the absorptive mucosa. The small intestine empties into the cecum, which is located at the base of the ascending colon. The ileocecal valve prevents retrograde movement of intestinal contents back into the small intestine.

The large intestine is the final portion of the gastrointestinal system and serves to manage waste excretion and fluid balance. The large intestine consists of the cecum, colon and rectum. In humans, the large intestine can be divided into four segments – the ascending colon, the transverse colon, the descending colon, and the sigmoid colon, which is directly followed by the rectum. In rodents, the colon has simply a distal and proximal part. The large intestine is noted for the prominent bands of longitudinal muscle running along the length of the colon called teniae coli. These bands of muscle are shorter than the length of the colon, which causes the colon to bunch. This bunching creates bulges called haustra. Additionally, the colon is histologically different than the small

intestine, containing no villi and instead having crypts of Lieberkühn, also called colonic glands. These glands contain predominately enterocytes, but also contain more mucus secreting goblet cells than the small intestine. Specialized Enteroendocrine cells are generally located in the bottom third of the colonic glands. Finally, the rectum is the last portion of the GI tract and connects to the anus. It stores intestinal contents until time for defecation.

## **B. Tissues of the Gastrointestinal System**

The wall of the GI tract can be divided into three layers: the mucosa, the submucosa and the muscularis propria. Starting most centrally, the epithelium of the gut is in contact with the contents of the lumen. Deep to the epithelium lies the lamina propria. Deep to the lamina propria lies the muscularis mucosa and then the submucosa. The submucosal ganglia are located within the submucosal space. Outside of the submucosa sits the muscularis propria which contains a layer of longitudinal and a layer of circular muscle, with the myenteric nerve plexus sitting in between.

### 1. *Mucosa*

The mucosal layer of the GI tract is comprised of three sublayers – the epithelium, lamina propria and muscularis mucosa. The epithelial layer is a heterogeneous mix of cell types, including enterocytes, Paneth cells, enteroendocrine cells, and goblet cells, with the latter two only present in the stomach and intestines. These cells have specialized functions and help to both protect the tissue, and maintain barrier function. The epithelial

layer in the oral cavity, pharynx, and esophagus is made of non-keratinized, stratified squamous epithelium, whereas the stomach and intestines contain simple columnar epithelium. Additionally, the epithelium of the stomach and intestines is organized into tubular glands, or folds. These folds help to not only increase surface area for nutrient absorption in the small intestine (plicae circulares), but also allow for expansion of the stomach to accommodate food intake (rugae), and restrict the movement of intestinal contents within the colon. The lamina propria is a layer of loose connective tissue that provides vascularization to the epithelium and houses mucosal glands and gut associated lymphoid tissues, including B and T lymphocytes and dendritic cells. Together, the epithelium and the immune cells in the lamina propria work to maintain barrier function and homeostasis within the gut. Lastly, the muscularis mucosa is a thin muscular layer beneath the lamina propria that modulates the surface area of the lumen. Contraction causes the rugae of the stomach and plicae circulares of the small intestine to decrease the available surface area, whereas relaxation of the muscularis mucosa causes those structures to expand, increasing the available surface area.

## 2. *Submucosa*

The submucosa is a rich connective tissue layer that houses the submucosal plexus of the enteric nervous system. In some areas of the gut, such as the duodenum, this layer contains glands that secrete contents into the lumen to aid in the digestive process. This layer is also rich in blood vessels and lymphatics. The submucosal plexus is responsible for coordinating secretory reflexes, as well as vasodilatory reflexes.

### 3. *Muscularis Externa*

The muscularis externa is comprised of two muscular layers and one ganglionated plexus. The myenteric plexus is sandwiched between a layer of circular muscle and a layer of longitudinal muscle. These two layers of muscle contract and relax in a coordinated fashion to produce motor patterns within the gut, including segmentation and peristalsis. Segmentation occurs when adjacent sections of gut contract and relax in a coordinated manner to allow for mixing of luminal contents. Peristalsis causes the forward movement of luminal contents by an upstream contraction and a downstream relaxation. These motor patterns are coordinated primarily by the myenteric plexus, with little extrinsic input from the central nervous system.

### 4. *Serosa*

The serosa, or adventitia layer, is a sheet like membrane covering the outside of the gut, and is made of a single layer of mesothelial cells. The parts of the GI tract that are intraperitoneal are covered with serosa, while the retroperitoneal parts, the esophagus, and the oral cavity are covered in adventitia. The serosa and the adventitia layer both serve the role of providing an external layer of tissue to these organs. The serosa is continuous with the visceral peritoneum lining the abdominal cavity. It contains blood vessels, lymphatics and nerves, and, within the intraperitoneal space, is connected to the mesentery.

## **II. Serotonin**

### **A. History**

The substance now known as serotonin was first isolated from rabbit gastric mucosa by the Italian pharmacologist Vittorio Erspamer in 1937 (Erspamer V, 1937). He named this bioactive amine, enteramine, as he identified it in the GI tracts of almost every vertebrate he studied. Later, a compound with vasoconstrictive properties was identified in bovine serum by researchers at the Cleveland Clinic. Maurice Rapport, Arda Green and Irvine Page called this compound serotonin due to its effect increasing vascular tone (Rapport et al., 1948). In time, these two molecules were discovered to be the same, 5-hydroxytryptamine, and the name serotonin was adopted for general use.

### **B. Gastrointestinal Mucosal Serotonin**

Serotonin (5-hydroxytryptamine, 5-HT) is an important signaling molecule for many physiological systems, and it plays a particularly crucial role within the GI tract (Mawe and Hoffman, 2013). The mucosa of the GI tract contains the body's largest store of 5-HT. It is synthesized from tryptophan, using the enzyme tryptophan hydroxylase-1 (TPH-1), by enterochromaffin (EC) cells within the gut epithelium. Once synthesized it is stored in intracellular granules along the basal side of EC cells until released into the lamina propria. Release can be stimulated by intraluminal changes in acidity, pressure, or increased nutrient concentration. Once within the lamina propria, 5-HT interacts with a variety of cell types, including enteric neurons, extrinsic neuron projections, epithelial

cells and immune cells. The precise action of 5-HT depends on the type of receptor that is expressed.

To terminate 5-HT signaling, molecules are removed from the extracellular space via the serotonin-selective reuptake transporter (SERT) protein. SERT is expressed on serotonergic enteric neurons and virtually all intestinal epithelial cells. Once 5-HT has been transported into cells by SERT, it is either degraded by intracellular degradative enzymes, such as monoamine oxidases, or is repackaged into vesicles. Any 5-HT not transported out of the interstitial space will enter the blood stream via the dense capillary bed in the lamina propria, and be taken up by blood platelets, which also express SERT.

Altered serotonin signaling within the GI tract is implicated in human inflammatory bowel diseases (IBD) such as Crohn's Disease and Ulcerative Colitis. Changes in GI serotonin content, EC cell density and SERT expression have been reported in both human disease and animal models of colitis (Coates et al., 2017). It is also known that serotonin can modify GI motility and that activation of epithelial serotonin receptors has protective epithelial actions and promotes recovery from colitis (Hoffman et al., 2012, Spohn et al., 2016). It should also be noted that serotonin exerts pro-inflammatory actions in the GI tract through activation of 5-HT<sub>7</sub> receptors on dendritic cells (Spohn and Mawe, 2017).

### **C. 5-HT<sub>4</sub> Receptor**

There are seven classes of 5-HT receptors (5-HT<sub>1</sub> – 5-HT<sub>7</sub>), with 13 distinct subtypes, 7 of which are expressed in the GI tracts of humans (5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>,

5-HT<sub>3</sub>, 5-HT<sub>4</sub>, and 5-HT<sub>7</sub>)(Mawe and Hoffman, 2013). They have been identified on a variety of cell types within the gut, including enterocytes, goblet cells, EC cells, neurons, immune cells, smooth muscle cells and interstitial cells of Cajal. One receptor subtype, the 5-HT<sub>4</sub> receptor, plays an important role in gastrointestinal homeostasis and motility. The 5-HT<sub>4</sub> receptor is a G-protein coupled receptor, that is G<sub>s</sub> coupled and activates the adenylate cyclase/cyclic adenosinemonophosphate (cAMP)/protein kinase A pathway. Thus, it plays a role in many different cellular functions including, but not limited to, prosecretory actions and facilitation of neurotransmitter release (Tonini et al., 1989, Pan and Galligan, 1994, Hoffman et al., 2012)

The 5-HT<sub>4</sub> receptor is expressed on enteric nerve terminals, where it helps to mediate neurotransmitter release through presynaptic facilitation (Pan and Galligan, 1994). It has also been demonstrated that 5-HT<sub>4</sub> receptor stimulation promotes enteric neuronal survival and neurogenesis (Liu et al., 2009). This receptor is also heavily expressed on colonic epithelial cells, where it helps to mediate 5-HT release from enterochromaffin cells, stimulates chloride secretion from enterocytes, and promotes goblet cell degranulation (Hoffman et al., 2012). Furthermore, activation of epithelial 5-HT<sub>4</sub> receptors has been shown to stimulate propulsive motility, decrease visceral hypersensitivity, promote epithelial proliferation and promote the integrity of the epithelial barrier (Spohn et al., 2016).

## **D. Mucosal 5-HT<sub>4</sub> Receptor Actions**

There is strong evidence for mucosal 5-HT<sub>4</sub> receptors mediating the prokinetic, anti-nociceptive and anti-inflammatory actions within the gastrointestinal tract. The 5-HT<sub>4</sub> receptor is specifically expressed by a multitude of cell types in the epithelium of the GI tract, including 5-HT containing enterochromaffin (EC) cells, mucin-2 containing goblet cells and enterocytes in human and mouse tissues (Hoffman et al., 2012, Spohn et al., 2016)

### *1. Prokinetic Actions*

5-HT<sub>4</sub> agonists have potent prokinetic actions that have been identified through multiple mechanisms and at multiple sites of action. Our lab previously reported that activation of EC cells with a 5-HT<sub>4</sub> agonist results in an increased release of 5-HT and increases in goblet cell cavitation and mucus release (Hoffman et al., 2012). It has also been shown that stimulation of the cAMP/PKA pathway in human colonic cell lines results in mucus secretion (Bradbury, 2000). This implies that mucus release could result from direct activation of mucosal 5-HT<sub>4</sub> receptors. Lastly, it is known that 5-HT increases chloride secretion in GI epithelium and that application of 5-HT<sub>4</sub> agonists to mouse colonic tissue increases the I<sub>SC</sub> current (Budhoo et al., 1996, Ning et al., 2004, Hoffman et al., 2012). Together, these three actions of 5-HT<sub>4</sub>R activation may contribute to increasing propulsive motility of the GI tract in the following ways: (1) Increases in 5-HT release can directly activate peristaltic reflex circuitry; (2) Increases in mucus secretion may help decrease intraluminal friction, easing the passage of luminal contents; and (3)

Increases in chloride secretion result in increased water content in the lumen, softening stool and allowing for ease of passage. Furthermore, 5-HT<sub>4</sub> agonists have been shown to directly increase colonic motility in healthy and inflamed colons of guinea pig and mice by directly activating peristaltic reflex circuitry (Grider et al., 1998, Jin et al., 1999, Spohn et al., 2016). Together, these results indicate a potent prokinetic effect of 5-HT<sub>4</sub> receptor activation.

## *2. Anti-nociceptive Actions*

Anti-nociceptive effects of 5-HT<sub>4</sub> agonists have been observed in rat models of hypersensitivity and in clinical trials in humans. The 5-HT<sub>4</sub> agonist tegaserod alleviates abdominal pain and discomfort in rats, as seen by a decrease in colonic hypersensitivity (Greenwood-Van Meerveld et al., 2006, Hoffman et al., 2012). Greenwood-Van Meerveld and colleagues demonstrated that rats with both acute and chronic hypersensitivity showed a decreased visceral motor response to colorectal distention after oral or intracolonic treatment with tegaserod. The response to intracolonic delivered tegaserod was blocked by pretreatment with the 5-HT<sub>4</sub> selective antagonist GR113808. Multiple clinical trials have reported a significant decrease in perceived abdominal pain, a decrease in straining and an improvement in stool consistency for IBS patients treated with tegaserod as compared to control patients (Novick et al., 2002, Chey et al., 2008). The exact mechanism by which 5-HT<sub>4</sub> activation alters visceral hypersensitivity is unknown.

### 3. *Protective Epithelial Actions*

Our lab has demonstrated the protective actions of mucosal 5-HT<sub>4</sub> receptors in a multitude of animal models and assays. Intracolonic tegaserod treatment decreased clinical and histological damage scores in both the 2,4,6-trinitrobenzene sulfonic acid (TNBS)(mouse and guinea pig) and dextran sodium sulfate (DSS)(mouse) models of colitis (Spohn et al., 2016). Intraperitoneal administration of a 5-HT<sub>4</sub> agonist did not provide a protective effect, and the protective and restorative effects of intracolonic treatment were blocked by antagonist treatment (Spohn et al., 2016). In addition, these effects were not present in 5-HT<sub>4</sub> deficient mice. These results suggest that activation of the 5HT<sub>4</sub> receptor is sufficient to induce a protective response in experimental colitis. Furthermore, tegaserod treatment in mice with DSS colitis improved epithelial barrier function as seen by a decrease in bacterial translocation to the spleen or liver (Spohn et al., 2016). Lastly, treatment of Caco-2 cells, a human epithelial colorectal adenocarcinoma cell line, with tegaserod resulted in increased resistance to oxidative stress, and an increased rate of cell migration following an *in vitro* scratch assay (Spohn et al., 2016). These results were blocked by antagonist treatment. Together, these actions suggest a potent protective effect of epithelial 5-HT<sub>4</sub> receptor activation *in vivo* and *in vitro*.

#### **E. Epithelial 5-HT<sub>4</sub> receptors as a therapeutic target**

The 5-HT<sub>4</sub> receptor has been identified as a potent therapeutic target for constipation relief in irritable bowel syndrome (IBS) and chronic constipation, as well as

pain relief in IBS; however, off-target cardiac effects have limited their use in clinical populations (Novick et al., 2002, Chey et al., 2008, Tack et al., 2012).

Agonists of the 5-HT<sub>4</sub>R, including cisapride and tegaserod, have had remarkable clinical success for patients with constipation and constipation predominant IBD; however, their use in patient populations was suspended or restricted after reports of adverse cardiovascular events (McCallum et al., 1988, Evans et al., 2007, Tack et al., 2012).

Tegaserod was removed from the market in 2007 by recommendation from the FDA based on evidence from pooled clinical trial data suggesting a risk of ischemic cardiovascular events (Wooltorton, 2004). However, it is unknown how tegaserod led to these adverse events. Tegaserod has a low affinity for the 5-HT<sub>1B</sub> receptor located on blood vessels, but causes vasodilation, and thus would not lead to ischemia. A large, matched-cohort study in 2010 detected no increase in ischemic cardiovascular events in tegaserod users (Loughlin et al., 2010).

Cisapride is a benzamide, with moderate affinity and poor selectivity for the 5-HT<sub>4</sub> receptor (Nagakura et al., 1999). It also blocks 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, and the human ether-a-go-go-related gene (hERG)- encoded K<sup>+</sup> channel (Nagakura et al., 1999, Potet et al., 2001). The hERG channel is present on cardiac smooth muscle cells, and it is thought that blockage of this channel led to a lengthening of the QT interval, causing the adverse cardiac events, particularly in individuals with long QT syndrome (Drolet et al., 1998). While 5-HT<sub>4</sub> agonists other than cisapride have shown to have low to no-binding capacity for the hERG-K<sup>+</sup> channel, or other K<sup>+</sup> or Na<sup>2+</sup> channels, there is still a stigma

and fear associated with serotonin receptor agonists as a therapeutic target (Tack et al., 2012). Since these side effects were a result of systemic availability, a therapeutic in which the active compound is not absorbed systemically could yield the same efficacy without the potential for dangerous off-target effects.

## **II. Inflammatory Bowel Disease**

Inflammatory Bowel Disease (IBD) is a condition of chronic or relapsing/remitting inflammation of the gastrointestinal tract. IBD is an umbrella term that includes Crohn's disease (CD) and Ulcerative Colitis (UC). This disease typically manifests between the second and third decade of life, and predominately affects women over men. According to the CDC, in 2015 the prevalence of IBD diagnosis was 1.3% (3.1 million) of U.S. adults, which increased from 0.9% (1.8 million) in 1999. This study showed a higher prevalence in adults over the age of 45, Hispanics, non-Hispanic whites, and adults with a less than high school education (Dahlhamer, 2016). However, other studies have shown increased prevalence at higher socioeconomic levels (Krishnan and Korzenik, 2002).

There is extensive physical and financial burden on those suffering from IBD. According to a 2008 study, the average annual cost for those suffering from IBD is between \$5000 and \$10,000 (Kappelman et al., 2008). Additionally, there is a higher hospitalization rate for individuals with IBD, and most patients will require surgery at least once in their lifetime (Bewtra et al., 2007). There is no current cure of IBD, only life-long palliative treatments.

The etiology of IBD is unknown. It is thought that a combination of altered gut microbiota and aberrant mucosal immune responses due to diet and environmental factors in genetically susceptible individuals leads to development of IBD (Matsuoka et al., 2018). There have been 163 susceptibility loci identified for IBD, including approximately 300 potential genes (Loddo and Romano, 2015). The first IBD associated gene discovered was for nucleotide-binding oligomerization domain containing 2 (NOD2), which codes for an intracellular pattern recognition receptor. Bacterial binding to this protein induces autophagy in dendritic cells, and mutations in this protein are associated with CD (Cooney et al., 2010). There is a prominent familial component to IBD, with relatives of CD or UC having an 8- to 10-fold greater risk for developing IBD, and there is concordance between twins (Cho and Brant, 2011). The immunopathogenesis of IBD is still being elucidated, and is vastly complex. Simply, it appears that CD is primarily T<sub>H</sub>1 mediated and UC is primarily T<sub>H</sub>2 mediated; however, there is significant overlap in their immunologic profiles (Wallace et al., 2014). CD and UC share overlapping etiologies, but they differ anatomically and histologically, as described below.

### **A. Crohn's Disease**

Crohn's disease (CD) can affect any part of the gastrointestinal tract, but commonly affects the distal small intestine, and the proximal colon. It is characterized by discontinuously affected areas of inflammation, transmural immune cell infiltration and fistulas. The major symptoms of Crohn's include diarrhea, abdominal

pain, weight loss, fever, and less frequently bloody or mucus covered stool.

Genetically, CD is primarily associated with genes for lymphocyte chemotaxis and trafficking genes (Franke et al., 2010).

## **B. Ulcerative Colitis**

Ulcerative colitis (UC) most prominently affects the colon, primarily the distal side, and has a continuous area of inflammation. Histologically, the inflammation only affects the mucosal layer, leaving the deeper layers of the gut unaffected, and often forms erosions. The major symptoms of UC include recurrent bloody diarrhea, abdominal pain and frequent bowel movements. Genetically, UC is associated with genes that regulate barrier function, including proteins involved in epithelial tight junction integrity (Anderson et al., 2011). UC is also associated with an increased risk in colon cancer.

## **C. Current Therapies**

The predominant current therapies for IBD include anti-inflammatory, immunosuppressive, and biologic therapies (Neurath, 2017). Anti-inflammatory drugs include 5-aminosalicylates (5-ASAs) and corticosteroids. While both are effective at inducing remission, only 5-ASAs help to maintain remission. Immunosuppressive therapies, including azathioprine, methotrexate and ciclosporin-A, have efficacy inducing and maintaining remission of both CD and UC. Lastly, biologic therapies, including monoclonal antibodies that target tumor necrosis factor (TNF)-alpha

(infliximab, adalimumab, golimumab and certolizumab pegol), have been used to induce and maintain remission in UC and CD. Studies have shown that combined immunosuppressive and biologic therapies are more effective at inducing and maintaining remission than either therapy alone for both UC and CD (Colombel et al., 2010, Panaccione et al., 2014).

Given the complex etiology and the varied cytokine profiles of IBD patients, it is challenging to find the appropriate therapeutic for a given individual. Approximately 30-50% of patients do not respond to anti-TNF agents, and there is a significant risk of relapse after discontinuation of anti-TNF therapy (40% in CD and 28% in UC) (Olesen et al., 2016, Neurath, 2017). To fill this void, many new therapeutic targets are currently being studied, including integrin blockers and modulators of T-cell trafficking (alpha-4beta-7, beta-7, MAdCAM1, and S1P receptor agonists), matrix metalloprotease inhibitors to mediate fibrosis and tissue remodeling (MMP 9 inhibitors CHST15 siRNAs), blockers of transcription factors and modulators of barrier function and anti-inflammatory pathways (Neurath, 2017).

The therapies described above are useful, but up to 40% of patients still experience other functional bowel symptoms, even when the disease is in remission (Coates et al., 2013, Vivinus-Nebot et al., 2014). These functional bowel symptoms include abdominal pain and changes in bowel habits that, while not inherently dangerous, severely affect the patient's quality of life. Traditional therapies are targeted at dampening the immunological component of IBD; however, there is a

clear need for novel therapies targeting these functional bowel symptoms experienced by most patients.

### **III. Animal Models of Colitis**

There is no perfect animal model that recapitulates all features of human inflammatory bowel disease; however, there are a few different models that have key features of IBD. These models include the chemically induced models: dextran sodium sulfate, TNBS, and oxazolone; and spontaneous colitis in interleukin(IL)-10 deficient mice (Kiesler et al., 2015, Wirtz et al., 2017). These methods all reproduce histological, pathological and immunologic hallmarks of inflammatory bowel diseases, the details of which are described below:

#### **A. Dextran Sodium Sulfate**

DSS colitis is induced by oral administration of the sulfated polysaccharide dextran sodium sulfate. This experimental colitis recapitulates the clinical and inflammatory features of ulcerative colitis (Okayasu et al., 1990). The exact mechanism is not entirely understood, but it is thought that the molecule is taken up into epithelial cells, altering tight junction proteins, and leading to compromised barrier function. The increased epithelial permeability allows luminal bacteria and their by-products to interact with mucosal and submucosal immune cells, resulting in rapid and intense inflammation. It has been hypothesized that DSS creates nano-lipocomplexes with fatty acids that fuse to the membranes of epithelial cells, and

penetrate the cell membrane, depositing DSS molecules intracellularly, thus affecting barrier permeability (Laroui et al., 2012). These changes in barrier function are likely due to alteration in tight junction protein expression. It has been shown that DSS can alter ribosome interactions (Miyazawa et al., 1967). Furthermore, it is known that the cytokines IL-1 $\beta$  and IL-18 play crucial roles in the development of DSS colitis, and that the NLRP3 inflammasome is necessary for DSS colitis to develop, indicating a role for the innate immune system (Bauer, 2010). Additionally, immune deficient mice are susceptible to DSS, showing that the adaptive immune system is not required for this immune response (Dieleman et al., 1994); however, T<sub>H</sub>1 and T<sub>H</sub>2 cytokines are present in experimental models of chronic colitis (Dieleman et al., 1998). DSS induced colitis is an acceptable model to study the features of ulcerative colitis, particularly impaired barrier function.

## **B. Trinitrobenzine Sulfonic Acid**

TNBS is a haptenizing agent that is administered intracolonicly with ethanol. The ethanol allows the TNBS to access the epithelial cells and penetrate the gut wall. TNBS colitis predominately recapitulates the features of CD, including infiltration of CD4<sup>+</sup> T-cells and neutrophils, transmural inflammation, spontaneous resolution of inflammation, fibrosis of the mucosal tissue, severe diarrhea, weight loss and rectal prolapse (Neurath et al., 1995, Kiesler et al., 2015). Additionally, TNBS colitis and CD are both characterized by a T<sub>H</sub>1 and T<sub>H</sub>17 CD4<sup>+</sup> T-cell dominant response, involving cytokines IFN- $\gamma$  and IL-17 (Strober and Fuss, 2011).

This experimental colitis model has been particularly useful in studying the role of the adaptive immune system and T cell responses in inflammatory bowel disease. However, the role of the innate immune system should not be understated, as immune deficient mice are still susceptible to TNBS administration (Fiorucci et al., 2002). This is because IL-12 production by innate cells is necessary to drive the T-cell response.

### **C. Other Models of IBD: IL-10 knockout and Oxazolone colitis**

Interleukin-10 knockout mice spontaneously develop colitis between 4 and 8 weeks of age, with inflammation featuring infiltration of lymphocytes, macrophages and neutrophils into the intestinal mucosa (Kuhn et al., 1993). This model has been useful for assessing immunoregulation and macrophage involvement in gut homeostasis. The inflammatory response in IL-10<sup>-/-</sup> mice is initially driven by T<sub>H</sub>1 cells and related cytokines; however, this response slowly subsides and a T<sub>H</sub>2, IL-4 and IL-13 driven response becomes the dominant profile (Spencer et al., 2002). This phenomenon gives insight into the dynamic changes in immunoregulation occurring in gut inflammation that may parallel human IBD.

Oxazolone is another haptening agent that, when applied intracolonicly induces colonic inflammation. The features of oxazolone colitis align with features of UC both morphologically and immunopathologically. It causes superficial inflammation of the mucosa within the distal colon, including infiltration of lymphocytes and neutrophils, and erosions (Boirivant et al., 1998). It is characterized

by a T<sub>H</sub>2 cytokine profile, including IL-13 secretion from CD4<sup>+</sup> natural killer T cells in the lamina propria (Heller et al., 2002). IL-13 is known to cause and increase epithelial cell apoptosis, an increase in the pore-forming tight junction protein claudin-2, a decrease in the tight junction protein tricellulin and ultimately decreased barrier function (Heller et al., 2005, Krug et al., 2017). This model is useful for studying the immunopathogenesis of UC.

#### **IV. Specific Aims**

Serotonin is a very important signaling molecule within the gastrointestinal (GI) tract. One receptor subtype, the 5-HT<sub>4</sub> receptor, has been identified as a potent therapeutic target for functional bowel disease and pain. Agonists of the 5-HT<sub>4</sub> receptor, including cisapride and tegaserod, have had remarkable clinical success as prokinetic agents; however, adverse side effects due to systemic availability led to their removal from the market. Our lab has recently discovered that 5-HT<sub>4</sub> receptors are highly expressed in the colonic epithelium, and that activation of these receptors promotes motility and induces protective epithelial actions (Hoffman et al., 2012, Spohn et al., 2016). We hypothesize that the administration of lumenally restricted 5-HT<sub>4</sub> agonists will accelerate recovery from established colitis by inducing protective epithelial actions and restoring normal motility patterns.

**Aim 1: Evaluate the effect of lumenally restricted 5-HT<sub>4</sub> agonists on disease severity in DSS colitis.** *Hypothesis: Administration of lumenally restricted 5HT<sub>4</sub> agonists by enema will exert protective epithelial actions in the murine model of DSS induced colitis.*

A recovery paradigm will be used to test this hypothesis. The recovery paradigm will test whether 5-HT<sub>4</sub> agonist treatment, initiated after DSS colitis is established, accelerates recovery from colitis. Outcome measures will include daily recordings of disease activity index, fecal lipocalin-2 levels, analysis of epithelial proliferation, and histological damage scores.

**Aim 2: Evaluate the effect of luminally restricted 5-HT<sub>4</sub> agonists on colonic motility in DSS colitis.** *Hypothesis: Application of a luminally restricted 5-HT<sub>4</sub> agonist will increase colonic motility in vivo.* This hypothesis will be tested using *in vivo* techniques to elucidate functional outcomes of the luminally restricted agonist. Two assays will be used to assess gastrointestinal function in DSS mice treated with the non-absorbable 5-HT<sub>4</sub> agonist: colonic motility and fecal output and consistency. These assays will be performed throughout the DSS recovery paradigm.

The proposed studies are designed to elucidate the effects of luminally restricted 5-HT<sub>4</sub> agonists on development of and recovery from colitis. We will use well validated *in vivo* assays to determine the protective epithelial actions of this compound on the gastrointestinal tract. These studies will examine the therapeutic potential of luminally restricted 5-HT<sub>4</sub> agonists for the treatment of IBD.

## Works Cited

- Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel J-F, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RSN, Floyd JAB, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot J-P, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms L, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra H-J, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskis L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamailard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD (2011) Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nature genetics* 43:246-252.
- Bewtra M, Su C, Lewis JD (2007) Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 5:597-601.
- Boirivant M, Fuss IJ, Chu A, Strober W (1998) Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *The Journal of experimental medicine* 188:1929-1939.
- Bradbury NA (2000) Protein kinase-A-mediated secretion of mucin from human colonic epithelial cells. *Journal of cellular physiology* 185:408-415.
- Budhoo MR, Harris RP, Kellum JM (1996) 5-Hydroxytryptamine-induced Cl<sup>-</sup> transport is mediated by 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors in the rat distal colon. *European journal of pharmacology* 298:137-144.
- Chey WD, Pare P, Viegas A, Ligozio G, Shetzline MA (2008) Tegaserod for female patients suffering from IBS with mixed bowel habits or constipation: a randomized controlled trial. *The American journal of gastroenterology* 103:1217-1225.

- Cho JH, Brant SR (2011) Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 140:1704-1712.
- Coates MD, Lahoti M, Binion DG, Szigethy EM, Regueiro MD, Bielefeldt K (2013) Abdominal pain in ulcerative colitis. *Inflammatory bowel diseases* 19:2207-2214.
- Coates MD, Tekin I, Vrana KE, Mawe GM (2017) Review article: the many potential roles of intestinal serotonin (5-hydroxytryptamine, 5-HT) signalling in inflammatory bowel disease. *Alimentary pharmacology & therapeutics* 46:569-580.
- Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P (2010) Infliximab, azathioprine, or combination therapy for Crohn's disease. *The New England journal of medicine* 362:1383-1395.
- Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A (2010) NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 16:90-97.
- Dahlhamer JZ, EP; Ward, BW; Wheaton, AG; Croft, JB (2016) Prevalence of Inflammatory Bowel Disease Among Adults Aged  $\geq 18$  Years — United States, 2015. *Morbidity and Mortality Weekly Report* 65:1166-1169.
- Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, Van Rees EP (1998) Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clinical and experimental immunology* 114:385-391.
- Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO (1994) Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 107:1643-1652.
- Drolet B, Khalifa M, Daleau P, Hamelin BA, Turgeon J (1998) Block of the rapid component of the delayed rectifier potassium current by the prokinetic agent cisapride underlies drug-related lengthening of the QT interval. *Circulation* 97:204-210.
- Erspamer V VM (1937) Ricerche sul secreto delle cellule enterocromaffini. *Boll d Soc Med-chir Pavia* 51:357-363.
- Evans BW, Clark WK, Moore DJ, Whorwell PJ (2007) Tegaserod for the treatment of irritable bowel syndrome and chronic constipation. *The Cochrane database of systematic reviews* Cd003960.

- Fiorucci S, Mencarelli A, Palazzetti B, Sprague AG, Distrutti E, Morelli A, Novobrantseva TI, Cirino G, Koteliansky VE, de Fougères AR (2002) Importance of innate immunity and collagen binding integrin  $\alpha 1\beta 1$  in TNBS-induced colitis. *Immunity* 17:769-780.
- Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Buning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossom A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panes J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhardt AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nature genetics* 42:1118-1125.
- Greenwood-Van Meerveld B, Venkova K, Hicks G, Dennis E, Crowell MD (2006) Activation of peripheral 5-HT receptors attenuates colonic sensitivity to intraluminal distension. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 18:76-86.
- Grider JR, Foxx-Orenstein AE, Jin JG (1998) 5-Hydroxytryptamine<sub>4</sub> receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. *Gastroenterology* 115:370-380.
- Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD (2005) Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 129:550-564.
- Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W (2002) Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 17:629-638.
- Hoffman JM, Tyler K, Maceachern SJ, Balemba OB, Johnson AC, Brooks EM, Zhao H, Swain GM, Moses PL, Galligan JJ, Sharkey KA, Greenwood-Van Meerveld B, Mawe GM (2012) Activation of Colonic Mucosal 5-HT<sub>4</sub> Receptors Accelerates

Propulsive Motility and Inhibits Visceral Hypersensitivity. *Gastroenterology* 142:844-854.e844.

Jin JG, Foxx-Orenstein AE, Grider JR (1999) Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT<sub>4</sub> and 5-HT<sub>3</sub> receptors. *The Journal of pharmacology and experimental therapeutics* 288:93-97.

Kappelman MD, Rifas-Shiman SL, Porter C, Ollendorf DA, Sandler RS, Galanko JA, Finkelstein JA (2008) Direct Health Care Costs of Crohn's Disease and Ulcerative Colitis in United States Children and Adults. *Gastroenterology* 135:1907-1913.

Kiesler P, Fuss IJ, Strober W (2015) Experimental Models of Inflammatory Bowel Diseases. *Cellular and molecular gastroenterology and hepatology* 1:154-170.

Krishnan A, Korzenik JR (2002) Inflammatory bowel disease and environmental influences. *Gastroenterology clinics of North America* 31:21-39.

Krug SM, Bojarski C, Fromm A, Lee IM, Dames P, Richter JF, Turner JR, Fromm M, Schulzke JD (2017) Tricellulin is regulated via interleukin-13-receptor alpha2, affects macromolecule uptake, and is decreased in ulcerative colitis. *Mucosal immunology*.

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75:263-274.

Laroui H, Ingersoll SA, Liu HC, Baker MT, Ayyadurai S, Charania MA, Laroui F, Yan Y, Sitaraman SV, Merlin D (2012) Dextran Sodium Sulfate (DSS) Induces Colitis in Mice by Forming Nano-Lipocomplexes with Medium-Chain-Length Fatty Acids in the Colon. *PloS one* 7:e32084.

Liu MT, Kuan YH, Wang J, Hen R, Gershon MD (2009) 5-HT<sub>4</sub> receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29:9683-9699.

Loddo I, Romano C (2015) Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. *Front Immunol* 6:551.

Loughlin J, Quinn S, Rivero E, Wong J, Huang J, Kralstein J, Earnest DL, Seeger JD (2010) Tegaserod and the risk of cardiovascular ischemic events: an observational cohort study. *Journal of cardiovascular pharmacology and therapeutics* 15:151-157.

- Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, Kato J, Kobayashi K, Kobayashi K, Koganei K, Kunisaki R, Motoya S, Nagahori M, Nakase H, Omata F, Saruta M, Watanabe T, Tanaka T, Kanai T, Noguchi Y, Takahashi KI, Watanabe K, Hibi T, Suzuki Y, Watanabe M, Sugano K, Shimosegawa T (2018) Evidence-based clinical practice guidelines for inflammatory bowel disease. *Journal of gastroenterology*.
- Mawe GM, Hoffman JM (2013) Serotonin Signaling in the Gastrointestinal Tract:: Functions, dysfunctions, and therapeutic targets. *Nature reviews Gastroenterology & hepatology* 10:473-486.
- McCallum RW, Prakash C, Campoli-Richards DM, Goa KL (1988) Cisapride. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use as a prokinetic agent in gastrointestinal motility disorders. *Drugs* 36:652-681.
- Miyazawa F, Olijnyk OR, Tilley CJ, Tamaoki T (1967) Interactions between dextran sulfate and Escherichia coli ribosomes. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 145:96-104.
- Nagakura Y, Akuzawa S, Miyata K, Kamato T, Suzuki T, Ito H, Yamaguchi T (1999) Pharmacological properties of a novel gastrointestinal prokinetic benzamide selective for human 5-HT<sub>4</sub> receptor versus human 5-HT<sub>3</sub> receptor. *Pharmacological research* 39:375-382.
- Neurath MF (2017) Current and emerging therapeutic targets for IBD. *Nat Rev Gastroenterol Hepatol* 14:269-278.
- Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W (1995) Antibodies to interleukin 12 abrogate established experimental colitis in mice. *The Journal of experimental medicine* 182:1281-1290.
- Ning Y, Zhu JX, Chan HC (2004) Regulation of ion transport by 5-hydroxytryptamine in rat colon. *Clinical and experimental pharmacology & physiology* 31:424-428.
- Novick J, Miner P, Krause R, Glebas K, Bliesath H, Ligozio G, Ruegg P, Lefkowitz M (2002) A randomized, double-blind, placebo-controlled trial of tegaserod in female patients suffering from irritable bowel syndrome with constipation. *Alimentary pharmacology & therapeutics* 16:1877-1888.
- Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98:694-702.

- Olesen CM, Coskun M, Peyrin-Biroulet L, Nielsen OH (2016) Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases. *Pharmacology & therapeutics* 159:110-119.
- Pan H, Galligan JJ (1994) 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons. *The American journal of physiology* 266:G230-238.
- Panaccione R, Ghosh S, Middleton S, Marquez JR, Scott BB, Flint L, van Hoogstraten HJ, Chen AC, Zheng H, Danese S, Rutgeerts P (2014) Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 146:392-400.e393.
- Potet F, Bouyssou T, Escande D, Baro I (2001) Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-a-gogo K(+) channel. *The Journal of pharmacology and experimental therapeutics* 299:1007-1012.
- Rapport MM, Green AA, Page IH (1948) Partial purification of the vasoconstrictor in beef serum. *The Journal of biological chemistry* 174:735-741.
- Silverthorn DJ, BR; Ober, WC; Garrison, CW; Silverthorn, AC (2013) *Human Physiology: and Integrated Approach*. Pearson.
- Spencer DM, Veldman GM, Banerjee S, Willis J, Levine AD (2002) Distinct inflammatory mechanisms mediate early versus late colitis in mice. *Gastroenterology* 122:94-105.
- Spohn SN, Bianco F, Scott RB, Keenan CM, Linton AA, O'Neill CH, Bonora E, Dicay M, Lavoie B, Wilcox RL, MacNaughton WK, De Giorgio R, Sharkey KA, Mawe GM (2016) Protective Actions of Epithelial 5-Hydroxytryptamine 4 Receptors in Normal and Inflamed Colon. *Gastroenterology*.
- Spohn SN, Mawe GM (2017) Non-conventional features of peripheral serotonin signalling - the gut and beyond. *Nat Rev Gastroenterol Hepatol* 14:412-420.
- Strober W, Fuss IJ (2011) Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140:1756-1767.
- Tack J, Camilleri M, Chang L, Chey WD, Galligan JJ, Lacy BE, Müller-Lissner S, Quigley EMM, Schuurkes J, Maeyer JH, Stanghellini V (2012) Systematic review: cardiovascular safety profile of 5-HT<sub>4</sub> agonists developed for gastrointestinal disorders. *Alimentary pharmacology & therapeutics* 35:745-767.

- Tonini M, Galligan JJ, North RA (1989) Effects of cisapride on cholinergic neurotransmission and propulsive motility in the guinea pig ileum. *Gastroenterology* 96:1257-1264.
- Vivinus-Nebot M, Frin-Mathy G, Bziouche H, Dainese R, Bernard G, Anty R, Filippi J, Saint-Paul MC, Tulic MK, Verhasselt V, Hebuterne X, Piche T (2014) Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* 63:744-752.
- Wallace KL, Zheng LB, Kanazawa Y, Shih DQ (2014) Immunopathology of inflammatory bowel disease. *World journal of gastroenterology* 20:6-21.
- Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF (2017) Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature protocols* 12:1295-1309.
- Wooltorton E (2004) Tegaserod (Zelnorm) for irritable bowel syndrome: reports of serious diarrhea and intestinal ischemia. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 170:1908.

## **CHAPTER II: PROTECTIVE ACTIONS OF LUMINALLY RESTRICTED 5-HT<sub>4</sub> RECEPTOR AGONISTS IN DEXTRAN SODIUM SULFATE INDUCED COLITIS**

### **I. Abstract**

**Background:** The 5-hydroxytryptamine receptor 4 (5-HT<sub>4</sub> receptor) is heavily expressed on colonic epithelial cells and has been targeted as a therapeutic for functional bowel symptoms and pain; however, adverse cardiac events related to 5-HT<sub>4</sub> agonist treatment limited their therapeutic use. Previous studies in the Mawe laboratory have demonstrated that intraluminal application of a 5-HT<sub>4</sub> agonist exerts protective epithelial actions in animal models of colitis, and accelerates recovery from colitis. The aim of this study was to test the effects of a luminally restricted 5-HT<sub>4</sub> agonist in a mouse model of experimental colitis.

**Methods:** The luminally restricted 5-HT<sub>4</sub> agonist SYR313832Z (Takeda Pharmaceuticals; 10 mg/kg) was administered to mice during active DSS colitis. Colitis activity was evaluated using disease activity index, a fecal lipocalin-2 assay, and histological damage scoring. Epithelial proliferation and colonic motility were also measured as readouts of the potential protective actions and colonic function, respectively.

**Results:** Oral gavage and intracolonic delivery of SYR313832Z had no detectable effect on recovery from colitis or colonic motility as compared to vehicle. Additionally, in positive control experiments, we failed to see an effect of the 5-HT<sub>4</sub>R agonist, tegaserod, on colitis severity or colonic motility in any of the measures tested.

**Conclusions:** In conclusion, it is unclear if the lumenally restricted 5-HT<sub>4</sub> agonist SYR313832Z has any effect on recovery from DSS colitis. Given inconsistencies with the model and lack of an effect of tegaserod, additional studies will be required, possibly involving different doses and time points, to fully assess the actions of SYR313832Z in colitis recovery.

## II. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important signaling molecule for many physiological systems, and it plays a particularly crucial role within the gastrointestinal (GI) tract. Within the GI tract, 5-HT acts as a neurotransmitter and paracrine signaling molecule to stimulate motility, secretion, vasodilation and sensation (Mawe and Hoffman, 2013). Altered serotonin signaling within the GI tract is implicated in human inflammatory bowel diseases (IBD), such as Crohn's Disease and Ulcerative Colitis (Coates et al., 2017). Serotonin receptors have been the target of therapies for functional gastrointestinal disorders and pain (Mawe and Hoffman, 2013). Unfortunately, use of many of these therapies was suspended or restricted due to reports of adverse cardiovascular events (De Maeyer et al., 2008).

Serotonin exerts its effects through activation of a family of G-protein coupled receptors. One receptor, the 5-HT<sub>4</sub> receptor, is a G-protein coupled receptor, that is G<sub>s</sub> coupled and activates the adenylate cyclase/cyclic adenosine monophosphate (cAMP)/protein kinase A pathway. The 5-HT<sub>4</sub> receptor, is expressed on enteric nerve terminals, where it helps to mediate neurotransmitter release through presynaptic

facilitation, and is amply expressed on colonic epithelial cells, where it mediates 5-HT release from enterochromaffin cells, stimulates chloride secretion and goblet cell degranulation (Pan and Galligan, 1994, Hoffman et al., 2012). Furthermore, activation of mucosal 5-HT<sub>4</sub> receptors has been shown to stimulate propulsive motility, decrease visceral hypersensitivity, promote epithelial proliferation and promote the integrity of the epithelial barrier (Spohn et al., 2016). In the outer layers of the intestines, 5-HT<sub>4</sub> agonist stimulation has been shown to promote neuronal proliferation and survival, and to enhance growth of nerve fibers (Liu et al., 2009, Matsuyoshi et al., 2010, Belkind-Gerson et al., 2015)

The 5-HT<sub>4</sub> receptor, has been identified as a potent therapeutic target for constipation relief in irritable bowel syndrome (IBS) and chronic constipation, as well as pain relief in IBS (Novick et al., 2002, Chey et al., 2008, Tack et al., 2012). Studies from our lab have shown that intracolonic administration of the 5-HT<sub>4</sub> agonist, tegaserod, improves colitis recovery over intraperitoneal administration in experimental models of colitis (Spohn et al., 2016).

This study examined the hypothesis that a lumenally restricted 5-HT<sub>4</sub> agonist, SYR313832Z, would exert protective epithelial actions and restore normal motility patterns in murine dextran sodium sulfate induced colitis. We used a recovery paradigm to investigate whether treatment of animals with active colitis would accelerate recuperation. Outcome measures included recording disease activity index, examining levels of fecal lipocalin-2, histological damage scoring, assessing epithelial proliferation,

and measuring colonic motility. The measures tested in this study reveal no effect of the luminally restricted agonist on colitis.

### **III. Methods**

#### **Animal Care and Use**

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Vermont. The animals used in this study were 8-10 week-old male CD-1 IGS mice (Charles River Laboratories, Saint-Constant, QC, Canada). Animals were euthanized using isoflurane overdose followed by exsanguination.

#### **Experimental Paradigms**

For these studies, the protective epithelial actions of the proprietary, luminally restricted 5-HT<sub>4</sub> agonist, SRY313832Z (832Z), were assessed in a paradigm modeling recovery from induced colitis. 832Z was given to us by Takeda Pharmaceuticals for the purposes of this study. Pharmacokinetics analyses by Takeda indicate that 832Z is not detectable in the blood serum and it is heavily detected in colonic tissue up to 8 hours after gavage administration.

Colitis was induced by addition of 3% or 4% DSS to the drinking water on days 0 through 5. On day 5, DSS water was replaced with tap water. Starting on day 5, mice received daily administration (gavage or enema) of 832Z, tegaserod (positive control) or vehicle for 10 consecutive days (see Table 1 for formulations). Mice were euthanized on

day 15, and colon sections taken for histology and immunohistochemistry. To assess levels of inflammation and colitis severity the following assays were used: disease activity index score, histological damage score, and fecal lipocalin-2 levels. The measures of colonic motility included fecal water content, fecal output and bead expulsion. Any differences in the paradigm or drug administration protocol are noted in the figure legends.

	<b>Gavage</b>	<b>Enema</b>
<b>Vehicle</b>	1% Tween80, 0.5% Methyl Cellulose in tap water	1% DMSO in 0.9% saline
<b>832Z</b>	10 mg/kg 832Z, 1% Tween80, 0.5% Methyl Cellulose in tap water	1 or 10 mg/kg 832Z, 1% DMSO in 0.9% saline
<b>Tegaserod</b>	N/A	1 mg/kg tegaserod, 1% DMSO in 0.9% saline

Table 1. Drug and vehicle formulations for 15 day recovery paradigm.

### **Disease Activity Index**

Disease severity was assessed using a rubric score throughout the course of the disease paradigm. This rubric includes the major pathological landmarks of colitis: weight loss, stool consistency, and fecal blood. Each symptom is rated on a scale from 0 to 4; with a score of 4 being the most severe. The exact scoring criteria were adapted from Cooper et. al. and is described in Table 2 (Cooper et al., 1993). This score was taken every other day through out the experimental paradigm to track the progression of the colitis. For the sake of this study, a score of 4-8 is considered moderate colitis, whereas a score greater than 8 is considered severe colitis.

<b>Score</b>	<b>Weight Loss</b>	<b>Stool Consistency</b>	<b>Occult/gross bleeding</b>
<b>0</b>	None	Normal	Normal
<b>1</b>	1-5%		
<b>2</b>	5-10%	Loose stool	Hemoccult +
<b>3</b>	10-20%		
<b>4</b>	>20%	Diarrhea	Gross bleeding

Table 2. Details of disease activity index rubric.

### **Fecal Lipocalin-2 ELISA**

Fecal pellets were collected over the course of the experiment and stored at -20 degrees Celsius. To prepare samples for the assay, fecal pellets were homogenized in 0.1% Tween20/PBS at 50 mg/mL, and then centrifuged for 5 minutes at 20,000 x g. Supernatant was collected and diluted from 1:5 to 1:6000 depending on the time point of the sample. Two dilutions were performed for each sample using the kit-recommended diluent (1% BSA in PBS). Lipocalin-2 levels were determined using a solid phase sandwich ELISA (Mouse Lipocalin-2/NGAL DuoSet ELISA kit, R&D Systems, Minneapolis, MN), and levels compared between the treatment and control groups at all time points. Dilutions that presented the most accurate duplicates and that fell within the standard curve of the ELISA were used for analysis.

### **Histological Damage Score**

Histological assessment of tissue damage is a classic method to determine the extent of colitis. For this measure, small pieces of distal colon were fixed in 4% PFA and 0.1M sodium phosphate, paraffin embedded, sectioned, and stained using a hematoxylin and eosin (H&E) stain. Sections were blindly scored according to a scoring rubric that is

based on the classic histological features of human inflammatory bowel disease (Villanacci et al., 2013, Spohn et al., 2016). Briefly, the rubric considers infiltration of immune cells, cytoarchitecture, crypt architecture, epithelial integrity and ulcerations. Scores were compared between treatment and control groups by an unpaired students t-test or one-way ANOVA, as appropriate.

### **Proliferation Assay**

Epithelial cell proliferation was assessed using immunohistological staining for the post-mitotic marker Ki-67, a technique that has been used previously to assess epithelial proliferation in human and animal models of colitis (Joly et al., 2009, Hoffman et al., 2012, Spohn et al., 2016). Paraffin-embedded sections of distal colon (same as used for H&E staining), were co-stained for Ki-67 and the nuclear marker DAPI. Within each crypt, the ratio of Ki-67<sup>+</sup> cells:DAPI<sup>+</sup> nuclei was counted. Three crypts were analyzed per tissue section, and then averaged to give a percent of Ki-67<sup>+</sup> cells per crypt. These numbers were compared between groups using an unpaired students t-test.

### **Colonic Motility**

The bead expulsion assay is an *in vivo* assay to assess colonic motility. This assay has been used previously to assess *in vivo* colonic motility in DSS colitis (McClain et al., 2014, Spohn et al., 2016). Briefly, the assay was performed by inserting a glass bead 2 cm into the rectum of a lightly anesthetized mouse and recording the latency from waking of the animal to expulsion of the bead. Bead expulsion data were collected from each

mouse before, during and after inflammation (approximately days 0, 5, and 15). This technique allowed for an *in vivo* measurement of colonic motility at multiple time points. Expulsion times shorter than 30 seconds and longer than 40 minutes were excluded. Expulsion times were either averaged and compared between groups using an unpaired students t-test, or normalized to the day 0 expulsion time, averaged and compared between groups over time using a two-way ANOVA.

### **Fecal Output and Water Content**

Fecal output is the measurement of number of fecal pellets produced during one hour. To measure fecal water content, all pellets were collected during the fecal output assay and immediately placed into closed Eppendorf tubes. At the end of one hour, the total wet weight of all pellets was recorded. Pellets were then dried overnight at 50 degrees Celsius, and the dry weight was obtained the following day. The longer it takes a pellet to pass through the colon, the dryer the pellet will be (i.e. more time for water absorption leads to lower water content in the feces). Additionally, inflammation of the colon affects the absorption and secretion action of the epithelium, and thus inflammation of the colon also leads to increased water content. Fecal output and fecal water content were assessed over the course of the experimental paradigm, and results were compared between vehicle and treatment groups.

## **Data Analysis**

Data are shown as mean +/- SEM for  $n$  number of animals. All data analysis was completed using GraphPad Prism software (version 7.0; GraphPad Software, La Jolla, CA). Data sets were examined before analysis to ensure that test assumptions were met (variability and  $n$  values per group). Significance was determined using unpaired students t-test, one-way ANOVA, or two-way ANOVA, with corrections for multiple comparison, where appropriate. Statistical significance was defined as  $P$  values of less than 0.05.

## **IV. Results**

### **Effect of Treatment on Disease Activity**

Disease activity index (DAI) is an *in vivo* assay of disease severity that can be taken at multiple time points throughout the course of colitis development and recovery. It is common practice in the field to utilize such a rubric to understand disease progression and recovery (Spohn et al., 2016, Wirtz et al., 2017). We tested whether intracolonic treatment of 832Z (10 mg/kg) would improve recovery from colitis, decreasing the DAI relative to the DAI of vehicle treated animals. This effect has previously been seen with intracolonic administration of tegaserod, was blocked by antagonist pre-treatment, and was not seen in 5-HT<sub>4</sub> knockout mice (Spohn et al., 2016).

In the current study, DAI was assessed approximately every other day over the 15-day paradigm (3% DSS for 5 days, 10 days treatment). There was no significant effect of either treatment (Figure 1A and 1B). Many other iterations of this paradigm were performed with different drug delivery methods, different concentrations of drug, and

different concentrations of DSS, and similar results were obtained (Supplemental Figures 1-4).

### **Effect of treatment on Histological Damage**

Clinically diagnosis of colitis typically involves histological evaluation of patient biopsies to determine the extent of inflammation. In experimental colitis, histological evaluation of mucosal architecture and neutrophil infiltration is a common method used to determine the extent of disease (Wirtz et al., 2017). Other methods, such as endoscopy, allow for longitudinal evaluation of mucosal health; however, evaluation of histological damage is the most robust readout. Given previous reports from our lab of improved histological damage with the 5-HT<sub>4</sub> receptor agonist tegaserod, we tested whether histological damage would be alleviated by the 5-HT<sub>4</sub> agonist 832Z.

We found no significant difference in histological damage scores between groups (Figure 1C). Many other iterations of this paradigm were performed with different drug delivery methods, different concentrations of drug, and different concentrations of DSS, and comparable results were obtained (Supplemental Figures 1-4).

### **Effect of treatment on Epithelial Proliferation**

Human and experimental colitis is associated with degradation of the epithelial barrier. It has previously been shown that treatment with a 5-HT<sub>4</sub> agonist promotes epithelial proliferation *in vitro* and *in vivo*, resulting in improved barrier function and improved histological appearance (Spohn et al., 2016). Thus, we expected that

intracolonic treatment with the lumenally restricted 5-HT<sub>4</sub> agonist would result in an increase of recently divided epithelial cells.

Our results indicate that there is no effect of treatment with 832Z on epithelial proliferation (Figure 1D). Many other iterations of this paradigm were performed with different drug delivery methods, different concentrations of drug, and different concentrations of DSS, and similar results were obtained (Supplemental Figures 1-4).

### **Effect of treatment on fecal lipocalin-2 levels**

Recently, lipocalin-2 (Lcn-2), also known as neutrophil gelatinase-associated lipocalin in humans, has been identified as a non-invasive and sensitive biomarker of intestinal inflammation in TNBS, DSS, and IL-10 knockout experimental colitis (Chassaing et al., 2012, Hsieh et al., 2016). Fecal lipocalin levels are correlated with disease severity and pro-inflammatory cytokine levels in DSS-induced colitis and spontaneous colitis in IL-10 deficient mice (Chassaing et al., 2012). We tested whether Lcn-2 levels increased with induction of colitis, and then decreased as mice recovered from colitis. Additionally, we tested whether Lcn-2 levels for animals treated with 832Z and tegaserod returned to baseline faster than vehicle treated animals. To our knowledge, this is the first time lipocalin levels have been tested in CD-1 mice in response to induction of DSS colitis, and more importantly, used to assess the efficacy of therapeutic administration.

We found there was an increase of Lcn-2 levels in response to 3% DSS administration, but no return to baseline during the recovery phase of the paradigm

(Figure 1E; significant effect of day,  $p=0.038$  by two-way ANOVA). With induction at 3% DSS, Lcn-2 levels increased to  $\sim 10,000$  ng/g feces by day 10 post-induction, and remained high through day 15. There were no significant differences between vehicle and agonist treated groups (Figure 1E). A preliminary study was conducted using 2.5% and 3% DSS, and assessing Lcn-2 levels in untreated mice out to day 25. By day 10, levels rose to  $\sim 7000$  ng/g<sup>25</sup>, and only dropped to  $\sim 1000$  ng/g by day 25 (Supplemental Figure 5; not significant). Many other iterations of this paradigm were performed with different drug delivery methods, different concentrations of drug, and different concentrations of DSS, and similar results were obtained (Supplemental Figures 1-4).

### **Effect of treatment on colonic motility**

It has been established that clinical IBD and experimental colitis models are associated with abdominal pain and changes in colonic motility and stool frequency (Linden et al., 2004, Hoffman et al., 2011, Vivinus-Nebot et al., 2014, Spohn et al., 2016). In animal models, it has been shown that colonic motility is decreased in colitis and is associated with inflammation induced neuroplasticity (Hoffman et al., 2011, Roberts et al., 2013). Additionally, it has been shown that treatment with a 5-HT<sub>4</sub> agonist rescues this slowing of colonic motility in mice and guinea pigs (Spohn et al., 2016). Thus, we tested whether treatment with 832Z affected colitis induced dysmotility.

We found no changes in latency of bead expulsion between vehicle and treatment groups when colitis was induced with 3% DSS (Figure 1F). Interestingly, we also did not see the expected slowing of motility from days zero to five in response to colitis

induction. This slowing of motility was noted in one experiment in response to 4% DSS, with a significant effect of treatment (Supplemental Figure 1E; significant effect of treatment,  $p=0.0098$ , by two-way ANOVA). Many other iterations of this paradigm were performed with different drug delivery methods, different concentrations of drug, and different concentrations of DSS, and similar results to that of 3% colitis induction were obtained (Supplemental Figures 1-4).

Fecal output and fecal water content were only assessed in the first iteration of this study (Supplemental Figures 1F and 1G). This experiment, along with others in our lab, revealed that CD-1 mice have relatively high fecal water content at baseline (~60%) as compared to C57BL6/J (data not shown). Given this finding, in addition to the fact that diarrhea is a primary read out of colitis, we decided to discontinue this assay in the colitis models. Additionally, bead expulsion appeared to be a more robust measure of colonic motility than fecal output. Therefore, this assay was discontinued as well.

## **V. Discussion**

This study tested the hypothesis that lumenally restricted 5-HT<sub>4</sub> agonists would induce protective epithelial actions in DSS-induced experimental colitis. The results of this study failed to support the hypothesis. With the conditions that were tested, the lumenally restricted 5-HT<sub>4</sub> agonist did not exert protective effects detectable by our outcome measures. Given previous research, these results are surprising and lead us to believe that this study may not have involved conditions optimal for activity of the lumenally restricted agonist 832Z.

Based on the results reported here, it appears that 832Z does not exert the same protective actions that have previously been published for the 5-HT<sub>4</sub> agonist tegaserod (Spohn et al., 2016). Theoretically, this compound should stimulate the 5-HT<sub>4</sub> receptor and initiate the same protective actions – including stimulating epithelial proliferation, mucus secretion and propulsive motility. Additionally, pharmacokinetic analysis of 832Z performed by Takeda, indicate that this compound is present in colon tissue and the colonic lumen up to 8 hours after gavage administration. While this does not indicate that the drug is active once it reaches the colon, it does imply that there is compound binding to receptors in the wall of the lumen. In our studies, delivery of the compound via enema ensured that the compound was reaching mucosal receptors without being altered by transit through the stomach and the small intestine. However, even with enema administration, no effects of receptor stimulation were seen. This lack of an effect could be explained by incorrect dosing of the compound or incompatible vehicle formulation. Further studies will be required to test these possibilities.

An alternative explanation is that the mechanism of protective actions of tegaserod are not due to activation of mucosal 5-HT<sub>4</sub> receptors. If this were the case, a lumenally restricted 5-HT<sub>4</sub> agonist would be ineffective. It is unknown if epithelial 5-HT<sub>4</sub> receptors are located on the apical or basal side of intestinal epithelial cells. If the receptor is located on the basal side of epithelial cells, an agonist would have to be present in the lamina propria to stimulate receptor actions. This would require a mechanism to transport a lumenally delivered compound from the lumen into the lamina propria. Under normal conditions, transport mechanisms are predominately expressed in

the small intestine, not colonic epithelial cells. However, the transporter protein hPept1 is aberrantly expressed in colonic epithelial cells of UC and CD patients, and have a role in transporting bacterial di- and tri-peptides across the epithelial barrier (Merlin et al., 2001). Another possible explanation is that tegaserod can diffuse across the compromised epithelial barrier known to occur in experimental colitis, and the lumenally restricted 5-HT<sub>4</sub> agonist cannot.

These explanations, while plausible, are unlikely. Previous results from our lab demonstrate that intracolonic administration of tegaserod was significantly more effective than systemic administration (IP) in reducing experimental colitis activity. Additionally, the effect of intracolonic tegaserod was blocked by intraluminal administration of a 5-HT<sub>4</sub> selective antagonist. These results indicate that luminal 5-HT<sub>4</sub> receptor activation is necessary to induce the protective actions, and that it is a 5-HT<sub>4</sub> receptor specific effect. Future investigations involving conditional knockdown of the 5-HT<sub>4</sub> receptor in epithelial cells might be necessary to confirm the involvement of epithelial receptors in the protective actions.

Tegaserod is also a 5-HT<sub>2B</sub> receptor antagonist. 5-HT<sub>2B</sub> receptors are located on longitudinal and circular gastrointestinal smooth muscle cells and neurons within the myenteric plexus (Engel et al., 1984, Borman et al., 2002). Additionally, tegaserod has similar affinity for the 5-HT<sub>2B</sub> receptor as for the 5-HT<sub>4</sub> receptor (Beattie et al., 2004). Therefore, it is possible that any effects of tegaserod on motility could have been complicated by 5-HT<sub>2B</sub> antagonism, but it is improbable that 5-HT<sub>2B</sub> receptor antagonism

would interfere with protective epithelial actions. Furthermore, it is unknown the affinity of the lumenally restricted 5-HT<sub>4</sub> agonist for the 5-HT<sub>2B</sub> receptor.

It is important to note that there were inconsistencies in the model of experimental colitis that was used. We had significant variability in the extent of colitis that was induced, and we were not able to reproduce the protective actions of tegaserod (Figure 1; Supplemental Figures 3 and 4). A possible explanation for the variability in the colitis model is due to the type of DSS used. Previous studies in our lab induced colitis with DSS from MP Biomedicals (Solon, OH; molecular weight: 36,000-50,000), while the current study used DSS from Affymetrix (Cleveland, OH; molecular weight: 40,000-50,000). It is known that dosing and the molecular weight of DSS affect the quality and severity of inflammation (Kitajima et al., 2000). Initial experiments in this study induced colitis with 4% DSS, which resulted in many mice developing severe colitis (histological damage scores >8 at day 5). For later studies, we induced colitis with 3% DSS to stimulate development of a more moderate colitis (histological damage scores 4-8 at day 5). However, even with this reduction in colitis severity, we failed to see a recovery effect of 832Z or tegaserod.

It was surprising that we were unable to recapitulate the effects of tegaserod. This may be attributed to the inconsistencies in the model; therefore, these findings do not overturn the previously reported data by our lab (Spohn et al., 2016).

Lastly, it is plausible that this particular lumenally restricted compound, 832Z, does not promote epithelial wound healing. Given that this is the first time 832Z has been tested *in vivo*, it is possible that we are not seeing effects because this drug does not exert

an effect on the mechanisms addressed in this study. Other research in our lab does demonstrate a prokinetic effect of 832Z administration in naïve animals, and this effect is blocked by a 5-HT<sub>4</sub> antagonist (data not shown). These results are in line with our hypothesis and indicate that the compound is active, and stimulates mucosal 5-HT<sub>4</sub> receptors when administered by gavage or enema.

While this study revealed no effects of the lumenally restricted 5-HT<sub>4</sub> agonist SYR313832Z, there is need for further study to confirm these findings. This study was complicated by inconsistencies in the animal model and a lack of effect of the positive control, tegaserod. While it is possible that this compound does not exert protective actions in colitis, there is not sufficient evidence to make a definitive conclusion.

### **Concluding Remarks**

This study tested the effects of a novel therapeutic agent, a lumenally restricted 5-HT<sub>4</sub> agonist, in an *in vivo* animal model of colitis. The results show that this novel compound does not exert protective epithelial actions or prokinetic effects in dextran sodium sulfate induced colitis. However, further studies need to be done to assess this novel compound with additional assays to make a definitive conclusion.

## VI. Works Cited

- Beattie DT, Smith JA, Marquess D, Vickery RG, Armstrong SR, Pulido-Rios T, McCullough JL, Sandlund C, Richardson C, Mai N, Humphrey PP (2004) The 5-HT<sub>4</sub> receptor agonist, tegaserod, is a potent 5-HT<sub>2B</sub> receptor antagonist in vitro and in vivo. *British journal of pharmacology* 143:549-560.
- Belkind-Gerson J, Hotta R, Nagy N, Thomas AR, Graham H, Cheng L, Solorzano J, Nguyen D, Kamionek M, Dietrich J, Cherayil BJ, Goldstein AM (2015) Colitis induces enteric neurogenesis through a 5-HT<sub>4</sub>-dependent mechanism. *Inflammatory bowel diseases* 21:870-878.
- Borman RA, Tilford NS, Harmer DW, Day N, Ellis ES, Sheldrick RL, Carey J, Coleman RA, Baxter GS (2002) 5-HT<sub>2B</sub> receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro. *British journal of pharmacology* 135:1144-1151.
- Chassaing B, Srinivasan G, Delgado MA, Young AN, Gewirtz AT, Vijay-Kumar M (2012) Fecal Lipocalin 2, a Sensitive and Broadly Dynamic Non-Invasive Biomarker for Intestinal Inflammation. *PloS one* 7:e44328.
- Chey WD, Pare P, Viegas A, Ligozio G, Shetzline MA (2008) Tegaserod for female patients suffering from IBS with mixed bowel habits or constipation: a randomized controlled trial. *The American journal of gastroenterology* 103:1217-1225.
- Coates MD, Tekin I, Vrana KE, Mawe GM (2017) Review article: the many potential roles of intestinal serotonin (5-hydroxytryptamine, 5-HT) signalling in inflammatory bowel disease. *Alimentary pharmacology & therapeutics* 46:569-580.
- Cooper HS, Murthy SN, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69:238-249.
- De Maeyer JH, Lefebvre RA, Schuurkes JA (2008) 5-HT<sub>4</sub> receptor agonists: similar but not the same. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 20:99-112.
- Engel G, Hoyer D, Kalkman HO, Wick MB (1984) Identification of 5HT<sub>2</sub>-receptors on longitudinal muscle of the guinea pig ileum. *Journal of receptor research* 4:113-126.
- Hoffman JM, McKnight ND, Sharkey KA, Mawe GM (2011) The relationship between inflammation-induced neuronal excitability and disrupted motor activity in the

guinea pig distal colon. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 23:673-e279.

Hoffman JM, Tyler K, Maceachern SJ, Balemba OB, Johnson AC, Brooks EM, Zhao H, Swain GM, Moses PL, Galligan JJ, Sharkey KA, Greenwood-Van Meerveld B, Mawe GM (2012) Activation of Colonic Mucosal 5-HT(4) Receptors Accelerates Propulsive Motility and Inhibits Visceral Hypersensitivity. *Gastroenterology* 142:844-854.e844.

Hsieh H, Morin J, Filliettaz C, Varada R, LaBarre S, Radi Z (2016) Fecal Lipocalin-2 as a Sensitive and Noninvasive Biomarker in the TNBS Crohn's Inflammatory Bowel Disease Model. *Toxicologic Pathology* 44:1084-1094.

Joly F, Mayeur C, Messing B, Lavergne-Slove A, Cazals-Hatem D, Noordine M-L, Cherbuy C, Duée P-H, Thomas M (2009) Morphological adaptation with preserved proliferation/transporter content in the colon of patients with short bowel syndrome. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 297:G116-G123.

Kitajima S, Takuma S, Morimoto M (2000) Histological analysis of murine colitis induced by dextran sulfate sodium of different molecular weights. *Experimental animals* 49:9-15.

Linden DR, Sharkey KA, Ho W, Mawe GM (2004) Cyclooxygenase-2 contributes to dysmotility and enhanced excitability of myenteric AH neurones in the inflamed guinea pig distal colon. *The Journal of physiology* 557:191-205.

Liu MT, Kuan YH, Wang J, Hen R, Gershon MD (2009) 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29:9683-9699.

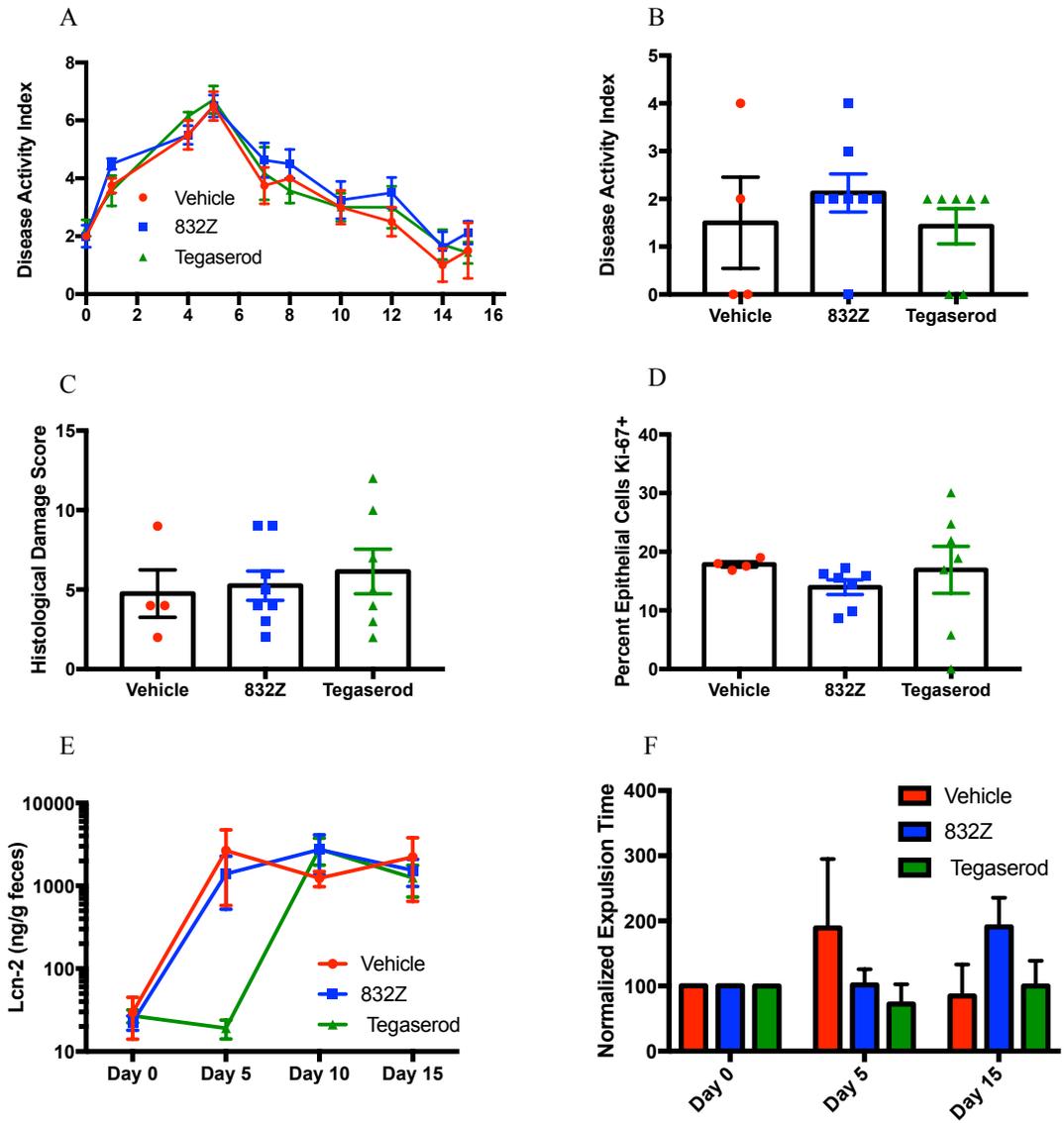
Matsuyoshi H, Kuniyasu H, Okumura M, Misawa H, Katsui R, Zhang GX, Obata K, Takaki M (2010) A 5-HT(4)-receptor activation-induced neural plasticity enhances in vivo reconstructs of enteric nerve circuit insult. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 22:806-813, e226.

Mawe GM, Hoffman JM (2013) Serotonin Signaling in the Gastrointestinal Tract:: Functions, dysfunctions, and therapeutic targets. *Nature reviews Gastroenterology & hepatology* 10:473-486.

McClain JL, Grubisic V, Fried D, Gomez-Suarez RA, Leininger GM, Sevigny J, Parpura V, Gulbransen BD (2014) Ca<sup>2+</sup> responses in enteric glia are mediated by

connexin-43 hemichannels and modulate colonic transit in mice. *Gastroenterology* 146:497-507.e491.

- Merlin D, Si-Tahar M, Sitaraman SV, Eastburn K, Williams I, Liu X, Hediger MA, Madara JL (2001) Colonic epithelial hPepT1 expression occurs in inflammatory bowel disease: Transport of bacterial peptides influences expression of MHC class 1 molecules. *Gastroenterology* 120:1666-1679.
- Novick J, Miner P, Krause R, Glebas K, Bliesath H, Ligozio G, Ruegg P, Lefkowitz M (2002) A randomized, double-blind, placebo-controlled trial of tegaserod in female patients suffering from irritable bowel syndrome with constipation. *Alimentary pharmacology & therapeutics* 16:1877-1888.
- Pan H, Galligan JJ (1994) 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons. *The American journal of physiology* 266:G230-238.
- Roberts JA, Durnin L, Sharkey KA, Mutafova-Yambolieva VN, Mawe GM (2013) Oxidative stress disrupts purinergic neuromuscular transmission in the inflamed colon. *The Journal of physiology* 591:3725-3737.
- Spohn SN, Bianco F, Scott RB, Keenan CM, Linton AA, O'Neill CH, Bonora E, Dickey M, Lavoie B, Wilcox RL, MacNaughton WK, De Giorgio R, Sharkey KA, Mawe GM (2016) Protective Actions of Epithelial 5-Hydroxytryptamine 4 Receptors in Normal and Inflamed Colon. *Gastroenterology*.
- Tack J, Camilleri M, Chang L, Chey WD, Galligan JJ, Lacy BE, Müller-Lissner S, Quigley EMM, Schuurkes J, Maeyer JH, Stanghellini V (2012) Systematic review: cardiovascular safety profile of 5-HT<sub>4</sub> agonists developed for gastrointestinal disorders. *Alimentary pharmacology & therapeutics* 35:745-767.
- Villanacci V, Antonelli E, Geboes K, Casella G, Bassotti G (2013) Histological healing in inflammatory bowel disease: a still unfulfilled promise. *World journal of gastroenterology* 19:968-978.
- Vivinus-Nebot M, Frin-Mathy G, Bziouche H, Dainese R, Bernard G, Anty R, Filippi J, Saint-Paul MC, Tulic MK, Verhasselt V, Hebutterne X, Piche T (2014) Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* 63:744-752.
- Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF (2017) Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature protocols* 12:1295-1309.

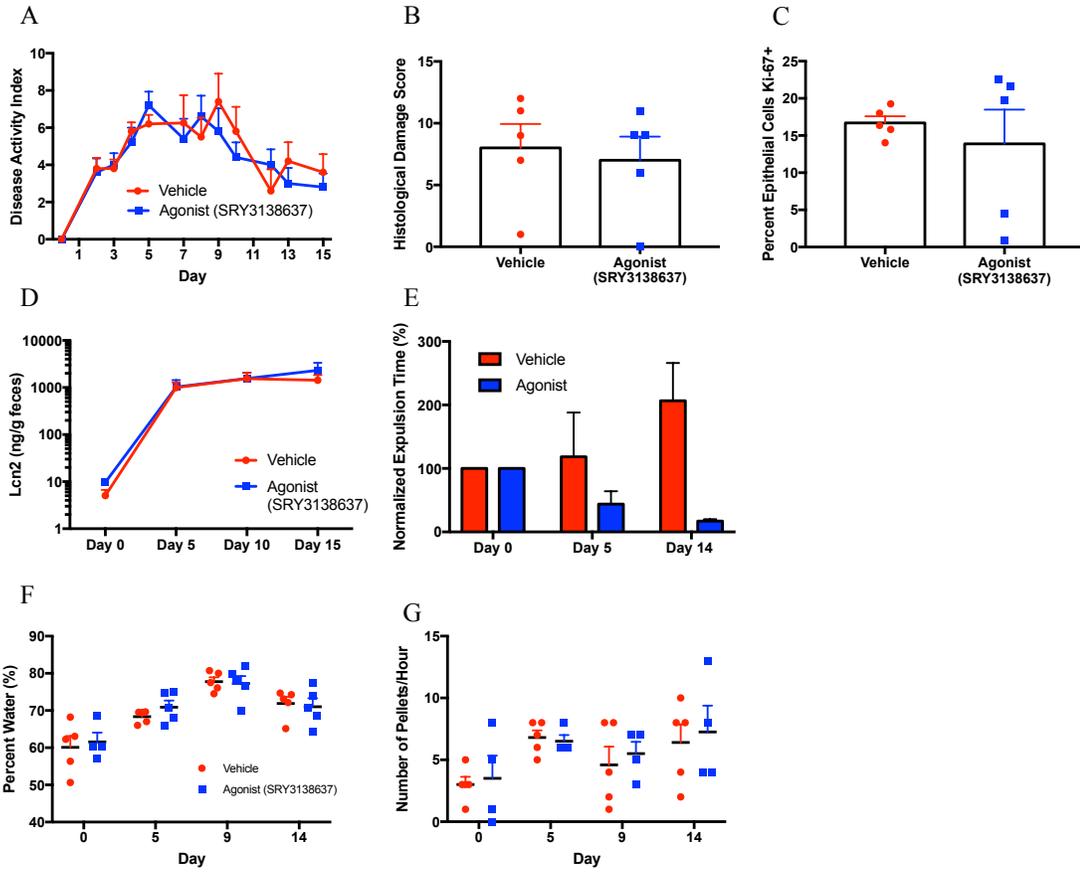


**Figure 1**

## Figure Legend

Figure 1. Results from a 15-day recovery paradigm. Male CD1 mice, 8-10 weeks old.

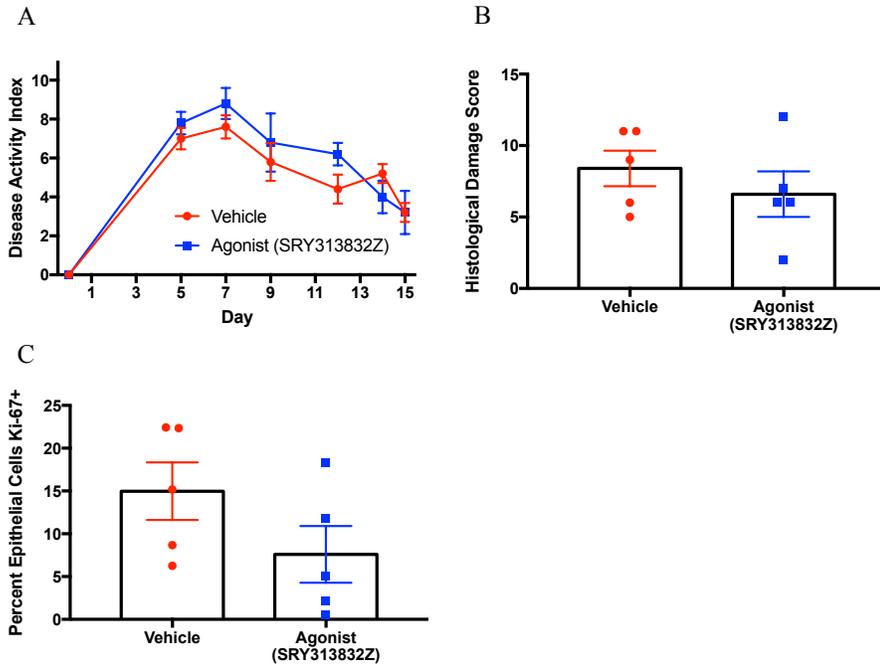
Vehicle n=5\*. 832Z n=8. Tegaserod n=7. Colitis induced with 3% DSS. Enema treatment with vehicle (1% DMSO/0.9% saline), SYR313832Z (10 mg/kg SYR313832Z, 1% DMSO/0.9% saline) or tegaserod (1 mg/kg tegaserod, 1% DMSO/0.9% saline). A) Disease activity index over the course of the paradigm. There were no significant differences in disease activity index ( $p=0.1598$ ), however there was a significant effect of day ( $p<0.0001$ ) via 2-way ANOVA. B) Disease activity index for day 15. C) Histological damage scores ( $p=0.7545$  by one-way ANOVA). D) Percent Epithelial cells Ki67<sup>+</sup>, reported as a ratio of Ki67<sup>+</sup> cells:DAPI stained nuclei. Each data point represents one animal, as an average of 3 crypts. ( $p=0.06217$  by one-way ANOVA). E) Fecal Lipocalin-2 levels for days 0, 5, 10 and 15. There is no significant effect of treatment ( $p=0.7123$ ), but there is a significant effect of day ( $p=0.038$ ) by two-way ANOVA. F) Bead expulsion times for Days 0, 5, and 15, reported as percent of day 0 expulsion time. All times are calculated as the latency from wake to expulsion, and the normalized to the day 0 expulsion time. There was no significant effect of treatment ( $p=0.4298$ ) or day ( $p=0.7280$ ) by two-way ANOVA. \*One mouse was euthanized on day 9 due to >30% weight loss and lethargy as per protocol.



Supplemental Figure 1

## Figure Legend

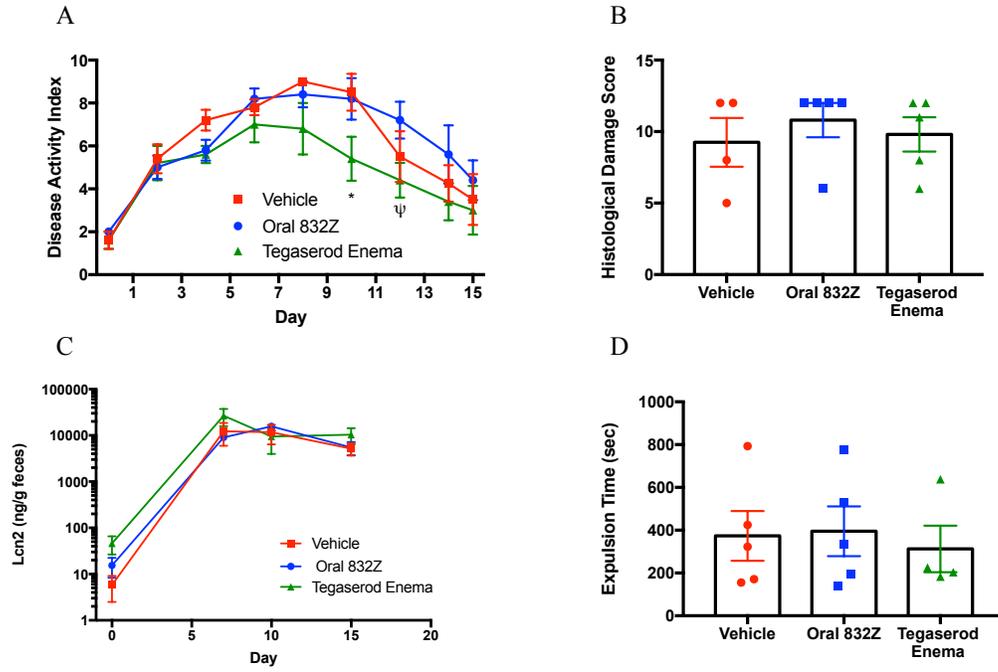
Supplemental Figure 1. Results from a 15-day recovery paradigm. Male CD1 mice, 8-10 weeks old, 5 mice per group. Colitis induced with 4% DSS. Gavage treatment with vehicle (0.5% methylcellulose, 50 mM citrate monohydrate in double distilled water, pH 3.0) or agonist (10 mg/kg SYR3138637, 0.5% methylcellulose, 50 mM citrate monohydrate in double distilled water, pH 3.0). A) Disease activity index over the course of the paradigm showed no significant effect of treatment ( $p=0.5103$ ), but there was a significant effect of day ( $p<0.0001$ ) by two-way ANOVA. Mice reached a peak average score of  $\sim 7$ , indicating moderate to severe colitis. B) Histological damage scores showed no significant differences between groups ( $p=0.7245$  by unpaired t-test). C) Bead expulsion times for Days 0, 5, and 14, reported as percent of day 0 expulsion time. All times are calculated as the latency from wake to expulsion, and the normalized to the day 0 expulsion time. Significant effect of treatment by two-way ANOVA ( $p=.0098$ ). D) Percent Epithelial cells Ki67<sup>+</sup>, reported as a ratio of Ki67<sup>+</sup> cells:DAPI stained nuclei. Each data point represents one animal, as an average of 3 crypts. There were no significant differences between groups ( $p=0.5644$  by unpaired t-test). E) Lcn-2 levels as detected by ELISA showed no significant differences between groups ( $p=0.5264$ ), but there as a significant effect of day ( $p=0.0052$ ) by two-way ANOVA. F) Fecal output showed no effect of treatment ( $p=0.6006$ ), but there was a significant effect of day ( $p=0.0356$ ) by two-way ANOVA. G) Fecal water content demonstrated to effect of treatment ( $p=0.6418$ ), but there was a significant effect of day ( $p<0.0001$ ) by two-way ANOVA.



**Supplemental Figure 2**

## Figure Legend

Supplemental Figure 2. Results from a 15-day recovery paradigm. Male CD1 mice, 8-10 weeks old, 5 mice per group. Colitis induced with 4% DSS. Gavage treatment with vehicle (1% Tween80/Tap water) or agonist (10 mg/kg SYR313832Z, 1% Tween80/Tap water). A) Disease activity index over the 15-day paradigm demonstrated no effect of treatment ( $p=0.2123$ ), but a significant effect of day ( $p<0.0001$ ) by two-way ANOVA. B) Histological damage scores demonstrated no significant differences between treatment ( $p=0.4011$ ) by an unpaired t-test. C) Percent Epithelial cells Ki67<sup>+</sup>, reported as a ratio of Ki67<sup>+</sup> cells:DAPI stained nuclei. Each data point represents one animal, as an average of 3 crypts. There was no significant difference between groups ( $p=0.1557$ ) by an unpaired t-test.

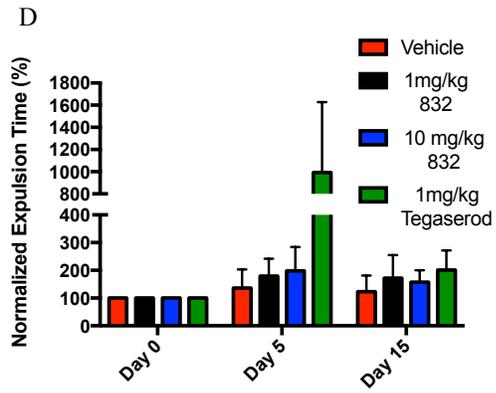
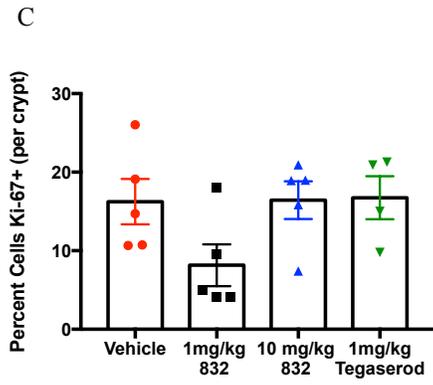
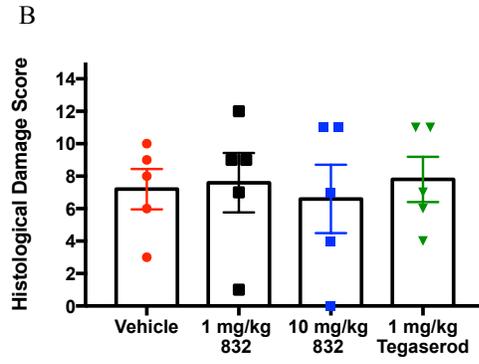
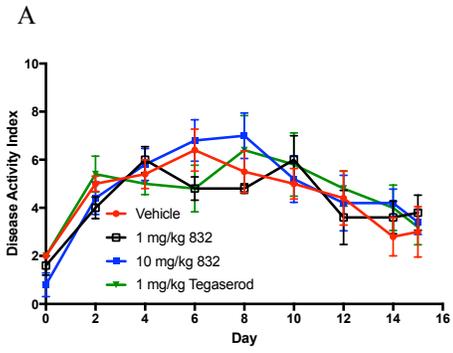


**Supplemental Figure 3**

## Figure Legend

Supplemental Figure 3. Results from a 15-day recovery paradigm. Male CD1 mice, 8-10 weeks old, 5 mice per group. Colitis induced with 4% DSS. Gavage treatment with vehicle (1% Tween80/Tap water), agonist (10 mg/kg SYR313832Z, 1% Tween80/Tap water) or enema treatment with tegaserod (1 mg/kg tegaserod, 1% DMSO/0.9% saline).

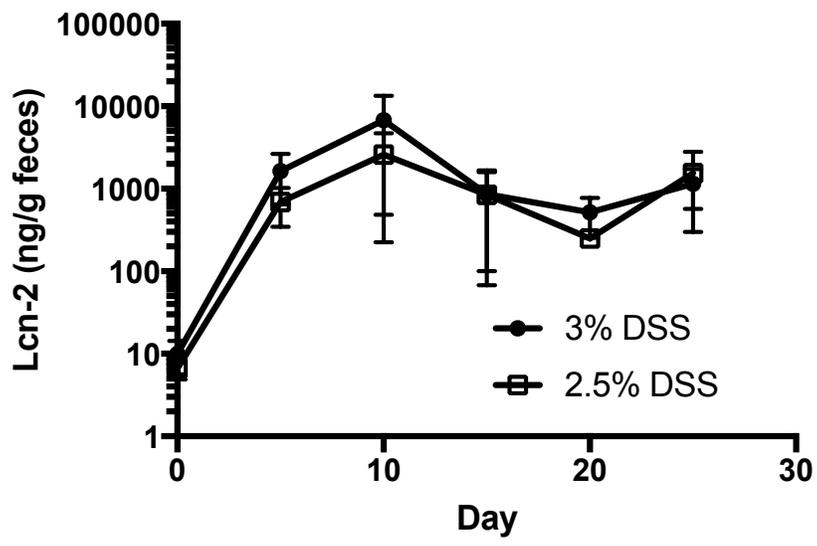
A) Disease activity index scores over the course of the paradigm. Tegaserod improved DAI quicker than oral 832Z or vehicle, as seen by significant difference on days 8 and 10 (\* $p < 0.05$  between Tegaserod and both vehicle and oral 832Z;  $\psi$   $p < 0.05$  between Tegaserod and oral 832Z; analysis by two-way ANOVA with multiple comparisons, Bonferroni corrections). B) Histological damage scores demonstrated no significant differences between treatments at day 15 ( $p = 0.7214$ ) by one-way ANOVA. C) Fecal Lipocalin-2 levels on days 0, 5, 7 and 15 as detected by ELISA demonstrated no effect of treatment ( $p = 0.3208$ ), but a significant effect of day ( $p = 0.0009$ ) by two-way ANOVA. D) Raw bead expulsion times, shown as latency from wake to expulsion in seconds demonstrate no significant effect of treatment ( $p = 0.4642$ ) or day ( $p = 0.9734$ ) by two-way ANOVA.



**Supplemental Figure 4**

## Figure Legend

Supplemental Figure 4. Results from a 15-day recovery paradigm. Male CD1 mice, 8-10 weeks old, 5 mice per group. Colitis induced with 4% DSS. Enema treatment with vehicle (1% DMSO/0.9% saline), SYR313832Z (10 mg/kg or 1 mg/kg SYR313832Z, 1% DMSO/0.9% saline) or tegaserod (1 mg/kg tegaserod, 1% DMSO/0.9% saline). A) Disease activity index scores over the course of the paradigm demonstrated no effect of treatment ( $p=0.6824$ ), but a significant effect of day ( $p<0.0001$ ) by two-way ANOVA. B) Histological damage scores demonstrate no effect of treatment ( $p=0.9594$ ) on day 15 by one-way ANOVA. C) Percent Epithelial cells Ki67<sup>+</sup>, reported as a ratio of Ki67<sup>+</sup> cells:DAPI stained nuclei. Each data point represents one animal, as an average of 3 crypts. There was no significant difference between groups ( $p=0.1017$ ) by one-way ANOVA. D) Bead expulsion times for Days 0, 5, and 15, reported as percent of day 0 expulsion time. All times are calculated as the latency from wake to expulsion, and the normalized to the day 0 expulsion time. There was no significant effect of treatment ( $p=0.1692$ ) or day ( $p=0.1116$ ) by two-way ANOVA.



Supplemental Figure 5

### **Figure Legend**

Supplemental Figure 5. Results from a preliminary experiment inducing colitis with either 3% or 2.5% DSS and collected fecal samples for Lcn-2 ELISA at days 5, 10, 15, 20 and 25. CD1 male mice, aged 8 weeks, 3 mice per group. There was no significant effect of treatment ( $p=0.4487$ ) or day ( $p=0.2090$ ) by two-way ANOVA.

## **CHAPTER III: FINAL CONCLUSIONS AND FUTURE DIRECTIONS**

### **I. Summary and Conclusions**

There is a drastic need for novel therapeutic agents for IBD. The current therapies, including anti-inflammatory agents, immunosuppressants, and monoclonal antibodies, do not serve all patient needs. A unique target for treatment in IBD is the epithelial barrier. Compromised barrier function, or “leaky gut”, is a common feature of IBD and may even contribute to the pathogenesis. This study aimed to explore mucosal 5-HT<sub>4</sub> receptor activation using a lumenally restricted 5-HT<sub>4</sub> agonist as a novel therapeutic target for IBD and other functional bowel disorders.

Results from my study reveal that the lumenally restricted 5-HT<sub>4</sub> agonist failed to exert protective effects in DSS induced colitis. These results were surprising and prompt the use of other paradigms and techniques to fully reveal the effects of this compound.

### **II. Future Directions**

A primary concern of this study was the inconsistencies and variability within the colitis model. It is known that induction of DSS colitis is dependent on both the mouse strain and the molecular weight of DSS used (Kitajima et al., 2000, Chassaing et al., 2014). I think it would be beneficial for future studies to utilize a different mouse strain or DSS from a different company to explore the protective actions of 832Z. C57BL/6 mice are another commonly used strain to study DSS colitis, and I think attempting a recovery paradigm with this strain would be beneficial. Additionally, other labs have shown recovery of fecal lipocalin-2 levels in C57BL/6 mice. Given the lack of return to

baseline of fecal lipocalin-2 levels in the CD-1 mice used in this study, it would be beneficial to utilize a strain where we know that this biomarker can indicate a reduction in inflammation. Furthermore, our lab has demonstrated prokinetic effects of 832Z in naïve C57BL/6 mice, but not CD-1 mice. We initially used CD-1 mice for the colitis studies based on the previous work in our lab showing protective epithelial actions of tegaserod in CD-1 mice (Spohn et al., 2016). However, the CD-1 mice used in this study had hemocult positive stool at baseline, and sometimes soft stool, indicating a slight dysregulation of epithelial barrier homeostasis prior to induction of colitis. We were unable to identify the cause of these symptoms. C57BL/6 mice concurrently used in our lab did not show the same symptoms. Therefore, I think it would be beneficial to test this same hypothesis using C57BL/6 mice given that they are healthy at baseline and we have seen effects of the compound on gastrointestinal motility. Additionally, I propose using a more sensitive disease activity index rubric for the daily disease scoring. A protocol update for chemically induced models of colitis included a disease activity index rubric with more detailed scoring of stool consistency and fecal blood (see table 3) (Wirtz et al., 2017). Utilization of this more detailed DAI rubric would take away some of the inherent subjectivity of scoring stool consistency.

<b>Score</b>	<b>Weight Loss</b>	<b>Stool Consistency</b>	<b>Occult/gross bleeding</b>
<b>0</b>	None	Normal	Negative hemocult
<b>1</b>	1-5%	Soft but still formed	
<b>2</b>	5-10%	Soft	Positive hemocult
<b>3</b>	10-20%	Very soft; wet	Blood traces in stool
<b>4</b>	>20%	Watery diarrhea	Gross rectal bleeding

Table 3. Scoring system for disease activity index from Wirtz et al. (2017)

Given the null results of this study in an *in vivo* model, I also suggest testing the actions of 832Z with *in vitro* and *ex vivo* assays that specifically test the effects on barrier function and wound healing. It is known that barrier function is disrupted in colitis and experimental models of colitis (Stein et al., 1998, Turner, 2009). One of the three primary actions of 5-HT<sub>4</sub> agonists identified by our lab is improvement of barrier function, facilitated by improved wound healing in colitis. Wound healing can be tested *in vitro* using a scratch assay in Caco-2 cells, which specifically measures epithelial migration. Caco-2 cells are a human epithelial adenocarcinoma cell line that are frequently used to assess properties of intestinal epithelial cells *in vitro*. Previous reports in our lab demonstrate that tegaserod improves closure of the scratch over vehicle control, and that this is blocked by co-administration of a 5-HT<sub>4</sub> selective antagonist (Spohn et al., 2016). Another important component of wound healing is the rate of apoptosis. Reactive oxygen species caused by oxidative stress are present in colitis and contribute to epithelial apoptosis (Shi et al., 2011, Novak and Mollen, 2015, Spohn et al., 2016). To assess the effect of 832Z on reactive oxygen species induced apoptosis, Caco-2 cells are pretreated with the compound, exposed to H<sub>2</sub>O<sub>2</sub>, and then the percent of cells undergoing apoptosis is measured. This question could also be answered *in vivo* by using a colitis prevention paradigm and performing a TUNEL assay on sections of colon after treatment. Barrier function or intestinal permeability can be specifically tested *ex vivo* or *in vitro* using Ussing chamber assays or measuring transepithelial resistance of cultured cells, respectively.

This study used one method to assess colonic motility in inflamed mice. There are many other assays, some more sensitive than bead expulsion, that can be utilized. Current data from our lab indicate prokinetic effects with acute treatment of 832Z in naïve mice using measures of whole gastrointestinal transit. Additionally, smaller motor events such as colonic migrating motor complexes and slow wave motor events can be video-captured from *ex vivo* colon preparations and analyzed to show changes in these more minute phenomena. These *ex vivo* assays can be performed after administration of compound to the animal, or with application in the *ex vivo* bath preparation. Lastly, the integrity of the neuromuscular junction can be assessed using intracellular electrophysiology to record junction potentials from longitudinal smooth muscle cells.

In conclusion, there are many other detection measures that can be utilized to assess the effects of the lumenally restricted 5-HT<sub>4</sub> agonist 832Z. While my study did not demonstrate any effects of this compound, it was the first study to explore the novel therapeutic mechanism of lumenally restricted compounds in a model of experimental colitis. Additionally, this was the first study to my knowledge assessing fecal lipocalin-2 levels in CD-1 mice in response to a therapeutic treatment in a model of colitis. These results will inform future studies into the protective epithelial actions of this and future lumenally restricted 5-HT<sub>4</sub> agonists.

## Works Cited

- Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M (2014) Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice. *Current protocols in immunology* / edited by John E Coligan [et al] 104:Unit-15.25.
- Kitajima S, Takuma S, Morimoto M (2000) Histological analysis of murine colitis induced by dextran sulfate sodium of different molecular weights. *Experimental animals* 49:9-15.
- Novak EA, Mollen KP (2015) Mitochondrial dysfunction in inflammatory bowel disease. *Frontiers in Cell and Developmental Biology* 3:62.
- Shi X-Z, Winston JH, Sarna SK (2011) Differential immune and genetic responses in rat models of Crohn's colitis and ulcerative colitis. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 300:G41-G51.
- Spohn SN, Bianco F, Scott RB, Keenan CM, Linton AA, O'Neill CH, Bonora E, Dickey M, Lavoie B, Wilcox RL, MacNaughton WK, De Giorgio R, Sharkey KA, Mawe GM (2016) Protective Actions of Epithelial 5-Hydroxytryptamine 4 Receptors in Normal and Inflamed Colon. *Gastroenterology*.
- Stein J, Ries J, Barrett KE (1998) Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. *The American journal of physiology* 274:G203-209.
- Turner JR (2009) Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 9:799-809.
- Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF (2017) Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature protocols* 12:1295-1309.

## COMPREHENSIVE BIBLIOGRAPHY

- Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel J-F, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RSN, Floyd JAB, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot J-P, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms L, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra H-J, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskis L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD (2011) Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nature genetics* 43:246-252.
- Beattie DT, Smith JA, Marquess D, Vickery RG, Armstrong SR, Pulido-Rios T, McCullough JL, Sandlund C, Richardson C, Mai N, Humphrey PP (2004) The 5-HT<sub>4</sub> receptor agonist, tegaserod, is a potent 5-HT<sub>2B</sub> receptor antagonist in vitro and in vivo. *British journal of pharmacology* 143:549-560.
- Belkind-Gerson J, Hotta R, Nagy N, Thomas AR, Graham H, Cheng L, Solorzano J, Nguyen D, Kamionek M, Dietrich J, Cherayil BJ, Goldstein AM (2015) Colitis induces enteric neurogenesis through a 5-HT<sub>4</sub>-dependent mechanism. *Inflammatory bowel diseases* 21:870-878.
- Bewtra M, Su C, Lewis JD (2007) Trends in hospitalization rates for inflammatory bowel disease in the United States. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 5:597-601.
- Boirivant M, Fuss IJ, Chu A, Strober W (1998) Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *The Journal of experimental medicine* 188:1929-1939.
- Borman RA, Tilford NS, Harmer DW, Day N, Ellis ES, Sheldrick RL, Carey J, Coleman RA, Baxter GS (2002) 5-HT<sub>2B</sub> receptors play a key role in mediating the

excitatory effects of 5-HT in human colon in vitro. *British journal of pharmacology* 135:1144-1151.

Bradbury NA (2000) Protein kinase-A-mediated secretion of mucin from human colonic epithelial cells. *Journal of cellular physiology* 185:408-415.

Budhoo MR, Harris RP, Kellum JM (1996) 5-Hydroxytryptamine-induced Cl<sup>-</sup> transport is mediated by 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors in the rat distal colon. *European journal of pharmacology* 298:137-144.

Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M (2014) Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice. *Current protocols in immunology* / edited by John E Coligan [et al] 104:Unit-15.25.

Chassaing B, Srinivasan G, Delgado MA, Young AN, Gewirtz AT, Vijay-Kumar M (2012) Fecal Lipocalin 2, a Sensitive and Broadly Dynamic Non-Invasive Biomarker for Intestinal Inflammation. *PloS one* 7:e44328.

Chey WD, Pare P, Viegas A, Ligozio G, Shetzline MA (2008) Tegaserod for female patients suffering from IBS with mixed bowel habits or constipation: a randomized controlled trial. *The American journal of gastroenterology* 103:1217-1225.

Cho JH, Brant SR (2011) Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 140:1704-1712.

Coates MD, Lahoti M, Binion DG, Szigethy EM, Regueiro MD, Bielefeldt K (2013) Abdominal pain in ulcerative colitis. *Inflammatory bowel diseases* 19:2207-2214.

Coates MD, Tekin I, Vrana KE, Mawe GM (2017) Review article: the many potential roles of intestinal serotonin (5-hydroxytryptamine, 5-HT) signalling in inflammatory bowel disease. *Alimentary pharmacology & therapeutics* 46:569-580.

Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P (2010) Infliximab, azathioprine, or combination therapy for Crohn's disease. *The New England journal of medicine* 362:1383-1395.

Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A (2010) NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 16:90-97.

Cooper HS, Murthy SN, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69:238-249.

- Dahlhamer JZ, EP; Ward, BW; Wheaton, AG; Croft, JB (2016) Prevalence of Inflammatory Bowel Disease Among Adults Aged  $\geq 18$  Years — United States, 2015. *Morbidity and Mortality Weekly Report* 65:1166-1169.
- De Maeyer JH, Lefebvre RA, Schuurkes JA (2008) 5-HT<sub>4</sub> receptor agonists: similar but not the same. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 20:99-112.
- Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, Van Rees EP (1998) Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clinical and experimental immunology* 114:385-391.
- Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO (1994) Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 107:1643-1652.
- Drolet B, Khalifa M, Daleau P, Hamelin BA, Turgeon J (1998) Block of the rapid component of the delayed rectifier potassium current by the prokinetic agent cisapride underlies drug-related lengthening of the QT interval. *Circulation* 97:204-210.
- Engel G, Hoyer D, Kalkman HO, Wick MB (1984) Identification of 5HT<sub>2</sub>-receptors on longitudinal muscle of the guinea pig ileum. *Journal of receptor research* 4:113-126.
- Erspamer V VM (1937) Ricerche sul secreto delle cellule enterocromaffini. *Boll d Soc Med-chir Pavia* 51:357-363.
- Evans BW, Clark WK, Moore DJ, Whorwell PJ (2007) Tegaserod for the treatment of irritable bowel syndrome and chronic constipation. *The Cochrane database of systematic reviews* Cd003960.
- Fiorucci S, Mencarelli A, Palazzetti B, Sprague AG, Distrutti E, Morelli A, Novobrantseva TI, Cirino G, Kotliansky VE, de Fougères AR (2002) Importance of innate immunity and collagen binding integrin  $\alpha 1\beta 1$  in TNBS-induced colitis. *Immunity* 17:769-780.
- Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Buning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T,

Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panes J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M (2010) Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nature genetics* 42:1118-1125.

Greenwood-Van Meerveld B, Venkova K, Hicks G, Dennis E, Crowell MD (2006) Activation of peripheral 5-HT receptors attenuates colonic sensitivity to intraluminal distension. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 18:76-86.

Grider JR, Foxx-Orenstein AE, Jin JG (1998) 5-Hydroxytryptamine<sub>4</sub> receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. *Gastroenterology* 115:370-380.

Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD (2005) Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 129:550-564.

Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W (2002) Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* 17:629-638.

Hoffman JM, McKnight ND, Sharkey KA, Mawe GM (2011) The relationship between inflammation-induced neuronal excitability and disrupted motor activity in the guinea pig distal colon. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 23:673-e279.

Hoffman JM, Tyler K, Maceachern SJ, Balemba OB, Johnson AC, Brooks EM, Zhao H, Swain GM, Moses PL, Galligan JJ, Sharkey KA, Greenwood-Van Meerveld B, Mawe GM (2012) Activation of Colonic Mucosal 5-HT<sub>4</sub> Receptors Accelerates Propulsive Motility and Inhibits Visceral Hypersensitivity. *Gastroenterology* 142:844-854.e844.

Hsieh H, Morin J, Filliettaz C, Varada R, LaBarre S, Radi Z (2016) Fecal Lipocalin-2 as a Sensitive and Noninvasive Biomarker in the TNBS Crohn's Inflammatory Bowel Disease Model. *Toxicologic Pathology* 44:1084-1094.

- Jin JG, Foxx-Orenstein AE, Grider JR (1999) Propulsion in guinea pig colon induced by 5-hydroxytryptamine (HT) via 5-HT<sub>4</sub> and 5-HT<sub>3</sub> receptors. *The Journal of pharmacology and experimental therapeutics* 288:93-97.
- Joly F, Mayeur C, Messing B, Lavergne-Slove A, Cazals-Hatem D, Noordine M-L, Cherbuy C, Duée P-H, Thomas M (2009) Morphological adaptation with preserved proliferation/transporter content in the colon of patients with short bowel syndrome. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 297:G116-G123.
- Kappelman MD, Rifas-Shiman SL, Porter C, Ollendorf DA, Sandler RS, Galanko JA, Finkelstein JA (2008) Direct Health Care Costs of Crohn's Disease and Ulcerative Colitis in United States Children and Adults. *Gastroenterology* 135:1907-1913.
- Kiesler P, Fuss IJ, Strober W (2015) Experimental Models of Inflammatory Bowel Diseases. *Cellular and molecular gastroenterology and hepatology* 1:154-170.
- Kitajima S, Takuma S, Morimoto M (2000) Histological analysis of murine colitis induced by dextran sulfate sodium of different molecular weights. *Experimental animals* 49:9-15.
- Krishnan A, Korzenik JR (2002) Inflammatory bowel disease and environmental influences. *Gastroenterology clinics of North America* 31:21-39.
- Krug SM, Bojarski C, Fromm A, Lee IM, Dames P, Richter JF, Turner JR, Fromm M, Schulzke JD (2017) Tricellulin is regulated via interleukin-13-receptor alpha2, affects macromolecule uptake, and is decreased in ulcerative colitis. *Mucosal immunology*.
- Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75:263-274.
- Laroui H, Ingersoll SA, Liu HC, Baker MT, Ayyadurai S, Charania MA, Laroui F, Yan Y, Sitaraman SV, Merlin D (2012) Dextran Sodium Sulfate (DSS) Induces Colitis in Mice by Forming Nano-Lipocomplexes with Medium-Chain-Length Fatty Acids in the Colon. *PloS one* 7:e32084.
- Linden DR, Sharkey KA, Ho W, Mawe GM (2004) Cyclooxygenase-2 contributes to dysmotility and enhanced excitability of myenteric AH neurones in the inflamed guinea pig distal colon. *The Journal of physiology* 557:191-205.
- Liu MT, Kuan YH, Wang J, Hen R, Gershon MD (2009) 5-HT<sub>4</sub> receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *The*

Journal of neuroscience : the official journal of the Society for Neuroscience  
29:9683-9699.

- Loddo I, Romano C (2015) Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. *Front Immunol* 6:551.
- Loughlin J, Quinn S, Rivero E, Wong J, Huang J, Kralstein J, Earnest DL, Seeger JD (2010) Tegaserod and the risk of cardiovascular ischemic events: an observational cohort study. *Journal of cardiovascular pharmacology and therapeutics* 15:151-157.
- Matsuoka K, Kobayashi T, Ueno F, Matsui T, Hirai F, Inoue N, Kato J, Kobayashi K, Kobayashi K, Koganei K, Kunisaki R, Motoya S, Nagahori M, Nakase H, Omata F, Saruta M, Watanabe T, Tanaka T, Kanai T, Noguchi Y, Takahashi KI, Watanabe K, Hibi T, Suzuki Y, Watanabe M, Sugano K, Shimosegawa T (2018) Evidence-based clinical practice guidelines for inflammatory bowel disease. *Journal of gastroenterology*.
- Matsuyoshi H, Kuniyasu H, Okumura M, Misawa H, Katsui R, Zhang GX, Obata K, Takaki M (2010) A 5-HT(4)-receptor activation-induced neural plasticity enhances in vivo reconstructs of enteric nerve circuit insult. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 22:806-813, e226.
- Mawe GM, Hoffman JM (2013) Serotonin Signaling in the Gastrointestinal Tract:: Functions, dysfunctions, and therapeutic targets. *Nature reviews Gastroenterology & hepatology* 10:473-486.
- McCallum RW, Prakash C, Campoli-Richards DM, Goa KL (1988) Cisapride. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use as a prokinetic agent in gastrointestinal motility disorders. *Drugs* 36:652-681.
- McClain JL, Grubisic V, Fried D, Gomez-Suarez RA, Leininger GM, Sevigny J, Parpura V, Gulbransen BD (2014) Ca<sup>2+</sup> responses in enteric glia are mediated by connexin-43 hemichannels and modulate colonic transit in mice. *Gastroenterology* 146:497-507.e491.
- Merlin D, Si-Tahar M, Sitaraman SV, Eastburn K, Williams I, Liu X, Hediger MA, Madara JL (2001) Colonic epithelial hPepT1 expression occurs in inflammatory bowel disease: Transport of bacterial peptides influences expression of MHC class 1 molecules. *Gastroenterology* 120:1666-1679.

- Miyazawa F, Olijnyk OR, Tilley CJ, Tamaoki T (1967) Interactions between dextran sulfate and Escherichia coli ribosomes. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 145:96-104.
- Nagakura Y, Akuzawa S, Miyata K, Kamato T, Suzuki T, Ito H, Yamaguchi T (1999) Pharmacological properties of a novel gastrointestinal prokinetic benzamide selective for human 5-HT<sub>4</sub> receptor versus human 5-HT<sub>3</sub> receptor. *Pharmacological research* 39:375-382.
- Neurath MF (2017) Current and emerging therapeutic targets for IBD. *Nat Rev Gastroenterol Hepatol* 14:269-278.
- Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W (1995) Antibodies to interleukin 12 abrogate established experimental colitis in mice. *The Journal of experimental medicine* 182:1281-1290.
- Ning Y, Zhu JX, Chan HC (2004) Regulation of ion transport by 5-hydroxytryptamine in rat colon. *Clinical and experimental pharmacology & physiology* 31:424-428.
- Novak EA, Mollen KP (2015) Mitochondrial dysfunction in inflammatory bowel disease. *Frontiers in Cell and Developmental Biology* 3:62.
- Novick J, Miner P, Krause R, Glebas K, Bliesath H, Ligozio G, Ruegg P, Lefkowitz M (2002) A randomized, double-blind, placebo-controlled trial of tegaserod in female patients suffering from irritable bowel syndrome with constipation. *Alimentary pharmacology & therapeutics* 16:1877-1888.
- Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98:694-702.
- Olesen CM, Coskun M, Peyrin-Biroulet L, Nielsen OH (2016) Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases. *Pharmacology & therapeutics* 159:110-119.
- Pan H, Galligan JJ (1994) 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors mediate inhibition and facilitation of fast synaptic transmission in enteric neurons. *The American journal of physiology* 266:G230-238.
- Panaccione R, Ghosh S, Middleton S, Marquez JR, Scott BB, Flint L, van Hoogstraten HJ, Chen AC, Zheng H, Danese S, Rutgeerts P (2014) Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology* 146:392-400.e393.

- Potet F, Bouyssou T, Escande D, Baro I (2001) Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-a-gogo K(+) channel. *The Journal of pharmacology and experimental therapeutics* 299:1007-1012.
- Rapport MM, Green AA, Page IH (1948) Partial purification of the vasoconstrictor in beef serum. *The Journal of biological chemistry* 174:735-741.
- Roberts JA, Durnin L, Sharkey KA, Mutafova-Yambolieva VN, Mawe GM (2013) Oxidative stress disrupts purinergic neuromuscular transmission in the inflamed colon. *The Journal of physiology* 591:3725-3737.
- Shi X-Z, Winston JH, Sarna SK (2011) Differential immune and genetic responses in rat models of Crohn's colitis and ulcerative colitis. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 300:G41-G51.
- Silverthorn DJ, BR; Ober, WC; Garrison, CW; Silverthorn, AC (2013) *Human Physiology: and Integrated Approach*. Pearson.
- Spencer DM, Veldman GM, Banerjee S, Willis J, Levine AD (2002) Distinct inflammatory mechanisms mediate early versus late colitis in mice. *Gastroenterology* 122:94-105.
- Spohn SN, Bianco F, Scott RB, Keenan CM, Linton AA, O'Neill CH, Bonora E, Dickey M, Lavoie B, Wilcox RL, MacNaughton WK, De Giorgio R, Sharkey KA, Mawe GM (2016) Protective Actions of Epithelial 5-Hydroxytryptamine 4 Receptors in Normal and Inflamed Colon. *Gastroenterology*.
- Spohn SN, Mawe GM (2017) Non-conventional features of peripheral serotonin signalling - the gut and beyond. *Nat Rev Gastroenterol Hepatol* 14:412-420.
- Stein J, Ries J, Barrett KE (1998) Disruption of intestinal barrier function associated with experimental colitis: possible role of mast cells. *The American journal of physiology* 274:G203-209.
- Strober W, Fuss IJ (2011) Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140:1756-1767.
- Tack J, Camilleri M, Chang L, Chey WD, Galligan JJ, Lacy BE, Müller-Lissner S, Quigley EMM, Schuurkes J, Maeyer JH, Stanghellini V (2012) Systematic review: cardiovascular safety profile of 5-HT(4) agonists developed for gastrointestinal disorders. *Alimentary pharmacology & therapeutics* 35:745-767.
- Tonini M, Galligan JJ, North RA (1989) Effects of cisapride on cholinergic neurotransmission and propulsive motility in the guinea pig ileum. *Gastroenterology* 96:1257-1264.

- Turner JR (2009) Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 9:799-809.
- Villanacci V, Antonelli E, Geboes K, Casella G, Bassotti G (2013) Histological healing in inflammatory bowel disease: a still unfulfilled promise. *World journal of gastroenterology* 19:968-978.
- Vivinus-Nebot M, Frin-Mathy G, Bziouche H, Dainese R, Bernard G, Anty R, Filippi J, Saint-Paul MC, Tulic MK, Verhasselt V, Hebuterne X, Piche T (2014) Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* 63:744-752.
- Wallace KL, Zheng LB, Kanazawa Y, Shih DQ (2014) Immunopathology of inflammatory bowel disease. *World journal of gastroenterology* 20:6-21.
- Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF (2017) Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature protocols* 12:1295-1309.
- Wooltorton E (2004) Tegaserod (Zelnorm) for irritable bowel syndrome: reports of serious diarrhea and intestinal ischemia. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 170:1908.