# A Prospective, Randomized, Multi-centered, Placebo-controlled, Clinical Trial of Oral Vancomycin in Adults with Primary Sclerosing Cholangitis

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# A Prospective, Randomized, Multi-centered, Placebo-controlled, Clinical Trial of Oral Vancomycin in Adults with Primary Sclerosing Cholangitis

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List of Changes Since Previous Version:

- Changed PI, as Dr Lindor is now retired
- Removed ASU as a study site
- Clarified Individual and Study Stopping criteria
- Added protocol if patient found to have received vancomycin outside of the study

**Previous Version(s)**
Version 1.0 – January 13, 2016
Version 2.0 – April 26, 2018
Version 2.1 – July 25, 2018
Version 3.0 – November 30, 2018
Version 3.1 – December 20, 2018
Version 3.2 – March 12, 2019
Version 3.3 – October 23, 2019
Version 3.4 – February 26, 2020
Version 3.5 – March 3, 2021
Version 3.6 – November 8, 2023

## I. Significance and Background

### A. Importance of Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is an idiopathic disease of the liver characterized by ongoing inflammation of the intrahepatic- and/or extrahepatic bile ducts, which can ultimately lead to cirrhosis and end-stage liver disease (ESLD). PSC also increases the risk for colorectal and hepatobiliary cancer.[1] The disease can affect both children and adults and is often associated with inflammatory bowel disease (IBD), namely ulcerative colitis (UC), with a reported IBD prevalence of 70%-80% in PSC patients.[2, 3] PSC is an important risk factor for cholangiocarcinoma (CCA), a bile duct cancer that carries a grave prognosis, and colorectal adenocarcinoma in patients with colitis.[1] Patients with PSC have a diminished life expectancy with a median survival of 17 years after diagnosis.[4] In addition, PSC is currently the fifth leading indication for liver transplantation in the United States, and is the leading indication for transplantation in some European countries.[5, 6] Liver transplantation, an invasive and expensive procedure, is currently the only life-extending therapy for patients with ESLD secondary to PSC,[6, 7] and patients with PSC complicated by CCA who meet specific criteria.[8] PSC recurs in the transplanted liver in up to 40% of patients.[9] Further complicating matters, CCA has been reported in liver allografts of patients transplanted due to ESLD secondary to PSC. Taken together, these data highlight the considerable disease burden and clinical impact of PSC on patients' outcomes.

Little is known about the epidemiology of PSC in the United States. The reported incidence and prevalence of PSC show substantial variation, depending on the criteria used for ascertainment of PSC cases, the population under assessment, and the geographical area(s) studied. The highest reported prevalence rate for PSC of 13.6 per 100,000 age- and sex-adjusted persons was reported in the year 2000 in Olmsted County, Minnesota, United States. Toy et al. reported a lower age-adjusted PSC prevalence of 4.15 per 100,000 in Northern California in the year 2005. In Alaska, no PSC patients were identified between 1984 and 2000. These studies support the notion that PSC is a rare disease. Based on our experience and the studies reported, we estimate a PSC prevalence of 50,000 in the United States.

Epidemiological studies have found the highest prevalence rates of PSC in Northern European countries and North America (United States and Canada) ranging between 3.85 to 16.2 per 100,000 persons.[7] In contrast, the reported prevalence of PSC in Southern Europe[8] and South East Asia[9] is nearly 70 times lower. Moreover, PSC is one of the most important indications for liver transplantation in Northern Europe[10]. Hence, we have selected sites with a preference for northern locations.

Despite the high mortality associated with PSC and the efforts to optimize its management, there is no medical therapy proven to halt the progression of PSC or prevent its serious complications (i.e., ESLD and CCA). Ursodeoxycholic acid (UDCA) has been extensively studied in PSC, however its use is in PSC is controversial. The Scandinavian high-dose UDCA clinical trial in PSC has shown that UDCA provided no clinical benefit on the outcome of liver transplant-free survival and development of CCA in PSC as compared to placebo.[11] However recent data has also shown that withdrawal of UDCA in PSC patients resulted in worsening of PSC-related symptoms and liver biochemistries, suggesting that its use may be beneficial in some patients.[12]Unfortunately, UDCA is associated with side effects, and a North American high-dose UDCA study in PSC was

prematurely terminated at 5 years because of an excess of serious adverse events in patients treated with UDCA as compared to placebo.[13]

B. Monitoring Therapeutic Response in PSC: Serum Alkaline Phosphatase and Non-Invasive Markers of Liver Fibrosis-Liver Stiffness Measurement

There has been a renewed interest in identifying disease-specific markers that could serve as surrogate endpoints in PSC. Among the most extensively studied is serum alkaline phosphatase (ALP). Our group at Mayo Clinic and others have reported an association between serum ALP and clinical outcomes in PSC. Specifically, normalization of serum ALP appears to be associated with long-term survival free of CCA, need for liver transplantation, liver-related death, and colorectal cancer (CRC). Persistent ALP elevation has been associated with worse outcomes in PSC (table 1). [14-18]

**Table 1: Relationship between alkaline phosphatase normalization and clinical endpoints**

| Study | # of subjects included in analysis | N (%) of patients with ALP normalization | N (%) of those with ALP normalization who reached an endpoint, * | N (%) of those with ALP elevation who reached an endpoint | P value |
|---|---|---|---|---|---|
| Stanich | 87 | 35 (40) | 5 (14) | 17 (33) | .02 |
| Hilscher | 86 | 38 (44) | 7 (18) | 24 (50) | .003 |

*Bile duct cancer, transplantation or death

Transient elastography (TE), a new technique that allows non-invasive assessment and follow up of liver fibrosis by measuring liver stiffness, differentiates between severe from non-severe fibrosis in PSC, and the rate of progression of liver stiffness measurement (LSM) assessed by TE correlates well with the rate of progression of fibrosis in PSC and with patients' clinical outcomes. [19] Corpechot et al. examined the clinical utility of LSM, using TE, in adults with PSC in the largest study of its kind to date. In this study, a strong association between the LSM and fibrosis has been reported, and baseline and rate of progression of LSM have been found to be strongly linked with clinical outcomes (n= 168). Among the 168 patients who were followed up for a minimum of 1 year, 23 (14%) patients experienced an adverse outcome (need for liver transplantation, development of hepatobiliary malignancy, cirrhosis, and portal hypertension) an average of 3.4 ± 1.8 years (range: 1-6.8 years) after baseline LSM. The estimated progression rates from METAVIR fibrosis stages 0, 1, 2, 3, and 4 were 0.47 ± 0.45 kilo pascals (kPa)/year, 0.25 ± 0.67 kPa/year, 1.64 ± 0.78 kPa/year, 3.4 ± 0.89 kPa/year, and 4.37 ± 0.76 kPa/year, respectively. Moreover, the average estimate of change in LSM in the group who had repeated LSMs over time (n= 130) was 1.5 ± 2.7 kPa/year (range: - 2.2 to 15.3 kPa/year), and the change in LSM has been found to be significantly linked to the risk of adverse clinical outcomes in their PSC cohort. These data clearly indicate that LSM, and thus fibrosis, can change annually in PSC patients, and these LSM changes are clinically meaningful.

A recent consensus statement by the International PSC Study Group recommended five of the following candidates as surrogate endpoints for measuring PSC progression: 1) ALP, 2) TE, 3) liver histology, 4) combination of ALP+histology, and 5) bilirubin. Of these, the experts' panel concluded that ALP, TE, and histology came out as the most promising, and combinations thereof as the most likely candidate surrogate markers for clinical trial design at this time. A recent FDA

workshop on PSC advised that PSC clinical trial designs should include both a biliary-specific blood test (ALP in adults) and TE as surrogate endpoints.

Based on these reports, the following surrogate markers where chosen to serve as endpoints for our proposed clinical trial:

1. ALP normalization
2. TE

We will assess liver biochemistries (including ALP) every 6 months, with potential escalating the oral vancomycin dose in non-responders, and TE every 6 months. This will allow us to examine any potential change in liver stiffness (and hence progression of liver disease) over the period of this study.

C. Preclinical Data on the Potential Relationship Between the Intestinal Microbiota and PSC

1. Association between PSC and IBD

The association between PSC and IBD has long been recognized, but is an area of limited research. The exact relationship between PSC and IBD remains unclear, and is currently an intensive area of research. There is mounting evidence postulating the involvement of intestinal microbiota in initiating and determining IBD phenotype.[20] In addition, evidence suggests a link between the intestinal microbiota and the hepatobiliary inflammation that occur in PSC.[21-23] Experiments with rats where induction of small bowel bacterial overgrowth secondary to bowel ligation exhibited intra- and extra-hepatic ductal dilatation and beading resembling PSC. Interestingly, daily treatment with antibiotics in these animal models resulted in histological improvement.[24, 25] Another study found that administration of peptidoglycans, microbial derivatives, results in irregularities of hepatic bile ducts and focal areas of narrowing consistent with cholangitis.[26] In another study, the administration of N-formyl L-methionine L-leucine L-tyrosine, another microbial derivative, rectally in rats results in inflammation of the portal triad and bile duct injury, similar to PSC.[27] More recently, germ-free mice have been found to have significantly more severe PSC-like histopathological abnormalities (i.e., ductopenia, fibrosis, and ductular reaction) compared to conventionally housed mice, suggesting that commensal microbes play an important role in protecting against biliary injury in PSC.[26]

2. Potential relationship between the intestinal microbiota and PSC

The etiology of PSC is poorly understood. A critical barrier to progress in the development of effective therapies for PSC is the lack of understanding the underlying mechanisms of liver tissue/bile duct damage that occur in PSC. Immune-mediated destruction of the liver tissue and accumulation of toxic bile acids in the liver tissue have been postulated. One emerging theory is that the intestinal microbiota (and/or related molecules) may play an important role in the etiopathogenesis of PSC.[31-33] Recently, researchers have begun to explore the potential relationship between the intestinal microbiota and PSC in humans. Preliminary studies suggest that PSC patients exhibit a distinct intestinal microbiota signature as compared to patients with IBD alone and healthy controls. The intestinal microbiota of patients with PSC has been found to be characterized by decreased microbiota diversity, and a significant overrepresentation of

Enterococcus, Fusobacterium, Lactobacillus, and Veillonella genera compared to patients with IBD alone and healthy controls.[28, 29] These observations suggest that: 1) the intestinal microbiota could be a contributing factor in PSC pathogenesis, and 2) alteration of the intestinal microbiota could be of therapeutic benefit in PSC.

D. Preliminary Data on the Efficacy, Safety, and Mechanism of Action of Oral Vancomycin in PSC

1. Efficacy and Safety

The relationship between intestinal microbiota and PSC suggest a role for antimicrobial agents as therapy for PSC. Vancomycin, a large molecular weight glycopeptide antibiotic, is one of the few studied antimicrobial agents in PSC. In a pilot study[30], 10 patients with PSC were given oral vancomycin (OV) 1 gram twice daily for 52 weeks; labs, liver biopsies and magnetic resonance cholangiograms (MRCPs) were assessed pre- and post-OV treatment. Seven patients completed 52 weeks of OV treatment; 1 patient stopped OV at 36 weeks due to abdominal pain, 1 patient stopped OV at 48 weeks during IBD flare, and 1 patient was lost for follow up.[30] Mean serum ALP decreased from 343.5±167.9[1] (at baseline) to 301.3±231.5 (end of study; % reduction range 13%-50%), and mean alanine aminotransferase (ALT) reduced from 80.3±34.6 (baseline) to 75.7±45.4 (end of study; % reduction range 14%-59%). In addition, an improvement in liver histology and MRCP was observed in 29% (2/7) and 44% (4/9), respectively. None had progression of disease on liver biopsy or MRCP, no serious adverse events were reported, and no patient developed vancomycin-resistant enterococci (VRE).[30]

In a randomized, double blinded clinical trial,[31] the safety and efficacy of OV was compared to oral metronidazole (an antibiotic commonly used for treatment of anaerobic infections) in adult patients with PSC (total n=35; 17 patients assigned to either low or high-dose OV). Treatment continued for 12 weeks with the primary endpoint being reduction in serum ALP. At 12 weeks, patients in the OV groups achieved the primary endpoint (reduction in serum ALP). Importantly, normalization of serum ALP occurred in 25% of patients in the low-dose OV group and OV was better tolerated than oral metronidazole.[31] Two patients in the OV groups discontinued vancomycin indefinitely (migraines and diarrhea in one, and increasing fatigue and diarrhea in the second), and 4 patients in the oral metronidazole groups discontinued metronidazole due to severe gastrointestinal side effects (nausea, dyspepsia, anorexia, and diarrhea).[30]

[1] Lab values in the Pratt study (reference # 30) are expressed as mean ± standard deviation

The experience of the Stanford University team in OV in pediatrics with PSC is similar; two studies on the use of OV in children with PSC reported normalization of serum gamma glutamyl-transpeptidase (GGTP, commonly used as a biomarker of cholestasis and liver injury in pediatric PSC) and improvement in biliary imaging and histological abnormalities.[32, 33] Liver enzymes' rose after treatment with OV was discontinued. It is noteworthy to mention that GGTP has been found to correlate well with long-term clinical outcomes in pediatric PSC (data presented at the 67th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2016).[34] Table 2 shows the percentage of PSC patients who experienced normalization of the cholestatic biomarker measured (ALP or GGTP) in all published clinical trials and case series of OV in PSC. Collectively, these data suggest that OV is safe and might be of therapeutic benefit in PSC.

Limitations of the open-label clinical trials of OV in PSC include: Small sample sizes, lack of a control group (i.e., placebo group), short treatment periods, incomplete clinical and laboratory follow up after completion of treatment, lack of follow up of disease progression, OV doses used across the pilot studies were inconsistent, and the relationship between the intestinal microbiota and PSC has not been examined.

## 2. Mechanism of Action

To understand how antibiotics work, we need to briefly understand the targets of antibiotics. There are three main targets for the antibacterial agents: 1) bacterial cell wall synthesis, 2) bacterial protein synthesis, and 3) bacterial DNA replication and repair.[35] The bulk of bacterial cell wall is composed of a complex network of peptide and glycans that are covalently cross- linked by the enzymatic actions of transpeptidases and transglycosylases, forming peptidoglycans. The mechanism of action of vancomycin has been extensively and well- studied. It targets the peptidoglycan call wall layer by sequestering the D-Ala-D-Ala termini of peptidoglycans, thereby preventing it from interacting with either transpeptidases or transglycosylases. The result is failure to produce peptidoglycan networks/crosslinks within the cell wall layer, which leads to a weaker cell wall, making the bacteria susceptible to killing by osmotic lysis.[35]

In PSC, however, the mechanism of action of vancomycin is unclear. The work by Abarbanel et al[36] sheds some light on the potential mechanism of action of vancomycin in PSC patients. In this study,[36] 14 children with PSC were treated with oral vancomycin (dose 40mg/kg body weight/day) for up to 12 months. GGTP and alanine aminotransferase (ALT) were abnormally high prior to oral vancomycin treatment, and normalized within 3 months of treatment and remained normal after 12 months of therapy.[36] This was concurrent with a significant decrease in peripheral white blood cells, neutrophils, and lymphocytes, and an increase in serum transforming growth factor-β (TGF-β). Moreover, the concentration of peripheral T regulatory cells (Treg) have been found to be significantly increased in OV-treated PSC patients, compared to those who were untreated. These changes continued to exist throughout the treatment period; studies from one case have confirmed that Treg levels fall after discontinuation of OV, and re-administration of OV therapy resulted in restoration of Treg to OV-treatment levels.[36] These data suggest that the therapeutic properties of OV in PSC may be linked to modulation of the Treg quantity/functions. Additional studies are needed to clarify the

role of OV in PSC.

From an immunologic perspective, treatment with OV has been shown to result in a reduction in white blood cells count driven by reduction in neutrophils and lymphocytes.[36] Transforming growth factor β (TGF-β), which plays critical role in the induction and function of regulatory T-cells (Treg), has also been found to be elevated after initiation of OV, without concurrent increase in cytokines associated with helper TH1 and TH2 responses (i.e. tumor necrosis factor α (TNF-α) and interleukins (IL) 4 and 13). The increase in number of Tregs in patients treated with OV supports a role for OV in modulating Tregs in controlling in inflammation at human mucosal surfaces.[36]

**Table 2: Summary of all *published* clinical trials and case series of OV in PSC**

| | Vancomycin, dose | Study Site/Year | Study population/age range OR median (interquartile range) | Type of clinical trial | No. of study subjects | Treatment duration | Cholestatic marker measured, at baseline | % reduction in cholestatic marker | N (%) of subjects who achieved normalization of cholestatic marker |
|---|---|---|---|---|---|---|---|---|---|
| Cox et al. (Ref # 33) | 375-1000mg daily in 3-4 divided doses | Stanford University/ 1998 | Children/14-15 | Open label | 3 | 6-20 months | **GGTP**[2] (510 818, and 291) | -84.7%, -89%, and -73.5% | 2/3 (66.7) |
| Davies et al. (Ref # 32) | 50mg/kg/day | Stanford University/ 2008 | Children/2-17 | Open label | 14 | 3-118 months | **GGTP**[3] (370±60) | -70.3% | 10/14 (71) |
| Abarbanel et al. (Ref # 35) | 50mg/kg/day, maximum daily dose: 1500mg/day | Stanford University/ 2013 | Children/2-18 | Open label | 14 | 12 months | **GGTP**[7](236±605) | -79.2% | 14/14 (100) |
| Tabibian et al. (Ref # 31) | 500mg daily in 4 divided doses | Mayo Clinic Rochester/ 2013 | Adults/35 (23–57) | Randomized | 8 | 12 weeks | **ALP**[4,5] (406 (210–545) | -46% | 2/8 (25) |

[2] Values in U/L
[3] Values expressed in mean± SD
[4] Values in U/L
[5] Values expressed in median (interquartile range)

E. Importance of Knowledge to be Gained

OV has been investigated in small pilot studies in PSC patients, and has been shown to improve biochemical markers of liver disease in PSC. This will be the first multicenter randomized placebo-controlled clinical trial examining the impact of daily OV, using a stepped-up dosing strategy with three increasing doses, on PSC disease symptoms, biochemical markers, and progression. Data collected from this study will help us determine if OV can be used as treatment option for PSC.

F. Availability and Acquisition of Study Agents for This Clinical Trial

OV kits and placebo diluents will be supplied by Azurity Pharmaceuticals, Inc., (Wilmington, Massachusetts, U.S.A.) sufficient for the 102 enrolled subjects. Azurity Pharmaceutical's Letter of Support is attached in the Appendix section of this application.

## II. INNOVATION

PSC currently has no medical therapy. This will be the first-of-its-kind larger-scale clinical trial of a newly applied drug, OV, for PSC patients, with treatment extending over an 18-month period, followed by a 6-month follow up period, using a dose-escalation method to help us determine an appropriate OV regimen in PSC. In addition, this will be the first study assessing the impact of daily OV dosing on PSC progression (by measuring liver stiffness), and quality of life measures related to PSC and IBD. If it is successful in stopping the progression of PSC, we will be also able to explore the mechanism of action. Data collected from this study may help us understand the interaction between the gut microbiome, bile acid metabolism, the immune system, and disease activity of PSC. Based on future mechanistic analyses and results from this study, it is possible that new treatments for PSC will emerge targeting specific areas of the gut microbiome, immune system, and/or bile acid metabolism.

## III. APPROACH

A. Overall Goal

The overall goal of this study is to examine the safety, tolerability and efficacy of daily dosing with OV, using a stepped-up dosing strategy with three increasing doses, on the clinical course and progression of PSC, and to compare the treated patients with those on placebo.

These results will help us:

1. Examine the potential therapeutic role of daily OV in PSC over an extended time period (18 months versus 3 months in the recent study[31]).
2. Determine an appropriate OV dosing strategy for PSC treatment.
3. If the study is positive, explore the potential relationship between the intestinal microbiota and PSC activity pre- and post- OV therapy, the effect of OV on the intestinal microbial environment in PSC, and changes in cytokine markers of immune function that may predict response to OV and elucidate mechanism of action by collecting and storing blood and stool samples. In addition, a comparison in the intestinal microbiota/microbiome between the treated versus placebo arms is of great interest.

B. Specific Aims of the Study

1. Determine if OV normalizes serum ALP in adults with PSC. Levels of serum ALP obtained at 6,12, and 18 months of OV treatment, and at 3, and 6 months post OV treatment will be compared to those obtained at baseline (month 0), and with values at the same study time points in the placebo arm.
2. Determine if OV stabilizes *or* improves liver fibrosis assessed by LSM using TE. Liver stiffness will be measured at 6, 12, and 18 months of OV treatment, and at 6 months post OV treatment, and values will be compared to those obtained at baseline (month 0), and with values in the placebo arm.
3. Determine the changes in the intestinal microbiota in relation to the use of OV, and study the correlation between the changes in the intestinal microbiota and the changes in 1) liver enzymes, particularly serum ALP, and 2) liver stiffness, assessed by LSM using TE.

C. Study Design and Methodology

This is an 18-month, randomized, placebo-controlled, multi-centered prospective clinical trial designed to assess the effects of different OV doses on the clinical and biochemical course in adult PSC. Subjects will be randomized to placebo or treatment and stratified by baseline presence of IBD and PSC severity. Subjects in the treatment group who do not meet the endpoints after 6 months of OV treatment will continue treatment at an increased OV dose, and those not meeting the endpoints after 12 months of OV treatment will continue treatment with OV at further increased dose, as outlined below:

1. Dosing protocol
   In this 18-months study, study subjects will be randomly assigned to one of the following two arms:
   i. OV group:
      Months 0-6 Clinical Trial (M0-6CT): 51 study subjects will be treated with OV 125mg four times a day for 6 months.
      Months 6-12 Clinical Trial (M6-12CT): Study subjects who meet the primary endpoint will continue OV treatment at the same dose (i.e., 125mg four times a day) for an additional 12 months. Those who do not meet the primary endpoint in the M0-6CT will have their OV dose increased to 250mg four times a day for months 6-12. The M6-12CT is designed to assess if non-responders in the M0-6CT will achieve the primary endpoint using a higher OV dose.
      Months 12-18 Clinical Trial (M12-18CT): Study subjects who meet the primary endpoint on M6-12CT will continue OV treatment at the same dose (i.e., 250mg four times a day) for an additional 6 months. Those who do not meet the primary endpoint in the M6-12CT will have their OV dose increased to 375mg four times a day for months 12-18. Similarly, the M12-18CT is designed to assess if non-responders in the M6- 12CT will achieve the primary endpoint using a higher OV dose.
   ii. Placebo group:
      51 study subjects will be treated with a placebo for 18 months. The placebo will be formulated to look like the active medication.

2. Endpoints
   The following endpoints assessed at month 6, 12, and 18 months during treatment, and at 6 months post-treatment, will be compared with baseline:
   i. Primary Endpoint:
      a. Normalization of serum ALP
   ii. Secondary Endpoints:
      a. Change in ALP at the different time points from baseline,
      b. Change in LSM at the different time points from baseline,
      c. Change in liver biochemistries (ALT, AST, GGTP, albumin, bilirubin, and International Normalized Ratio (INR)) at the different time points from baseline,
      d. Change in 5-D itch scale[37] at the different time points from baseline,
      e. Change in primary biliary cholangitis (PBC)-40 health-related quality of life (HRQoL) survey[38] at the different time points from baseline,
      f. Changes in proinflammatory cytokines' profile (mentioned in Specific aim 3) from baseline values, and
      g. Difference in number of cholangitis episodes from baseline.
   iii. Other Endpoint:
      a. Improvement in IBD symptoms

3. Rationale for dose selection and vancomycin brand to be used for this study
   Based on our recent paper,[31] adult patients with PSC were randomly assigned to receive low-dose (125mg four times a day) versus high-dose (250mg four times a day) OV. Both groups achieved significant improvement in serum ALP. These changes were more prominent in the low dose group. Pediatric studies with OV dosed at 50 mg/kg/day with a maximum dose of 1,500 mg/day (500 mg three times a day) were associated with significant improvement in GGTP.[32, 33, 36] This helps to establish the upper limit of the dose range in this study. This study will determine if increasing the treatment period and the dose for non-responders will result in additional patients meeting the endpoints.

   For this study, we will use compounded oral vancomycin hydrochloride solution, compounded by a licensed pharmacist in accordance with Section 503A of the United States Food, Drug and Cosmetic Act. The placebo will be composed of a compounded formula of the diluent without the drug substance. Oral Vancomycin and placebo will be provided by Azurity Pharmaceuticals, Inc. [redacted] The compounded product needs to be stored in a refrigerator between doses.

   All study drugs will be labeled with "Caution: New Drug--Limited by Federal (or United States) law to investigational use" per 21 CFR 312.6.

B. Institutions Participating in this Research Project and number of study subjects to be recruited from each
   1. Mayo Clinic, Rochester, Minnesota (54 subjects)
   2. Mayo Clinic, Phoenix, Arizona (17 subjects)
   3. Mayo Clinic, Jacksonville, Florida (31 subjects)
   4. TOTAL SUBJECTS = 102

   Number of subjects enrolled at each site may be adjusted at investigator discretion to facilitate total recruitment.

C. Inclusion Criteria
1. Male or female subject age 18-76 years
2. Diagnosis of PSC consistent with the guidelines published by the American Association for the Study of Liver Diseases (AASLD).[39] All subjects *must have* an elevated serum ALP of at least 1.5 times upper limit of normal at baseline *plus* cholangiographic evidence of PSC, as demonstrated by magnetic resonance imaging, endoscopic retrograde cholangiography, direct cholangiography, or liver biopsy.
3. Total bilirubin at screening must be ≤ 2 times upper limit of normal
4. An ultrasound (or equivalent imaging modality) that excludes biliary obstruction and malignancy within 6 months of study entry,
5. If a patient is on any of the following medications and/or supplements, he or she is expected to remain on the same daily dose through the treatment period: UDCA, azathioprine, prednisone (or an equivalent steroid compound), methotrexate, a 5-aminosalicylic acid, biologic therapy, and/or a probiotic.
6. If a patient has been on obeticholic acid or other experimental therapies for PSC, they must complete a 3 month washout period before study entry
7. PSC with or without inflammatory bowel disease, such as ulcerative colitis or Crohn's disease
8. Must agree to comply with the study protocol and provide informed consent.

D. Exclusion Criteria
1. Administration of an antibiotic within 3 months prior to the study,
2. Pregnancy or attempting to become pregnant or breastfeeding,
3. Presence of any of the following:
   i. Hepatitis B infection
   ii. Hepatitis C infection (antibody positive); patients with a history of hepatitis C infection will be eligible for this study if they have undetectable levels of HCV RNA
   iii. Other cholestatic liver diseases such as primary biliary cholangitis and cholestatic diseases of pregnancy
   iv. Metabolic liver diseases such as Wilson's disease and hemochromatosis
   v. Inherited diseases of the liver such as α-1 antitrypsin deficiency
   vi. Immunoglobulin G4-related cholangitis
   vii. PSC with concomitant autoimmune hepatitis (AIH) and/or primary biliary cholangitis (previously known as primary biliary cirrhosis)
   viii. Secondary sclerosing cholangitis (SSC),
   ix. Active acute ascending cholangitis requiring antibiotics
   x. CCA (malignant biliary stricture, neoplasm, and cytology/histopathology or positive fluorescence in situ hybridization (FISH) consistent with adenocarcinoma of the bile duct)
   xi. A liver biopsy, if one has been previously obtained, which showed non-alcoholic steatohepatitis (NASH). Patients with suspected fatty liver by imaging will not be excluded
   xii. Presence of decompensated cirrhosis such as hepatic encephalopathy, hepato-renal syndrome and hepato-pulmonary syndrome,
   xiii. History of liver transplantation, anticipated need for liver transplantation within 12 months from randomization, or a Model of End Stage Liver Disease (MELD) score of ≥15
   xiv. Ongoing alcohol abuse (>4 drinks per day for men, and >2 drinks per day for women)
   xv. History of allergic reaction to vancomycin,
   xvi. Moderate-to-severe renal impairment with a calculated creatinine clearance of < 60mL/min
   xvii. HIV/AIDS
   xviii. Any other conditions or abnormalities that, in the opinion of the investigator, may compromise the safety of the subject or interfere with the subject participating in or completing the study.

E. Visit Schedule for this Clinical Trial

1. Study Participation Period (table 3):
   For each study participant, the study participation period will consist of a screening period of up to 1 month, treatment period for 18 months, and a follow-up period of up to 6 months.
2. Screening & Recruitment Period:
   All study participants are expected to be recruited for this study within 12-18 months. Screening and Baseline Visits may be done on the same day. Any procedures done to meet inclusion/exclusion criteria at Screening do not need to be repeated for the Baseline values at the discretion of the investigator.
3. Assessments:
   All study participants will be treated for 18 months; 51 with OV and 51 with placebo. Study subjects will be seen in clinic at baseline, and thereafter every 6 months (baseline, 6, 12, 18, and 24 months). During these visits, safety monitoring, clinical, laboratory and imaging evaluations will be obtained. Further, at 3, 9, 15, and 21 months laboratory evaluations will also be obtained, and at 2, 4, 8, 10, 14, 16, 20, and 22 months phone calls will made to each study subject to assess safety and adverse events.
   i. During the treatment phase the following will be assessed:
      a. Assess adherence to OV during all clinic visits and telephone calls
      b. Assess the general well-being during all clinic visits and telephone calls
      c. Assess for side effects and adverse events related to the study agent during all clinic visits and telephone calls
      d. Administer 5-D itch scale and PBC-40 HRQoL during all clinic visits,
      e. Assess for PSC-related complications, such as episodes of bacterial cholangitis and development of portal hypertension (ascites, esophageal varices, etc.) during all clinic visits and telephone calls
      f. Assess IBD symptoms by administering the Short Inflammatory Bowel Disease Questionnaire (SIBDQ)[40] and the Ulcerative Colitis Clinical Score (UCCS)[41] during all clinic visits and telephone calls
      g. Measure levels of liver biochemistries (aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, GGTP, albumin, INR, and total bilirubin), at baseline, 3, 6, 9, 12, 15, and 18 months. INR is only required if drawn at Mayo Clinic study site.
      h. Assess liver stiffness measurement by transient elastography during all clinic visits
      i. Records findings from standard of care imaging (MRCP, ERCP, transient elastography, MR, or US) since previous visit (if any)
      j. Assess for administration of any antibiotic/probiotic other than OV during all clinic visits and during phone calls the following data will be recorded (if applicable): name of antibiotic, indication, dose, frequency, and duration of treatment
      k. If the study agent is discontinued the following data will be recorded: reason for discontinuation (such as allergic reaction; intolerable side effect(s); noncompliance; prolonged hospitalization; development of adverse outcomes such as cholangiocarcinoma; need for liver transplantation; varicella bleed, etc.), date of discontinuation of the study agent and date of resuming the study agent (if applicable)
      l. Collect blood and stool samples for future studies (if indicated by the results of this study) during all clinic visits. Testing will include, but is not limited to cytokines, bile acids, and microbiome analysis,
      m. Monitor CBC and renal function tests (calcium, carbon dioxide, chloride, creatinine, glucose, phosphate, potassium, sodium, BUN)

n. Assess if additional clinical or laboratory testing is indicated during all clinic visits and telephone calls.

ii. Follow-up Period:
   a. General well-being and vital signs,
   b. Side effect(s) and adverse events related to the study agent during clinic visits and telephone calls, 5-D itch scale and PBC-40 HRQoL at clinic visits
   c. Assessment for PSC-related complications during clinic visits and telephone calls
   d. Assessment of IBD (using the SIBDQ and UCCS) during all clinic visits and telephone calls
   e. Measure levels of liver biochemistries (AST, ALT, ALP, GGTP, albumin, INR, and total bilirubin) at 21 and 24 months. INR is only required if drawn at Mayo Clinic study site.
   f. Liver stiffness measurement by transient elastography at clinic visits
   g. Records findings from standard of care imaging (MRCP, ERCP, transient elastography, MR, or US) since previous visit (if any)
   h. Blood and stool samples' collection for cytokine, bile acids, and microbiome analysis (for future studies if indicated by the results of this study) at clinic visits
   i. Monitor CBC and renal function tests (calcium, carbon dioxide, chloride, creatinine, glucose, phosphate, potassium, sodium, BUN)
   j. Additional laboratory or clinical testing may be performed as clinically indicated.

**Table 3: Schedule of events: showing all planned study visits and safety, clinical, laboratory, and imaging assessments that will be performed during the clinical trial**

| Procedures | Screening | Enrollment/Baseline Treatment Period; Month 0 (visit 1) | Telephone Calls at Months 2, 4, 8, 10, 14, 16, 20, and 22 | Treatment Period; Month 3 | Treatment Period; Month 6 (Visit 2) | Treatment Period; Month 9 | Treatment Period; Month 12 (Visit 3) | Treatment Period; Month 15 | Treatment Period; Month 18 (Visit 4) | Follow-up; Month 21 | Final Study follow-up; Month 24 (Visit 5) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days |
| Informed Consent | X | | | | | | | | | | |
| **Clinical** | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | |
| Medical History, initial | X | | | | | | | | | | |
| Physical Examination, initial | X | X | | | | | | | | | |
| MRI/MRCP, ERCP and/or liver biopsy to confirm/rule out PSC (from medical record) | X | | | | | | | | | | |
| Randomization | | X | | | | | | | | | |
| Clinic Visits (includes limited physical exam after Baseline Visit) | X | X | | | X | | X | | X | | X |
| Review concurrent medications | X | X | X | | X | | X | | X | | X |
| Vital signs | X | X | | | X | | X | | X | | X |
| Adherence to study agent | | X | X | | X | | X | | X | | |
| 5-D Itch Scale | | X | | | X | | X | | X | | X |
| PBC-40 HRQoL | | X | | | X | | X | | X | | X |
| Assess for PSC-related complications | X | X | X | | X | | X | | X | | X |
| Assess for IBD-related symptoms and/or complications (SIBDQ & UCCS) | | X | X | | X | | X | | X | | X |
| Adverse events' evaluation | | | X | | X | | X | | X | | X |
| Hearing evaluation (only as indicated in Section F(3)(viii) | | X | | | X | | X | | X | | |
| **Laboratory** | | | | | | | | | | | |
| Liver biochemistries | X | X | | X | X | X | X | X | X | X | X |
| Blood sample collection for potential future studies | | X | | | X | | X | | X | | X |
| Stool sample collection for potential future studies | | X | | | X | | X | | X | | X |
| Prothrombin time and INR (only required if drawn at Mayo Clinic study site) | X | X | | X | X | X | X | X | X | X | X |
| Urine pregnancy test (if necessary) | X | X | | | X | | X | | X | | |
| CBC & renal function panel | X | X | | X | X | X | X | X | X | X | X |
| **Imaging** | | | | | | | | | | | |
| LSM by transient elastography | | X | | | X | | X | | X | | X |
| **Other study procedures** | | | | | | | | | | | |
| Screen for VRE | | Only if indicated (discussed in Section F(3)(iii)). | | | | | | | | | |

| | | |
|---|---|---|
| Serum vancomycin trough | | 2 weeks after OV therapy in subjects at risk for increased systemic exposure to OV (as discussed in Section F(3)(vi)), then if indicated per Section F(3)(vii). |

F. Potential problems related to the use of OV that can occur during the clinical trial, and ways to assess, track, and mitigate these potential risks

1. Overgrowth of vancomycin-resistant enterococci (VRE)
Enterococci are normal inhabitants of the alimentary canal, and can cause urinary tract infections, bacteremia (blood stream infection), and endocarditis. *Enterococcus faecalis* and *Enterococcus faecium* cause the majority of enterococcal infections overall. Enterococci can develop resistance (ability of the bacteria to withstand the effects of an antibiotic) to a number of glycopeptide antibiotics, such as vancomycin. Enterococci that gained resistance to vancomycin are called vancomycin-resistant enterococci (aka VRE).[42]

Risk factors for VRE infections are:
   i. Subjects receiving antibiotics
   ii. Immunocompromised
   iii. In-hospital use of antibiotics
   iv. Contact with subjects infected/colonized with VRE
   v. Subjects with medical devices that stay in place for a prolonged time such as urinary catheters and central intravenous (IV) catheters
   vi. Colonization with VRE, the most important and feared complication of use of OV is VRE colonization, which can subsequently cause VRE infection

The most common sites of VRE infections are:
   i. Urinary tract infections especially in subjects with catheters and/or central IV catheters
   ii. Intrapelvic infections (rare, but has been reported in some instances in subject post-abdominal/pelvic surgical procedures
   iii. VRE endocarditis (infection of ≥1 heart valve)
   iv. Bacteremia (blood stream infection caused by VRE).[43-47]

2. Other potential adverse events related to the use of vancomycin are nausea, abdominal cramps, diarrhea, rash, hematological abnormalities (anemia, leukopenia, and thrombocytopenia), renal insufficiency, and hearing deficits.[48] IBD flare up due to OV has rarely been reported.[30] Systemic absorption of OV has been reported in elderly patients and in those with active clostridium difficile colitis. Vancomycin may modulate the drug-metabolizing enzymes located in the gastrointestinal tract. This may result in an increase in serum concentrations of drugs with narrow therapeutic indices, which can potentially lead to undesired side effects or serious adverse events.

3. Ways to assess and track potential risks
We will use the following procedures to assess, track, and mitigate the potential risks of OV use:
   i. Telephone calls every two months, asking about abdominal pain (unexplained), urinary symptoms (dysuria, urgency, frequency, cloudy urine, foul-smelling urine), new onset chest pain, palpitations, and shortness of breath that cannot be explained by any ongoing/chronic cardiorespiratory illness. In addition, study subjects will be asked to report to the study coordinator/investigator if they are experiencing any new-onset hearing difficulties and ringing sensation in ears. Moreover, subjects will be asked about symptoms/signs of IBD flare.
   ii. Collect and store stool samples every 6 months.

iii. Will screen for VRE by PCR if a subject reports persistent increase in stool frequency of greater than 3 times normal frequency for more than 7 days, lower abdominal pain, or fever of unclear etiology.

iv. Clinic visits, as proposed in the clinical protocol (table 3), asking specifically about symptoms/illnesses mentioned in Section F, point (1) & (2).

v. Blood tests every 3 months to monitor complete blood count (CBC) with differential and renal function panel.

vi. Initial serum trough levels of vancomycin will be measured after 2 weeks of OV therapy in ALL the following clinical scenarios:
   a. Subjects ≥65 years old,
   b. Subjects recovering from a severe IBD flare episode (2 weeks after clinical remission, defined as UCCS of 0 or 1 and endoscopic remission documented in the clinical records[49]),
   *c.* Subjects recovering from clostridium difficile colitis (2 weeks after last diarrheal bowel movement), *and*
   d. Subjects with mild renal impairment (calculated creatinine clearance 60-75mL/min).

vii. Serum vancomycin trough levels will be measured:
   a. If a subject develops a severe IBD flare during OV treatment,
   *b.* If a subject develops clostridium difficile colitis during OV treatment, *and*
   c. If a subject is found to have an increase in serum creatinine that is unexplained

viii. Hearing evaluation will be performed, per the guidelines published by the American Speech-Language-Hearing Association50, 51, at baseline, 6, 12, and 18 months during the treatment period in:
   a. Subjects ≥70 years old,
   b. Subjects with severe IBD and
   c. Subjects with more frequent episodes of IBD flare

ix. Study subjects who are on concomitant drugs that are substrates for P-glycoprotein (P-gp) and cytochrome P450 (CYP3A) with a narrow therapeutic index will be closely monitored. An extensive list of these substrates/drugs can be found at (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/Dr ugInteractionsLabeling/ucm093664.htm#table3-1)

4. Ways to mitigate risk
   Participation of study subjects will be terminated if they meet ANY of the following:

   i. Development of VRE infection.

   ii. Development of VRE colonization and the infectious disease specialist/infection control units recommends stopping OV.

   In cases of VRE colonization and/or infection, study subjects will undergo the necessary clinical and microbiological evaluations predefined by the local authorities in the Infection Control Units and the infectious diseases specialists in the participating clinical trial sites.

   IBD flare in PSC subjects on OV has rarely been reported.[30] In fact, pediatric clinical reports on the use of OV in children with PSC suggest that OV resulted in amelioration of IBD symptoms.[32, 33, 36, 52]

5. Drug-induced liver injury (DILI)
   i. If a patient meets the individual drug discontinuation criteria (as noted in the protocol) and is excluded from the trial.
      a. Repeat liver profile and PT/INR within 48-72 hours and place patient under "close observation" as defined in the DILI guidance until the liver enzymes "normalize or stabilize". Depending on the patient's progression, consider initiating potential DILI evaluation for competing etiologies. Consider re-starting study medication only if a definitive alternative etiology is identified and liver tests return to baseline.
   ii. With any elevation of bilirubin by 0.3mg/dl or more, regardless of ALT or AST levels, and in the presence of indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis):
      a. Interrupt vancomycin.
      b. Initiate potential DILI evaluation for competing etiologies and repeat liver profile and PT/INR within 48-72 hours and place patient under "close observation" as defined in the DILI guidance.
      c. Study medication can be restarted only if a definite alternative etiology is identified and the liver tests return to baseline.
   iii. If a patient lives in a remote area, they can be tested locally and the results should be promptly communicated to the investigator site.
   iv. The investigational agent must be discontinued and the patient must be followed until the clinical and laboratory abnormalities stabilize or normalize if the following criteria are met:
      a. Close monitoring of a patient is not possible.
      b. If any degree of total bilirubin, ALT, or AST elevation recurs following re-challenge with the study drug.

G. Potential problems that could occur during the clinical trial as part of the natural course of PSC and/or Inflammatory Bowel Diseases

1. Problems related to PSC

   Biliary strictures. Most PSC patients have strictures in the biliary tree. Dominant strictures can induce stagnation of bile, resulting in bacterial colonization and secondary bacterial cholangitis.[39] Acute ascending cholangitis is a gastrointestinal emergency in the spectrum of acute biliary infection with high mortality rates (if untreated).[53] This condition requires immediate attention.[54] In severe complicated cases, subjects may present with mental status changes as well as shock indicative of ongoing biliary sepsis (Reynold's pentad). The management is directed at treating the infection with broad spectrum antibiotics (antibiotics active against gram negative rods, anaerobic bacteria), and biliary drainage (endoscopic, percutaneous, or surgical). The Tokyo guidelines are commonly used for the diagnosis of acute ascending cholangitis.[55]

   The Tokyo criteria are:
   i. Two of three (fever, right upper quadrant pain, and jaundice), plus
   ii. Systemic inflammation (elevated white blood count and/or C-reactive protein), plus
   iii. Abnormal liver function tests, plus
   iv. Imaging detection of biliary dilatation and etiology (stone, stricture, stent, neoplasm)

   Bacterial cholangitis. Study subjects will be instructed to report any symptoms suggestive of acute ascending cholangitis or biliary tree stricture (new onset jaundice,

abdominal pain, dark urine, light-colored stools) to the study coordinator. The study coordinator shall discuss these findings with the investigator. Study subjects will be advised to come to the clinic for evaluation (during the week days) or visit the nearest emergency room for consultation with an emergency room physician. Subject's records will be obtained and reviewed by the investigator to determine course of action (antibiotics' administration, percutaneous, endoscopic, or surgical intervention as well as length of hospital stay, if applicable).

Cholangiocarcinoma. Cholangiocarcinoma is a feared complication of PSC. Annual cross-sectional imaging of the liver is currently recommended as standard of care for all PSC patients to screen for cholangiocarcinoma. We recommend liver ultrasound every 6-12 months in all PSC patients. Magnetic resonance imaging of liver (MRI) is permissible, but not required.

2. Problems related to IBD

PSC is often associated with inflammatory bowel diseases, namely ulcerative colitis (UC).[3] Study subjects will undergo clinical assessment for their IBD at clinic visits during the treatment period (at 6, 12, and 18 months), then 6 months after the last dose of the study agent. The UCCS and the SIBDQ[40] will be used for clinical assessment of IBD.

Study subjects will be instructed to call the study coordinators/investigators to report any new symptoms that could be due to flare up of IBD. Common symptoms that suggest IBD flare up are abdominal pain, fever, bloody stools, and an increase in frequency of bowel movements. In addition, courtesy calls will be made by the study coordinators every two months to inquire about the presence of any of these symptoms.

If a study subject calls and reports any of these symptoms, the study subject will be instructed to come to visit the study coordinator for a clinical evaluation. The study coordinators shall communicate these findings to the clinical site's investigator to determine if further diagnostic workup (laboratory and/or imaging) is necessary.

In subjects with UC, we will use the Ulcerative Colitis Clinical Score[56] to assess the disease activity/severity. This score, a modification of the well-known Mayo Score,[57] consists of 4 items: stool frequency, rectal bleeding, subject's functional assessment, and physicians' global assessment (PGA). Scores range from 0 to 12 points, with higher scores meaning more active disease. The Short inflammatory Bowel Disease Questionnaire (SIBDQ) is a health-related quality of life HRQoL tool measuring physical, social, and emotional status in IBD patients.[58] The SIBDQ contains 10 items with 4 dimensions: bowel symptoms (3 items); systemic symptoms (2 items); emotional function (3 items); and social function (2 items). The total score ranges from 10 (worst health) to 70 (best). The SIBDQ can be administered and scored quickly and easily.[58]

In the event of an IBD flare, study subjects may take up to 20 mg of Prednisone per day without jeopardizing their participation in the study. If a higher dose of prednisone or a biological agent is needed, then the subject's participation in the study will be terminated.

Clostridium difficile colitis has been reported in IBD patients. If a study subject develops clostridium difficile colitis, other appropriate therapies will be used.

Colon cancer is an important endpoint in IBD, particularly in patients with PSC and UC.

Currently, screening colonoscopy is recommended annually in patients with PSC *and* IBD.

H. Potential Problems related to Data Management and Electronic Medical Records (EMR)

Breach of data or EMR by unauthorized personnel is a potential problem. Data will be stored in a locked cabinet and in a password secured internet database. Only personnel authorized by the Principal Investigator will be allowed to access study subjects' data

I. Biological Sample Storage

During all clinic visits (at baseline, treatment and follow-up period), blood and stool samples will be collected from study subjects and stored for potential future analysis of the microbiome, cytokine profile, and bile acids profile, if indicated by the results of this study.

Blood samples: a total of 2ml of serum will be collected and stored in aliquots at each study site where the samples have been collected. After study completion, these samples will be shipped to Dr. Tom Karlsen at University of Oslo, Norway, for future microbiome-related studies.

Stool samples: study subjects will be instructed to bring a stool sample (fresh sample, defined as collected < 24 hours "stored in the refrigerator at home" before their clinic visit) in collection tubes; these will be stored at the subjects' study site. After study completion, these samples will be shipped to Dr. Tom Karlsen at University of Oslo, Norway, for future microbiome- related studies.

J. Procedures related to this Clinical Trial

1. Liver biopsy
   Liver biopsy is rarely used in the management of PSC. Side effects include pain at the needle site, and rarely bleeding requiring blood transfusion and intra-abdominal infections.

2. Magnetic resonance imaging (MRI)
   MRI is commonly used for the diagnosis of PSC, but not routinely used for follow up. It is a noninvasive radiological test that uses magnetic field and radio waves to create images of the liver and bile ducts. MRI exams are often done using contrast. Individuals who suffer renal function impairment are at risk for systemic fibrosis. There is a very slight increased risk of allergic reaction to the contrast material used in MRI exams. Implanted medical devices may malfunction during the MRI exams.

3. Endoscopic retrograde cholangiopancreatography (ERCP)
   ERCP is an endoscopic technique used to highlight the anatomy of bile ducts. This is an invasive procedure. Adverse events related to ERCP include pancreatitis, excessive bleeding, puncture of the gastrointestinal (GI) tract or bile ducts, and cholangitis. This procedure is typically performed in an in-patient setting, after excluding predefined contra-indications (such as use of blood thinners, clinically significant or uncorrectable coagulopathy, anatomical abnormalities that would impede endoscopic access to the biliary tree, or unstable medical condition precluding safe administration of sedatives or general anesthesia).[66]

4. Colonoscopy
   Colonoscopy is an endoscopic technique used to visualize the interior of the colon. Patients with PSC and IBD are at high risk for colon cancer.[68] Annual colon surveillance is strongly recommended.[69] Adverse events related to colonoscopy include bowel perforation, bleeding, and infection.[70]

5. Ultrasonography of the liver
   This is a noninvasive technique that uses high-frequency sound waves to view the liver. Unlike X-ray imaging, there is no ionizing radiation exposure associated with ultrasound imaging. Ultrasound imaging has an excellent safety record.

6. Transient Elastography measurement by Fibroscan
   This is a new technique. The fibroscan device works by measuring shear wave velocity. In this technique, a 50-MHz wave is passed into the liver from a transducer (probe), which measures the stiffness of the liver. This is a painless, and safe exam, with no reported adverse events.
7. Phlebotomy
   This is a minimally invasive procedure used to gain access to a peripheral vein to draw blood. Risks include pain, bruising, lightheadedness, and fainting. These are often mild and tolerable.

8. Stool collection
   Not invasive without any reported adverse events.

K. Stopping Rules

1. Common Terminology Criteria for Adverse Events
   We will use the Common Terminology Criteria for Adverse Events (CTCAE. latest version) grading system to monitor the clinical trial's potential adverse events. These could include diarrhea, abdominal cramping, flatulence, bad taste, nausea, hematological, (anemia, leukopenia, and thrombocytopenia, occurring in <3%)[59], allergic reactions (rash, urticaria, edema, hypotension, respiratory distress/failure), renal insufficiency, hearing deficits, phlebitis[60], hepatotoxicity (rare)[61, 62], and overgrowth of VRE. The CTCAE is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term. The CTCAE displays grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline (table 4).

**Table 4: Common Terminology Criteria for Adverse Events (CTCAE) grading system developed by the National Cancer Institute (NCI)/National Institutes of Health (NIH)**

| Grade | Description |
|---|---|
| 1 | Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| 2 | Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL). |
| 3 | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting self-care ADL. |
| 4 | Left-threatening consequences; urgent intervention indicated. |
| 5 | Death related to AE. |

2. Study Stopping Rule
   i. Individual Subject Stopping Rule during the treatment phase (first 18 months) of the trial:

a. Increase in serum ALP ≥ 3 times the baseline or any treatment period value,
b. Increase in serum AST and/or ALT ≥ 3 times the baseline or any treatment period value,
c. Doubling of the total bilirubin level from baseline or any treatment period value,
d. IBD flare requiring > 20mg of prednisone per day or a biological agent,
e. Serious allergic reaction, or
f. Pregnancy and breastfeeding.

ii. The participation of a study subject may be terminated by the principal investigator if the subject meets ANY of the following criteria/conditions:
a. Non-adherence to study protocol and study procedures,
b. Noncompliance with study agent,
c. Need for liver transplantation
d. Liver decompensation (development of esophageal varices or variceal hemorrhage, hepatic encephalopathy, hyponatremia, development of hepatorenal syndrome),
e. Diagnosis of CCA,
f. Intolerable side effects that cannot be resolved with a drug holiday

iii. Global Study Stopping Rule
**a.** 3 subjects develop the same grade 3 CTCAE (excepting PSC-related complications), **or**
**b.** 2 subjects develop any grade 4 CTCAE (excepting PSC-related complications) **or**
**c.** 1 subject develops a grade 5 CTCAE (excepting PSC-related complications)**or**
d. 2 subjects develop ≥ 1 of the following IBD-related complications (IBD refractory to the standard medical treatment, development of colon cancer, **or** toxic megacolon)
e. > 5% of subjects develop ≥ 1 of the following PSC-related complications (variceal hemorrhage, bacterial peritonitis, acute ascending cholangitis requiring hospitalization, **or** development of cholangiocarcinoma)

The study may also be suspended or terminated based on the review criteria outlined in the Data Safety Monitoring Plan. The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures. The study would only be resumed after a thorough review of the incidents and once corrective and preventative actions have been put in place, in consultation between the study team and the IRB.

3. Drug induced liver injury procedure
The following applies to patients with suspected drug-induced liver injury (DILI):
i. If a patient meets the individual drug discontinuation criteria (as noted in the protocol) and is excluded from the clinical trial, liver profile and PT/INR shall be repeated within 48-72 hours, and the patient shall be placed under “close observation” as defined in the DILI guidance until the liver enzymes “normalize or stabilize”. Depending on the patient’s progression, consider initiating potential DILI evaluation for competing etiologies. Consider re-starting study medication only if a definitive alternative etiology is identified and liver tests return to baseline.
ii. With any elevation of bilirubin of 0.3mg/dl or more, regardless of ALT or AST levels, and in the presence of indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis):
a. Interrupt vancomycin.
b. Initiate potential DILI evaluation for competing etiologies and repeat liver profile and PT/INR within 48-72 hours and place patient under “close observation” as defined in the

DILI guidance.

c. Study medication can be restarted only if a definite alternative etiology is identified and the liver tests return to baseline.

iii. If a patient lives in a remote area, they can be tested locally and the results should be promptly communicated to the investigator site.

iv. The investigational agent must be discontinued and the patient must be followed until the clinical and laboratory abnormalities stabilize or normalize if the following criteria are met:
   a. Close monitoring of a patient is not possible.
   b. If any degree of total bilirubin, ALT, or AST elevation recurs following re-challenge with the study drug.

v. In the case of a study subject inadvertently prescribed oral or IV Vancomycin during the treatment phase of the trial, a trough vancomycin level will be drawn to exclude vancomycin toxicity. Subjects will be withdrawn from the trial if their clinicians believe that ongoing vancomycin is necessary for treatment of a medical condition. If the inadvertent use of vancomycin is < 14 days, investigator may opt to continue subject in the trial, with a note to file regarding vancomycin exposure.

## L. Sample Size Calculation and Statistical Analysis of Data

The power calculation is based on an intention to treat analysis. The sample size is calculated based on the assumption that 40% of PSC patients treated with OV will experience serum ALP normalization, and only 15% of those treated with placebo will experience ALP normalization. Assuming a two-sided type I error of alpha = 0.05, at least 80% (.8) power, 93 subjects are required for this study. We conservatively presume a 10% dropout rate (due to longevity of the study). Although we will treat dropouts as treatment failures in an intention-to-treat manner, we will increase our starting sample size to 102 to ensure sufficient participants complete the clinical trial. We will enroll (51 in OV group, and 51 in the placebo group). As shown in the table below, this sample size will provide 80% power for a range of potential response percentages.

**Table 5: The total sample size required for each scenario to achieve 80% power with 5% type I error**

| | Placebo percent with normalizing ALP | | |
|---|---|---|---|
| Vancomycin percent with normalizing ALP | 10% | 12% | 15% |
| 40% | 54 | 67 | **93** |
| 50% | 31 | 37 | 48 |

The difference in percent response we anticipate (15% on placebo vs. 40% on vancomycin) is based upon a conservative assessment of the literature to date on ALP normalization. Furthermore, this corresponds to a meaningful difference that would yield changes to clinical care based upon study results.

Patients will be included in the primary analysis on the basis of intention-to-treat (ITT). The proportion of patients with normalization of serum ALP will be compared between the treatment and placebo arms using the Pearson chi-square test. We will also explore the effect of ALP as a percentage reduction from baseline compared between the treatment and placebo arms using generalized estimating equations to cluster measurements over time from within the same patient;

this analysis will be repeated using the absolute ALP as an outcome and the baseline ALP as a predictor. Patients on OV who respond to OV at different doses will be compared descriptively to inform future studies regarding which patients require higher dosing. We will also compare study arms with Kaplan-Meier curves and the Cox proportional hazards model using OV as a fixed and time dependent predictor while adjusting for the stratification variables. Within the same series of Cox models, we will assess IBD and severity of PSC as effect modifiers for the effect of OV on ALP normalization. Descriptive statistics (median, mean, standard deviation, interquartile range, frequency and percentages) will be reported for the two arms at each of study time points. Mean change in hepatic biochemistries and difference in number of cholangitis episodes from baseline will be compared between the two arms by using analysis of variance or the Kruskal-Wallis test as appropriate. Changes in hepatic biochemistries at each of the study time points will be measured and compared between the two arms, and stratified by regimen (placebo vs. OV). The incidence, type and relatedness of serious adverse events and adverse events leading to discontinuation of the study agent will be reported for each group and compared between the two arms and regimens using chi-square tests.

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