Investigation of the Activity of Vidofludimus Calcium, a Novel, Orally Available, Small Molecule Inhibitor of Dihydroorotate Dehydrogenase, as a Treatment for Primary Sclerosing Cholangitis (PSC)

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Primary sclerosing cholangitis (PSC) is an idiopathic disease of the liver with a prevalence of approximately 50,000 patients in the United States. To date, **no medical therapies other than liver transplantation** (LT) have been shown to modify the natural course of PSC. The median time from diagnosis of PSC to death or LT is only 8 years. The investigational product **vidofludimus calcium (VC)** is a novel compound that selectively inhibits dihydroorotate dehydrogenase (DHODH), the enzyme that catalyzes the rate-limiting step in pyrimidine synthesis. Through a unique dual mechanism, VC **inhibits proliferating lymphocytes** and **reduces release of the proinflammatory cytokines IL-17 and IFNγ**.

Inflammatory bowel disease (IBD) is frequently seen in patients with PSC. Studies have indicated a central role for IL-17 and IL-17-producing lymphocytes in the pathogenesis of both diseases. Clinical data has also shown vidofludimus to be **effective in the treatment of IBD** while exhibiting a **highly favorable safety profile**.

Given this train of evidence, we are proposing a small, exploratory clinical trial investigating the role of VC in PSC. The trial will be conducted by investigators with long careers dedicated to the study of PSC and other hepatobiliary disorders. The trial will be executed at two sites that are both tertiary referral centers for PSC.

I. Goal:

The overall goal of this study is to examine the safety, tolerability, and efficacy of daily dosing with vidofludimus calcium over a 6-month period on the clinical course and progression of primary sclerosing cholangitis.
The results will help us:

- Establish proof-of-concept that VC may show activity for the treatment of PSC
- Gain insight into the effect of VC on profiles of the proinflammatory cytokines IL-17 and IFNγ
- Establish data to design more comprehensive clinical studies

II. Impact of Study:

This open-label, single-arm pilot study will give us the opportunity to explore the potential of this novel drug to show activity in patients suffering from PSC, a rare but devastating disease with no known effective treatment.

III. Expected Outcome:

We hypothesize that in PSC, daily treatment with vidofludimus calcium will result in reduction of serum ALP, a surrogate marker that has been shown to strongly correlate with positive outcomes in PSC. Further, we hypothesize that treatment will result in a decrease in certain proinflammatory cytokines, particularly IL- 17 and IFNγ. We expect based on a wealth of preclinical and clinical evidence that treatment with vidofludimus calcium will be safe and well tolerated.

IV. Specific Aims of the Study:

1. Determine if VC reduces serum alkaline phosphatase (ALP) in adults with PSC. Levels of serum ALP after 3 and 6 months of VC treatment will be compared with baseline (month 0).
2. Assess other liver biochemistries (an indication of general liver health) at 3 and 6 months of VC treatment and compare to baseline (month 0).
3. Determine if changes in the proinflammatory cytokines IL-17 and IFNγ predict

response to VC. Cytokines will be measured at baseline, 4 weeks, and 6 months.

If successful, this low-risk, high-reward study will lay the groundwork for larger, more comprehensive clinical studies investigating the efficacy of VC in PSC. This could ultimately lead to the development of a successful therapy for PSC and significantly improve patient outcome and quality of life.

RESEARCH STRATEGY

Significance

Primary sclerosing cholangitis (PSC) is an idiopathic disease of the liver with incidence and prevalence rates ranging from 0-1.3 per 100,000 inhabitants/year and 0-16.2 per 100,000 inhabitants, respectively, although these rates appear to be trending upward[1]. Based on our experience and reported studies, we estimate a PSC prevalence of approximately 50,000 patients in the United States. PSC is 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[2]. The disease can affect both children and adults and is frequently associated with inflammatory bowel disease (IBD), namely ulcerative colitis (UC)[3-7]. PSC is an important risk factor for cholangiocarcinoma (CCA), a bile duct cancer that carries a grave prognosis, and for colorectal adenocarcinoma in patients with colitis[2].

To date, **no medical therapies other than liver transplantation** (LT) have been shown to modify the natural course of PSC. The median time from diagnosis of PSC to death or LT is only 8 years[8] with the most recent modification of the Mayo PSC risk score being a helpful tool to predict prognosis[9]. An invasive and expensive procedure, LT is currently the only life-extending therapy for patients with ESLD secondary to PSC and patients with PSC complicated by CCA who meet specific criteria[1,10,11]. PSC is currently the fifth leading indication for LT in the United States, and is the leading indication for transplantation in some European countries.[10,12] Nevertheless, even after LT, *de novo* IBD and CCA have both been reported[13,14] and PSC recurrence rates as high as 20-40% have been seen.[15,16] Taken together, these data highlight the considerable disease burden, cost to the healthcare system, and clinical impact of PSC.

Only a handful of therapeutic agents for PSC have undergone clinical trials, possibly due to low disease prevalence. None of the agents studied have shown a beneficial effect on clinically important endpoints and survival in PSC.[17] Ursodeoxycholic acid (UDCA) is the single most extensively studied agent in PSC. However, large randomized and controlled prospective clinical trials have failed to demonstrate that UDCA can positively affect patient outcome.[18,19] Further, the safety of long term use of high-dose UDCA in PSC patients has been questioned, as it has been associated with increased rates of serious adverse events.[19,20] The American Association for the Study of Liver Disease (AASLD) recommends against the use of UDCA in PSC patients.[7]

Liver transplantation remains the sole treatment for end-stage PSC. There are several current avenues of research addressing treatment strategies for PSC. One target is the farnesoid X receptor, which plays a key role in bile acid homeostasis. Other evidence suggests a link with the intestinal microbiota, [21] leading to the investigation of fecal transplantation and probiotics as therapeutic approaches. A very promising therapy is the use of oral vancomycin. In addition to its potential effects on microbiota, work by Abarbanel *et al.*[22] suggests that its therapeutic properties in PSC are linked to immunomodulatory effects on regulatory T cells.

PSC is thought to arise from a combination of genetic and environmental factors, with increased prevalence among first-degree relatives indicating a strong genetic component.[23] One of the more intriguing aspects of PSC is the striking frequency of comorbidity with IBD, particularly ulcerative colitis. PSC is frequently seen in patients with underlying IBD[3,24] and is the most common hepatobiliary manifestation seen in those patients.[25] Conversely, **IBD is present in 70–80% of PSC patients**, up to 90% of that in the form of UC.[26-29]The link between PSC and IBD is not yet understood, but given the intimate relationship and shared antigen exposure between the gut and hepatobiliary systems (gut-liver crosstalk), it is not surprising to find PSC to be an extraintestinal manifestation of IBD. Recent genotyping studies suggest common genetic underpinnings, with three UC susceptibility loci also found to be associated with PSC.[30] Beyond genetic linkage, two emerging theories, which are not mutually exclusive, have been proposed to contribute to this close association. In one hypothesis, lymphocytes activated in the inflamed gut of IBD patients are aberrantly recruited to the liver via enterohepatic circulation. The work of Adams and colleagues[31-36] suggests that this is due to expression in the liver of adhesion molecules that are normally restricted to the gut. This aberrant homing results in the infiltration of the liver by T cells, including T helper 17 (Th17) cells, resulting in inflammation leading to PSC.[37-38] In the other, the so-called "leaky gut" hypothesis, the increased permeability of the chronically inflamed bowel facilitates translocation of bacterial components to the liver and bile duct via the enterohepatic circulation, contributing to the pathogenesis of PSC. In support of this, intraportal injection of enteric bacteria in rabbits[39] and intestinal leakage of bacteria in rats[40] have both been shown to lead to PSC-like symptoms.

The proinflammatory cytokine interleukin 17 (IL-17) is a key mediator of a variety of immune related human diseases[41] and Th17 lymphocytes, T helper cells defined by their production of IL-17, are found in chronically inflamed human tissues.[42] In IBD, Th17 cells massively infiltrate the inflamed intestine[43] and UC patients demonstrate greatly increased expression of IL-17 in colonic mucosa.[44] Perhaps not surprisingly, recent studies indicate a **central role of IL-17 in the pathogenesis of PSC**. Significant increases in IL-17-expressing lymphocytes are found in the livers of PSC patients compared to other types of liver inflammation. IL-17- expressing T cells aggregate around damaged bile ducts and in areas of neoductular proliferation.[45] PBMCs from PSC patients manifested significantly **increased frequencies of Th17 cells** compared to both healthy and cholestatic controls in response to pathogen stimulation.[46] CARD9, one of the UC susceptibility loci that has also been associated with PSC, encodes a gene product that has been shown to be involved in the induction of Th17 responses to some pathogens.[47] These results are all consistent with the strong clinical linkage between UC and PSC, the key role of IL-17 as a target for potential therapeutic intervention, and speak to the strong possibility of an overlap in therapeutic approaches to the two diseases.

The investigational product **vidofludimus calcium (VC)** is a novel compound that blocks the *de novo* synthesis of pyrimidines by **selectively inhibiting human dihydroorotate dehydrogenase (DHODH)**, the enzyme that catalyzes the rate-limiting step in this synthesis pathway. Proliferating or activated lymphocytes require this *de novo* pathway to meet their needs for pyrimidines, an essential component of nucleic acids, with DHODH inhibition in these cells leading to cell cycle arrest and apoptosis. On the other hand, resting lymphocytes do not depend on *de novo* synthesis, instead satisfying their pyrimidine requirements through the DHODH-independent salvage pathway. Thus, **VC selectively affects activated, rapidly proliferating lymphocytes**. Metabolic stress following inhibition of pyrimidine synthesis induces upregulation of the transcriptional regulator HEXIM1 which acts by two independent mechanisms[48] to modulate IL-17-mediated inflammation. By sequestering the pTEFb complex, it inhibits transcription of specific inflammatory signaling molecules, including IL-17.

Additionally, with prolonged pyrimidine depletion, HEXIM1 binds to and stabilizes apoptotic gene transcripts, leading to cell death in activated cells (*e.g.* Th17 lymphocytes). Through these two molecular mechanisms, DHODH leads to a reduction of proinflammatory cytokine release **including IL- 17 and interferon-gamma (IFNγ)**. Preclinical studies confirm these effects of vidofludimus. In two different mouse models of colitis, oral vidofludimus not only evoked substantial improvements in clinical and histological colitis scores and reduced lymphocyte infiltration, but also effected a significant reduction of IL-17 and IFNγ in the colon.[49] Similarly, in a rat model of colitis, vidofludimus improved inflammation and inhibited IL-17 production.[50]

Vidofludimus calcium constitutes a novel chemical class with no structural similarity to other known pharmaceuticals and the calcium salt is a more readily absorbable form of vidofludimus free acid. Its blood half-life of approximately 30 hours makes it suitable for once daily oral treatment. Currently, **VC is being developed as a treatment for patients with ulcerative colitis and Crohn's disease (CD)**. Two Phase 2 clinical trials examining the safety and efficacy of vidofludimus in rheumatoid arthritis (RA) have previously been completed (see Table 1). Although the primary endpoint was not met, the trials demonstrated consistent numerical superiority of vidofludimus over placebo. More importantly, these large RA trials established the safety of this compound, with an adverse event profile no different from placebo. Most notable are the results from a Phase 2a study that explored the efficacy, safety, and tolerability of vidofludimus in steroid-dependent IBD (both UC and CD). Overall, 88.5% of subjects responded following 12 weeks of treatment: 54% achieved complete response (complete weaning from steroids) and a further 35% were able to reduce steroid usage.[51] Further, favorable safety and tolerability profiles were demonstrated. Taken together, the safety data from these trials indicate that **vidofludimus at therapeutic doses is safe and well tolerated in humans**; a conclusion supported by a wealth of non-clinical pharmacology, pharmacokinetics, and toxicology data. These previous clinical studies have established a safe and effective dose of VC to be used in this proposed pilot study in patients with PSC as well as a more extensive Phase 2 trial in UC.

Given the favorable safety data, the demonstrated reduction of tissue IL-17 levels and inhibition of Th17 cells, the efficacy established in early clinical trials of patients with IBD, and the strong link between UC and PSC, vidofludimus calcium is a **low risk, high reward candidate** for therapeutic intervention in rare but devastating disease PSC. This pilot study is designed to show a first indication of activity of VC in patients with PSC using surrogate endpoints. If successful, this study will lay the groundwork for larger more comprehensive clinical studies investigating the efficacy of VC in PSC. Development of a successful therapy to reduce or halt the progress of this disease, for which there is currently no effective treatment, will significantly improve patient outcome and quality of life. Further, as PSC is one of the leading indications for liver transplantation in the US and Europe, it would significantly reduce the requirement for this expensive and risky procedure and lessen the burden on the transplant system, reducing wait times for others in need of LT.

Phase	Study Description
1	SC12267-1-2003: Single dose escalating Phase 1 study in 57 male volunteers investigating single doses of vidofludimus up to 350 mg *(complete)*
1	SC12267-2-2004: Multiple dose escalating Phase 1 study in 32 male volunteers investigating multiple doses of vidofludimus up to 70 mg *(complete)*
2a	SC12267-3-2005: Phase 2a study in 116 RA patients evaluating efficacy and safety of vidofludimus (20 mg or 35 mg) monotherapy or placebo *(complete)*
2a	SC12267-4-2008: Phase 2a study in 34 IBD patients evaluating efficacy and safety of vidofludimus (35 mg) ("ENTRANCE") *(complete)*
2	SC12267-5-2009: Phase 2 study in 241 RA patients evaluating efficacy and safety of vidofludimus (35 mg) or placebo on methotrexate background therapy ("COMPONENT") *(complete)*
1	P1-IMU-838-SAD: Open-Label, Single Ascending Dose, Phase 1, Study to Assess the Safety, Tolerability and Pharmacokinetics of Single Oral Doses of IMU-838 in Healthy Male Subjects Including Assessment of a Possible Food Effect *(complete)*
1	P1-IMU-838-MAD: Double-blind, Placebo Controlled, Ascending Dose, Phase 1, Study to Assess the Safety, Tolerability and Pharmacokinetics of Multiple Oral Doses of IMU-838 in Healthy Male Subjects *(ongoing)*
2	P2-IMU-838-UC: A phase 2, multicenter, randomized, double-blind, placebo-controlled, dose-finding study to evaluate the efficacy and safety of IMU-838 for induction and maintenance therapy in moderate-to-severe ulcerative colitis *(planned)*

Table 1. Completed, ongoing and planned clinical studies

Innovation

Vidofludimus calcium constitutes a novel chemical class and has no structural similarity to other known pharmaceuticals. Its chemical structure is considerably different from other DHODH inhibitors[49,52] such as leflunomide (Arava®, approved for use in RA and psoriatic arthritis) and its active metabolite teriflunomide (Aubagio®, approved for use in multiple sclerosis). Compared to a number of existing IBD therapeutic agents that might be considered as candidates for treatment of PSC, data so far indicates that **VC is likely to exhibit a superior safety and tolerance profile**. Thiopurine analogues azathioprine and mercaptopurine are in common use for managing UC and CD, but at least one third of patients fail to respond to therapy and up to 28% of patients stop medication due to intolerable side effects.[53] Methotrexate, the established second-line immunosuppression in CD, exhibits a clinically relevant toxicity profile.[54,55] Approved TNFα blockers, such as infliximab and adalimumab, have demonstrated excellent clinical response in UC and CD, however, major infections have been linked to TNFα-inhibitor treatments.[56] The DHODH inhibitor leflunomide has shown efficacy in CD, however its side effect of diarrhea is not acceptable in an IBD patient population.[57]

Vidofludimus is a **potent inhibitor of human DHODH**, three times more effective than teriflunomide. A second important and distinguishing property of vidofludimus is its **potent inhibition of IL-17 and IFNγ release** from stimulated immune cells. Inhibition of IL-17 production was found to be independent of its effects on lymphocyte proliferation and it is believed that both these independent activities of vidofludimus contribute to its therapeutic effects in IBD.[50,58] This **dual mechanism of action** confers a unique advantage over other DHODH inhibitors and makes vidofludimus a promising therapeutic option for both UC and PSC, diseases with frequent comorbidity and a common pattern of Th17 lymphocytic infiltration. With good clinical data for UC already in hand and a more comprehensive UC study in the works, a pilot trial for PSC is warranted. Success would result in a major step towards an effective

treatment for PSC where one does not currently exist.

Approach

We are proposing to test the hypothesis that vidofludimus calcium, a novel drug product, will be an effective treatment strategy for the rare but devastating disease primary sclerosing cholangitis. The rationale for this is based on **i) the strong linkage between ulcerative colitis and PSC; ii) the known involvement of Th17 lymphocytes and IL-17 in both disorders; iii) the demonstrated effect of vidofludimus on lymphocyte proliferation and IL-17 production; and iv) clinical evidence for the efficacy of vidofludimus in patients with UC**. Our approach is to investigate this hypothesis using a small two- center pilot study employing serum alkaline phosphatase, a surrogate marker having a well-established association with clinical outcome, as our primary endpoint. We further hypothesize that treatment with VC will affect profiles of proinflammatory cytokines and will assess IL-17 and IFNγ levels as a potential early indicator of improvements in ALP.

The Specific Aims of the study are:

1. Determine if VC reduces serum alkaline phosphatase (ALP) in adults with PSC. Levels of serum ALP after 3 and 6 months of VC treatment will be compared with baseline (month 0).
2. Assess other liver biochemistries (an indication of general liver health) at 3 and 6 months of VC treatment and compare to baseline (month 0).
3. Determine if changes in the proinflammatory cytokines IL-17 and IFNγ predict response to VC. Cytokines will be measured at baseline, 4 weeks, and 6 months.

These results will help us:

- Establish feasibility of the hypothesis that VC will play a therapeutic role in treatment of PSC
- Gain insight into the effect of VC on profiles of the proinflammatory cytokines IL-17 and IFNγ
- Pave the way for more comprehensive clinical studies

<u>Study Design and Methodology</u>

This pilot study will be a 6-month, 30-subject clinical trial to assess the effects of VC on a surrogate biochemical endpoint known to have relevant association with clinical outcomes in PSC. All endpoint tests chosen are rapid, inexpensive, and minimally invasive. Due to the low prevalence of PSC, the study will be a single-arm, open-label study. The study will be undertaken at two sites, Mayo Clinic, Rochester, Minnesota (20 subjects) and Mayo Clinic, Phoenix, Arizona (10 subjects), both of which are tertiary referral centers for PSC. Immunic AG, a biotechnology company based in Munich, Germany, will supply the study agent in sufficient quantities for the study (see Letter of Support from Immunic AG). We have confirmed through consultation with NIH personnel that this is permissible under this funding opportunity.

During the 6-month treatment period, subjects will receive 30 mg VC orally once daily, a dose determined to be both safe and therapeutically relevant based on previous clinical studies (see Table 1). This will be preceded by a lead-in dosing period where subjects will receive 15 mg VC once daily for 1 week. Lead-in dosing is used to minimize the risk of drug intolerance during the initial treatment period. While no clinically relevant adverse events have been noted at 30 mg once daily dosing, in rare instances potentially drug-related hematuria has been observed in the first few days of treatment at higher doses (≥70 mg/day). As a precaution, subjects will be monitored for two weeks after initial drug

exposure for elevated erythrocytes in the urine by a dipstick test. It is not anticipated that hematuria will be seen if it has not developed within the first two weeks of drug exposure.

The following endpoints will be assessed at various times during the study and compared with baseline:

i. Primary endpoint:
    a. Improvement of serum alkaline phosphatase (ALP) [baseline, 3 months, and 6 months]
ii. Secondary endpoints:
    a. Bilirubin, Total and Direct [baseline, 3 months and 6months]
    b. AST and ALT (aspartate and alanine transaminases) [baseline, 3 months, and 6 months]
    c. Proinflammatory cytokine profile (IL-17 and IFNγ) [baseline, 4 weeks, and 6 months]

The most extensively studied surrogate endpoint in PSC 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, liver transplantation, liver-related death, and colorectal cancer and persistent ALP elevation has been associated with worse outcomes in PSC.[59-61] Therefore, we have chosen this simple, rapid, inexpensive, and minimally invasive test as our primary endpoint. AST, ALT, and bilirubin will be monitored to assess general liver health during the treatment. AST, ALT, or total bilirubin elevation can signal increased liver damage or liver cell necrosis, while elevated direct bilirubin may indicate bile duct obstruction. Further, it is hypothesized that treatment with VC will result in a reduction of IL-17 and IFNγ production. Levels of these cytokines will be measured at 4 weeks and at 6 months (end of study) and compared to baseline. The early 4-week time point will be used to determine whether early changes in these cytokines may predict improvements in ALP.

A 25% improvement in ALP with no more than a 33% worsening of AST after 6 months of treatment will be assessed as a positive outcome. While full normalization of ALP levels is unlikely over the short course of this pilot study, a 25% improvement would be deemed significant, indicating that VC has a positive effect on the progression of PSC. A less than 33% increase in AST levels concomitant with this ALP reduction would be acceptable, as it would be considered only a mild elevation. While a recent consensus statement from the International PSC Study Group indicates that transient elastography (TE), a new technique that allows non- invasive assessment of liver fibrosis, is one of the top three most promising surrogate endpoints for PSC (along with ALP and liver histology),[62] it is unlikely that significant changes in TE would manifest in the 6-month course of this study. Thus, TE will not be included as an endpoint. Reduction of proinflammatory cytokines IL- 17 and IFNγ at the early 4-week time point concomitant with reduced ALP at the end of study would indicate a role for cytokine levels as an early predictor of improvements.

At the end of the 6-month treatment, any subjects demonstrating benefit may optionally continue VC treatment under a single-patient IND. Subjects without benefit will return to standard of care for PSC patients. All subjects will receive a post-study follow-up assessment at 30 days to capture any remaining adverse events.

Timeline and Schedule

For each subject, study participation will consist of a screening period of up to 1 month, a lead-in dosing period of 1 week, treatment period of 6 months, and a follow-up period of up to 1 month. A recruitment period of no more than 6 months is anticipated. Subjects will be seen in clinic at baseline and at the end of the treatment period, where safety, clinical and laboratory evaluations will be performed. 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. Every other day during the first two weeks, urine dipstick testing for hematuria will be performed. Between the clinical visits, patients will have laboratory tests and be monitored by phone call every 4 weeks throughout the treatment period, and again at 1 month after completion of the treatment period for safety, adverse events, subject health, and adherence to the study protocol. Safety monitoring tests will include AST, ALT, ALP, TBIL, DBIL. Cytokines (IL-17 and IFN-ɣ) will be tested at Baseline, 4 weeks, and 6 months. Blood will be collected at Baseline, 4 weeks, 3 months, and 6 months and stored for potential future analysis. Images from the MRCP and results from the Fibroscan, both done as standard of care, will be recorded at Screening /Baseline and at 6 months. If images from the MRCP or results from the Fibroscan are not performed as standard of care or are not available from the medical record, they do not need to be performed for the study. Women of childbearing capability will undergo monthly pregnancy tests. It will be required that both patient and partner use a barrier method as well as hormonal contraception while on study. If patient or partner has had onset of menopause, a hysterectomy, tubal ligation, or vasectomy, no other contraception is required. Those with a confirmed positive test will be removed from the study.

Pharmacokinetic Sample

Those patients who experience a Serious Adverse Event (SAE) suspected of being related to the study drug during the treatment period will be asked to provide a single pharmacokinetic (PK) blood sample at discretion of the clinician. Ideally this should be drawn before the drug is taken that day. The sample may still be taken if the patient has taken their study drug within 1.5 hours of the PK blood draw. If it has been longer than 1.5 hours since the study medication was taken, the PK draw should be postponed until the next morning; however, if a draw the next day is not possible, then the blood sample may be collected and timing noted on the accompanying paperwork to the lab. All samples will be processed by a central laboratory.

Risk Mitigation and Alternative Strategies

We have structured the study to minimize risk to the subjects and have included ways to assess, track, and mitigate these potential risks. We will use a dosing scheme that has been shown convincingly to be safe and well tolerated. We will include a lead-in dosing period with testing for drug-induced hematuria during the early part of the study despite the fact that this adverse event has only been demonstrated to occur at higher doses. During clinical trials, the one serious adverse event reported was in a subject with Gilbert syndrome, a genetic disease characterized by a drastic reduction in the activity of UGT1A1. As VC is known to be a moderate inhibitor of UGT1A1, patients with Gilbert syndrome are excluded from this study.

We have included liver function testing to monitor general liver health and to indicate potential hepatobiliary issues. Frequent assessments of general health will be performed and subjects will be instructed to alert the study coordinator of any potentially drug-related complications, particularly abdominal pain. Standard drug discontinuation criteria will be included in the protocol based on the investigators' knowledge of PSC and PSC- related clinical trials, information derived from previous vidofludimus clinical trials, and the extensive data contained within the drug manufacturer's

Investigator's Brochure.

Due to the relatively low prevalence, it is possible that 30 subjects meeting inclusion criteria cannot be recruited within the planned 6-month recruitment period or that they are not split roughly equally between the two sites. In that case, we will extend the recruitment period and if required, we will execute the study with as few as 20 subjects, regardless of the distribution between sites. After the study is complete, further research may reveal other potentially interesting biomarkers that should be examined and thus we have included storage of extra blood draws during the study. Given the pilot nature of this study, it is possible that benefits are seen even though results are not conclusive. Study data may be examined more carefully to determine if benefits occur in a distinct subpopulation of subjects (*e.g.* in PSC patients with UC versus those without).

Overall, considering the known safety data, the implemented risk minimization measures, the expected benefits in the target population, and the unmet medical need for treatment in PSC, the risk-benefit evaluation is considered favorable.

PROTECTION OF HUMAN SUBJECTS
This Human Subjects Research meets the definition of a clinical trial.

I. Risks to Human Subjects

a) Human Subjects Involvement, Characteristics, and Design: A total of 30 adults (age range 18-75 years) who meet the criteria for diagnosis of primary sclerosing cholangitis (PSC), as defined by the American Association for the Study of Liver Diseases (AASLD) published guidelines, will be enrolled into this study from two centers. PSC patients with and without inflammatory bowel disease (IBD) will be included. Other inclusion and exclusion criteria are detailed below:

Inclusion Criteria

a. Male or female subject age 18-75 years
b. Diagnosis of PSC consistent with the guidelines published by the AASLD. All subjects must have an elevated serum ALP of at least 1.5 times upper limit of normal (ULN) at baseline plus cholangiographic evidence of PSC (MRI, endoscopic retrograde cholangiography, or direct cholangiography).
c. Indirect bilirubin <1.2 times the ULN
d. An ultrasound (or equivalent imaging modality) that excludes biliary obstruction and malignancy within 6 months of study enrollment
e. PSC with or without inflammatory bowel disease, such as ulcerative colitis or Crohn's disease
f. Must agree to comply with the study protocol and provide informed consent

Exclusion Criteria

a. Pregnancy, attempting to become pregnant, or breastfeeding
b. Active hepatitis A or B infection
c. Active 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
d. HIV/AIDS (per medical record or HIVAb/HIV antigen) , tuberculosis, or positive interferon-gamma assay (IGRAs) for *Mycobacterium tuberculosis*
e. Other cholestatic liver disease such as primary biliary cholangitis and cholestatic diseases of pregnancy
f. History of metabolic liver diseases such as Wilson's disease, Gilbert's syndrome or hemochromatosis

g. Serum uric acid levels at Screening >1.2 ULN
h. Inherited diseases of the liver such as α-1 antitrypsin deficiency
i. Immunoglobulin G4-related cholangitis
j. PSC with concomitant autoimmune hepatitis (AIH) and/or primary biliary cholangitis
k. Secondary sclerosing cholangitis (SSC)
l. Active acute ascending cholangitis requiring antibiotics
m. CCA (malignant biliary stricture, neoplasm, and cytology/histopathology or positive fluorescence in situ hybridization (FISH) consistent with adenocarcinoma of the bile duct)
n. 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.
o. Presence of complications of advanced PSC such as hepatic encephalopathy, portal hypertension, hepato-renal syndrome, and hepato-pulmonary syndrome
p. History of liver transplantation, anticipated need for liver transplantation within 12 months from randomization, a Model of End-stage Liver Disease (MELD) score of ≥15, or a Child Pugh score >6
q. Ongoing alcohol abuse (>4 drinks per day for men, and >2 drinks per day for women)
r. Moderate-to-severe renal impairment with a calculated creatinine clearance of <60mL/min
s. 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
t. Evidence of, or treatment for, *C. difficile* infection within 30 days before the initiation of the study drug
u. Evidence of active *C. difficile* infection during the screening phase confirmed by a positive *C. difficile* toxin B
v. Subjects who have been treated for intestinal pathogens other than *C. difficile* infection within 30 days prior to study drug initiation
w. Received or plan to receive live vaccine within 30 days prior to, and through the end of the study
x. Use of methotrexate at dose ≥17.5mg/week
y. Rosuvastatin exceeding 10 mg daily.

PSC is a rare idiopathic liver disease associated with significant morbidity and mortality. There is no cure for PSC. Recent clinical evidence suggests that vidofludimus calcium (VC) might be of therapeutic benefit in ulcerative colitis (UC). The strong linkage between UC and PSC, commonalities in the underlying immunological characteristics of the two diseases, and the known methods of action of VC combine to make a compelling case for the first ever clinical trial of VC as a therapeutic agent for PSC. The excellent safety profile of VC, derived from clinical and non-clinical studies, further validate this case. This study will help us determine whether VC demonstrates feasibility for use as treatment for PSC.

Study subjects will be recruited and enrolled into the clinical trial from the following research sites:

Study Site	Investigator	Number of Enrollees
Arizona State University (ASU)	Dr. Keith Lindor (Principal Investigator)	Not Applicable
Mayo Clinic Rochester (MCR)	Dr. John Eaton (Co-Investigator)	20
Mayo Clinic Arizona (MCA)	Dr. Elizabeth Carey (Co-Investigator)	10
Total		30

Table 2. Study sites, recruitment and enrollment

These sites have been selected because of their interest, experience, and engagement with substantial populations of PSC patients. Overall recruitment for the study will be 30 patients, but number of enrollees at each site may be adjusted at discretion of the investigators to facilitate recruitment.

Each site (except ASU) will be responsible for:

- Recruiting and screening subjects
- Enrolling subjects into the clinical trial
- Administering the study agent
- Safety monitoring, clinical and laboratory assessments at the defined study time points
- Reporting progress of study, side effects, and adverse events to the local Institutional Review Board (IRB) /clinical trial monitor

We anticipate a recruitment period will be no longer than 6 months and a screening period of one month. Before beginning treatment of subjects with full dose of the drug, a lead-in dosing period where subjects will receive 15 mg VC once daily (half the treatment dose) for one week. Lead-in dosing is used to minimize the risk of drug intolerance during the initial treatment period. During the 6-month treatment period, subjects will be treated with 30 mg VC orally once daily, a dose determined to be both safe and therapeutically relevant based on results from previous clinical studies. Throughout the clinical trial, study subjects will undergo safety monitoring, clinical and laboratory evaluations. These will include:

- Primary endpoint testing (serum alkaline phosphatase, ALP) at baseline, 3 and 6 months
- Secondary endpoint testing (AST/ALT and bilirubin, total and direct) at baseline, 3 and 6 months
- Cytokine level testing (IL-17 and IFNγ) at baseline, 4 weeks, and 6 months
- Extra blood draws, to be stored for potential future studies, will be obtained at baseline, 4 weeks, 3 months, and 6 months
- Subjects will be seen in clinic at enrollment and at the end of study
- Phone monitoring will be conducted every 4 weeks during the study, beginning at the end of the lead-in dosing period. We will assess subject health and general well-being, including PSC-related symptoms and IBD-related complications, safety, adverse events, and adherence to the study protocol,
- Subjects will be contacted by phone one month after the end of the study as a follow-up to assess health and well-being and to capture any additional adverse events

At the end of the 6-month treatment, any subjects demonstrating benefit may optionally continue VC treatment under a single-patient IND. Subjects without benefit will return to standard of care for PSC patients. All subjects will receive a post-study follow-up assessment at 30 days to capture any remaining adverse events (see Figure 1 for an overview of the clinical trial design).

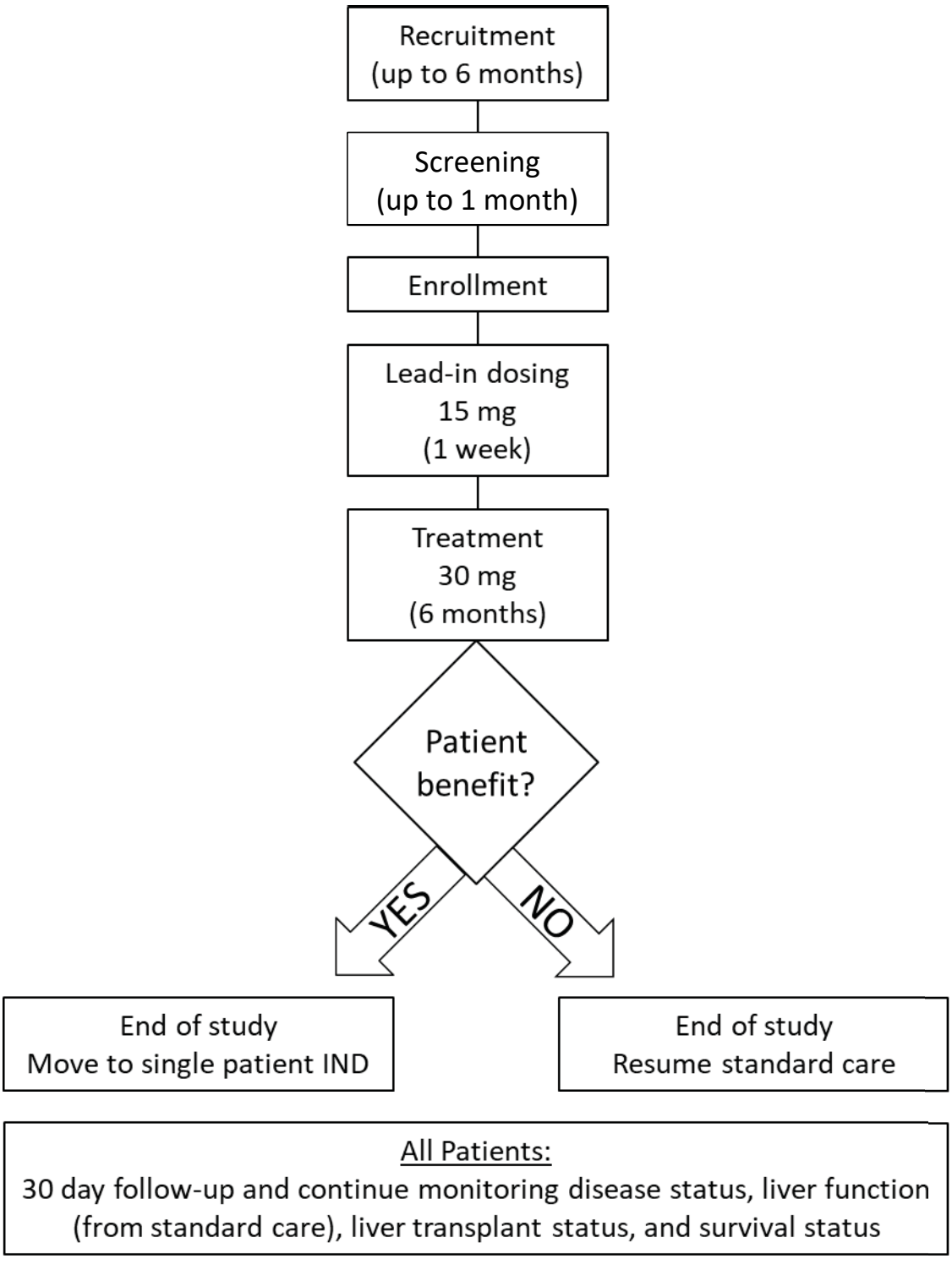

Figure 1. Clinical trial design

A dosage of 30 mg vidofludimus calcium has been chosen based on data from previous clinical trials. Vidofludimus has been investigated in two Phase 1 trials. A single dose study (escalating 17.5 - 350 mg) and a multiple dose study (35 and 70 mg once daily over 14 consecutive days) were performed in nearly 90 healthy male volunteers. In these studies, vidofludimus demonstrated robust pharmacokinetics with a t_{max} at 4 hours, a half-life of about 36 hours, and a reasonable dose linearity, which justifies once daily dosing. At a dose of 35 mg vidofludimus, plasma trough levels between 4.4 and 5.8 µg/ml were achieved, so that throughout the dosing interval plasma levels of vidofludimus were constantly above the IC_{50} values for inhibitory effects on stimulated human peripheral blood lymphocytes in vitro and for DHODH inhibition. In a Phase 2a study, vidofludimus (35 mg) demonstrated efficacy in patients with Crohn's disease and ulcerative colitis, with 88.5% of subjects responding to treatment.

Serum alkaline phosphatase (ALP) is one of the most extensively studied surrogate endpoint in PSC. Our group at Mayo Clinic and others have reported an association between serum ALP and clinical outcomes in PSC. Normalization of serum ALP appears to be associated with long-term survival free of cholangiocarcinoma, liver transplantation, liver-related death, and colorectal cancer, whereas persistent ALP elevation has been associated with worse outcomes in PSC. Therefore, we have chosen this simple, rapid, inexpensive, and minimally invasive test as the primary endpoint. Aspartate transaminase (AST), alanine transaminase (ALT), and bilirubin will be monitored to assess general liver health during the treatment. AST, ALT, or total bilirubin elevation can signal increased liver damage or liver cell necrosis, while elevated direct bilirubin may indicate bile duct obstruction. Part of the hypothesis being tested is that treatment with VC will result in a reduction of IL-17 and IFNγ production. Levels of these cytokines will be measured at 4 weeks and at 6 months and compared to baseline to determine whether early changes in these proinflammatory cytokines may predict improvements in ALP.

A 25% improvement in ALP with no more than a 33% worsening of AST after 6 months of treatment will be assessed as a positive outcome. While full normalization of ALP levels is unlikely over the short course of this pilot study, a 25% improvement would be deemed significant, indicating that VC has a positive effect on the progression of PSC. A less than 33% increase in AST levels would be considered only a mild elevation and as such would be tolerable when accompanied by a concomitant reduction of ALP. While a recent consensus statement from the International PSC Study Group indicates that transient elastography (TE), a new technique that allows non-invasive assessment of liver fibrosis, is one of the top three most promising surrogate endpoints for PSC (along with ALP and liver histology), it is unlikely that significant changes in TE would manifest in the 6 month course of this study. Thus, TE will not be included as an endpoint.

Vidofludimus calcium will be supplied by Immunic AG, Munich, Germany throughout the full course of the clinical trial. It has been confirmed through consultation with NIDDK personnel that this is permissible under this funding opportunity and does not constitute undue involvement by a foreign component. As this is a single- arm pilot trial, no placebo will be used. All study medication will be manufactured, tested, and released according to current Good Manufacturing Practice guidelines and local requirements. The active ingredient is vidofludimus calcium. Inactive ingredients are microcrystalline cellulose EP, polyvidon K25, crospovidone EP type A, talc, and magnesium stearate. The tablet formulation consists of a specific polymorph of vidofludimus calcium. Tablets are white, uncoated, biconvex tablets with a diameter of 8 mm. The dosage strength will be 15 mg VC per tablet. Subjects will take one tablet once daily during lead-in dosing period and two tablets, once daily during the treatment period. A licensed pharmacist (designated by the co-investigator) at each research site's

pharmacy will be responsible for dispensing study medication. 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) **Sources of Materials:** Please refer to Table 3 for details on the data that will be collected from study subjects. Each study subject will be assigned a study identification number. No protected health information (PHI) will be collected. Blood samples, collected and stored at the respective research sites, will be labeled using the subject's unique study identification numbers. Study documents and generated data will be stored securely in a password-protected database and in locked cabinets in locked offices. Only research team members authorized by the principal investigator will have access to study subject's data.

Table 3 – Data Collection Schedule

	Screening[a]	Enrollment/ Baseline[a]	Lead-in Dosing Period	Treatment Period							Follow-up Period
Procedures	**Up to Month -1**	**Day 0**	**Week 1 ± 1d**	**W2 (D14) ±3d**	**W4 ± 7d**	**W8 ± 7d**	**W12 [M3] ± 7d**	**W16 ± 7d**	**W20 ± 7d**	**W24 [M6] ± 7d**	**W28 ± 7d**
Informed Consent	x										
CLINICAL											
Demographics	x										
Medical History, initial	x										
Review inclusion and exclusion criteria	x	x									
Clinic Visit		x								x	
Physical Examination		x								x	
Vital signs		x								x	
MRCP (images from medical record)[b]		X								X	
Transient elastography (from medical record)[b]		X								X	
Telephone evaluations	x			x	X	x	x	X	x		x
Review any concurrent medications	x	x		x	X	x	x	X	x	x	x
Assess for PSC-related complications	x	x		x	X	x	x	X	x	x	x
Assess for IBD-related symptoms or complications	x	x		x	X	x	x	X	x	x	x
Adherence to study protocol				x	X	x	x	X	x	x	x
Adverse events evaluation				x	X	x	x	X	x	x	x
Dispense Study Medication		x[c]									
LABORATORY											
Hematuria testing by urine dipstick (patient self- administered every other day during lead-in period and during first week of Treatment			x	x							
HepBsAg, , Anti-HCV (from medical record)	X[d]										
HepBcAb, Anti-HAVIgM, (only if available from medical record)	X										
Screening for HIV and TB	x										
Creatinine with Estimated GFR (from medical record)	X										
Uric acid	X										
Urine pregnancy testing (Monthly during treatment period for women of child-bearing potential)	x	x			X	x	x	X	x	x	
Pharmacokinetics, if possible for those experiencing SAE		X									
ALP		x		X	X	X	x	X	X	x	X
Bilirubin, Total and Direct/Indirect		x		X	X	X	x	X	X	x	X
AST/ALT		x		X	X	X	x	X	X	x	X
Blood sample collection for potential future analysis frozen at -70°c		x			x		x			x	
Cytokine testing (IL-17 and IFN-ɣ)		x			x					x	

***a** 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.**b** If not available from medical record, does not need to be performed for the study. **c** Study may be mailed to participant after Baseline Visit once all test results available. d Will be tested at screening if unavailable from medical record*

c) Potential Risks:

1. Risks related to the study drug (VC)

Risks associated with the use of VC include hematuria early in the course of treatment. Although no clinically related adverse events were observed at doses of vidofludimus >70 mg, at higher doses potential drug-related increases in urine red blood cells (RBCs) and hematuria were observed. The underlying mechanism leading to increased red blood cells in urine appears to be increased uric acid elimination during the initial days after drug administration. Inhibition of the urate transport system URAT1 by VC may decrease the tubular re-uptake of uric acid in kidneys, leading to increased uric acid in the urine. This may in turn result in microcrystallization of uric acid in acidic urine and may lead to the occurrence of red blood cells in urine. Although this may not normally lead to clinically relevant adverse events at therapeutic doses, it may be important for patients with a risk factor of increased serum uric acid.

During clinical trials using vidofludimus, one serious adverse event of hepatitis was reported in a subject with Gilbert syndrome. Gilbert syndrome, which is associated with elevated blood levels of unconjugated bilirubin, is a genetic disease characterized by an up to 70–80% reduction in the glucuronidation activity of UGT1A1. The fact that VC is a moderate inhibitor of UGT1A1 may have contributed to this adverse event. Thus, patients with known or suspected Gilbert syndrome or with elevation of indirect (unconjugated) bilirubin above 1.2 time upper limit of normal (ULN) will be excluded from this clinical study.

Biliary hyperplasia, or increased cell proliferation in bile ducts, was seen when VC was tested in dog and rat models. In humans, there is no clinical means to test for this effect. When VC was stopped in either animal the bile duct cell proliferation stopped. This side-effect, which was only visible under the microscope, was only seen when the blood levels of VC were significantly higher than what we anticipate in human subjects who enter this clinical trial.

2. Risks related to the disease to be studied (PSC either in the absence or the presence of IBD)

Potential problems that may occur during the clinical trial as part of the natural course of PSC and/or PSC- associated IBD include 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. This biliary infection, acute ascending cholangitis, is a gastrointestinal emergency with high mortality rates if left untreated. The management is directed at treating the infection with broad-spectrum antibiotics and biliary drainage (endoscopic, percutaneous, or surgical).

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 (liver MRI) is permissible, but not required.

PSC is often associated with inflammatory bowel diseases, namely ulcerative colitis. Study subjects will undergo clinical assessment for IBD at clinic visits. Study subjects will be instructed to call the study coordinators/investigators to report any new symptoms that may 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, phone monitoring will be performed periodically throughout the study to specifically inquire about the presence of any of these

symptoms. If symptoms are reported, the clinical site investigator may request further diagnostic testing and a clinical evaluation will be required. As symptoms of *Clostridium difficile* colitis may resemble those of an IBD flare-up, *Clostridium difficile* infection needs to be ruled out.

Clostridium difficile colitis has been reported in IBD patients. If a study subject develops symptoms of *Clostridium difficile* colitis, *C. difficile* infection will be confirmed. The IBD status of the patient should be considered when weighing treatment options as recommended treatments may differ between the two patient populations.

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.

3. Risks related to the confidentiality of medical records

We take very seriously the issue of patient confidentiality and potential problems related to data management and Electronic Medical Records (EMR). Nonetheless, 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 subject's data.

II. Adequacy of Protection against Risks

a) Recruitment and Informed Consent

Institutional Review Board (IRB) review and approval of the study protocol, informed consent form, recruitment material, and all study subjects' materials will be obtained prior to initiation of the study. Any amendments to the study protocol will require review and approval by the IRB before changes are implemented to the study. All changes to the informed consent form will be IRB reviewed and approved; a determination will be made regarding whether previously consented study subjects need to be reconsented.

Subjects will be recruited at the participating clinical research centers. Potential subjects will be identified and contacted by the individual site investigator and/or study coordinator. If the patient agrees, consent for participation will be obtained. The site investigator will be responsible for explaining the purpose of the study, goals, risks, benefits, study procedures, expectations from study subjects, and expected outcomes and/or results. The site investigator will also answer any questions that may arise. Study subjects will have the opportunity to carefully review the consent and study materials and ask questions prior to signing. The study subjects will have the opportunity to discuss the study with their family members, friends, caregiver, and healthcare providers and/or think about the study prior to agreeing to participate. The study subject will sign the informed consent prior to any procedures being done specifically for the study. The informed consent will be stored as part of the subject's medical records for future reference, and a copy of the informed consent will be given to the study subjects for their records. Study subjects will be able to withdraw from the study at any time, and their lack of participation will have no effect on the care they receive. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

b) Protections Against Risk

1. Protections against risks related to the study drug (VC)

Risks associated with the use of VC include hematuria early in the course of treatment, believed to be due to microcrystallization of excess uric acid in the urine. As a precaution against this risk, patients with serum uric acid levels >1.2 ULN will be excluded from the study. Additionally, study subjects will be advised to drink sufficient fluids during the study to ensure adequate urine flow. Further, the trial will include a one week lead-in period where subjects receive half the treatment dose to minimize the risk of drug intolerance during the initial treatment period. Subjects will be monitored for two weeks after initial drug exposure for elevated RBCs in the urine by a urine dipstick test. It is not anticipated that hematuria will be seen if it has not developed within the first two weeks of drug exposure. Positive results will be immediately reported to the study coordinator and standard medical care will be provided. Three consecutive positive readings will require microscopic assessment of urine for sediment. All findings of RBCs in the urine will be listed as urinalysis abnormalities but will only be recorded as an adverse event when deemed by the investigator to be clinically significant. The investigator will assess whether there are more probable alternatives to explain this finding (*e.g.* evidence of contamination, evidence of infection not considered secondary to a drug induced damage, other benign causes such as menstruation, vigorous exercise, viral illness, trauma, or infection). If moderate or severe hematuria (see Table 4) cannot be remedied, the subject will discontinue VC treatment.

Due to an occurrence of a potentially drug-related adverse event of hepatitis in a subject with Gilbert syndrome, patients with known or suspected Gilbert syndrome or with elevation of indirect (unconjugated) bilirubin above 1.2 ULN will be excluded from this clinical study

To protect pregnant women, fetuses, and breastfeeding children, women who are pregnant, attempting to become pregnant, or breastfeeding will be excluded from the study. For female subjects of childbearing age, some form of contraception will be required during the study. A urine pregnancy test will be performed during each clinic visit and once a month during the entire treatment period. Subjects with a positive pregnancy test will discontinue the study immediately.

Classification	Symptoms
Mild	Asymptomatic hematuria: clinical or diagnostic observations only
Moderate	Symptomatic hematuria: with moderate flank pain (and including short-term [treatment for <24 hours], standard dose therapy with oral nonsteroidal anti-inflammatory drugs, oral acetaminophen or oral aspirin), interfering with but not limiting activities of daily living
Severe	Gross or macrohematuria; Any hematuria in connection with severe flank pain limiting activities of daily living. Any hematuria requiring additional treatment (e.g. oral anti-emetics or muscle relaxants, around the clock narcotic analgesics, use of narcotics or any intravenous treatment) or procedures for maintaining adequate urinary flow (e.g. urinary catheter or bladder irrigation).

Table 4. Classification of symptoms of hematuria

To date, no clear indication for drug-induced hepatic effects has been identified in clinical trials with vidofludimus. Nevertheless, liver function monitoring (ALP, AST, ALT and bilirubin) will be included as part of this clinical trial. Further liver function testing will be initiated in the event of any of the following:

a. Elevated ALP ≥3 x baseline
b. Elevated AST and/or ALT ≥ 3x baseline
c. Elevated total bilirubin ≥ 2x baseline
d. 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 (*e.g.* rash or >5% eosinophilia), or appearance of nausea, vomiting, and right upper quadrant pain (symptoms consistent with clinical hepatitis)
e. other suspected drug-induced liver injury(DILI)

Testing of all liver parameters (ALP, ALT, AST, total and indirect bilirubin) will be repeated within 48 to 72 hours. If repeat testing still shows an elevated liver function test, the patient will be placed under close observation, as defined in the DILI guidance, until the liver enzymes return to within 10% of baseline. This includes, but is not limited to, repeating liver function tests 2 or 3 times weekly, detailed evaluation of medical history and concomitant drug use, ruling out other causes of liver enzyme increases, and further liver function tests as considered appropriate by the investigator.

Table 5 – Algorithm for monitoring and interrupting study drug for Hepatocellular DILI signals in clinical trials evaluating drugs for patients with PBC and PSC without advance cirrhosis[a] and Normal Baseline ALT Values (Palmer et al 2019)

Treatment emergent ALT	**Bilirubin**	**Symptoms[b]**	**Action**
ALT ≥ 5x ULN	Normal Gilbert's syndrome or haemolysis: No change in baseline total bilirubin	None	Blood tests should be repeated 2-5 days[c] Follow-up for symptoms
ALT ≥ 8x ULN	Normal or elevated	None or present	Interrupt study drug Blood tests houdl be repeated within 2-5 days [c] Initiate close monitoring and workup for competing aetiologies Study drug can be restarted only if another aetiology is identified and liver enzymes return to baseline. Drug cannot be restarted if hepatic decompensation occurred.[d]
ALT ≥ 3x ULN	Total bilirubin ≥ 2x baseline Gilbert's syndrome or haemolysis: direct bilirubin > 2x baseline if baseline > 0.5 mg/dL	None or present	Interrupt study drug Blood tests should be repeated within 2-5 days[c] Initiate close monitoring and workup for competing aetiologies. Study drug can be restarted only if another aetiology is identified and abnormalities return to baseline. Drug cannot be restarted if hepatic decompensation occurs[d].
ALT ≥ 5x ULN	Normal or elevated	Present	Interrupt study drug

			Repeat blood tests in 2-5 days[c] Initiate close monitoring and workup for competing aetiologies. Study drug can be restarted only if another aetiology is identified and abnormalities return to baseline. Drug cannot be restarted if hepatic decompensation occurs[d].

[a]Advanced Cirrhosis indicated Child Pugh B and C
[b]Liver-related symptoms (eg, severe fatigue, nausea, new onset of or worsenting or pruritus, right upper quandant pain); Immunologic reaction (eg, rash, >5% eosinophilia); New onset of or increase of pruritus; or hepatic decompensation
[c]The specific interval between tests should also be determined based on the patient's clinical condition.
[d]The study subject will require close follow-up monitoring to exclude recurrence of liver injury after restarting the study drug.

Consider re- starting study medication only if a definitive alternative etiology is identified and liver tests return to within 10% of baseline. Discontinue the drug if total bilirubin, ALP, ALT, or AST elevation recurs following re-challenge.

Overall, the safety profile at the dosage level to be used in this trial is quite positive. An in-depth analysis of the entire clinical database demonstrates that vidofludimus at daily doses below 70 mg is not associated with an increased rate of clinically relevant adverse reactions, including hematuria, relative to placebo. These studies include 233 subjects with rheumatoid arthritis, Crohn's disease, or ulcerative colitis who received daily doses of vidofludimus 20 mg (~3000 treatment days) or vidofludimus 35 mg (~16,500 treatment days) in 3 clinical trials with individual treatment durations of 12-13 weeks.

The investigator will open an IND for the proposed clinical trial. Immunic AG, the drug supplier, will allow the investigator to reference Immunic's IND for vidofludimus calcium (see Letter of Support from Immunic).

2. Protections against risks related to the disease to be studied (PSC either in the absence or the presence of IBD): Acute ascending cholangitis, an acute biliary infection with high mortality rates, is a risk in patients with PSC. Study subjects will be asked about symptoms/signs of ascending cholangitis during clinic visits and phone calls, and subjects will be provided with a list of symptoms and instructed to report the symptoms to the study coordinator immediately if they arise. Symptoms suggestive of acute ascending cholangitis or biliary tree stricture include new onset jaundice, abdominal pain, dark urine, and light- colored stools. Study subjects will be advised to come to the clinic for evaluation or visit the nearest emergency room for consultation with an emergency room physician. If clinical suspicion for acute ascending cholangitis is high, the course of action may include aggressive antibiotic therapy and relief of biliary obstruction (via percutaneous, endoscopic, or surgical intervention), with or without a hospital stay. The Tokyo guidelines are commonly used for the diagnosis of acute ascending cholangitis.

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)

Cholangiocarcinoma (CCA) is one of the most serious complications of PSC. Screening for CCA is currently recommended every 6-12 months (by liver ultrasound or MRI) as part of standard of care for PSC patients. Patients with CCA at screening will be excluded. If during the course of the study CCA is suspected, subjects will undergo the necessary evaluations (which is part of standard of care) to exclude CCA.

As PSC is frequently associated with IBD, study subjects will undergo clinical assessment for IBD at clinic visits and by phone between these visits. Study subjects should report any new symptoms of an IBD flare to the study coordinator. These symptoms include abdominal pain, fever, bloody stools, and an increase in frequency of bowel movements.

In subjects with UC, we will use the Ulcerative Colitis Clinical Score to assess the disease activity/severity. This score, a modification of the well-known Mayo Score, consists of 4 items: stool frequency, rectal bleeding, subject's functional assessment, and physician's 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. 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.

In cases of confirmed *Clostridium difficile* colitis, any antibiotics the patient is already taking that are not specifically directed at *Clostridium difficile* will be reduced or stopped. Antibiotics such as vancomycin or other appropriate antibiotics specifically directed at *Clostridium difficile* will be administered. The use of immunosuppressive drugs should be minimized, but if required, patient will be closely monitored.

IBD, particularly ulcerative colitis, is an important risk factor for colorectal cancer. Currently, screening colonoscopy is recommended annually in patients with PSC and IBD.

3. **Protections against risks related to the confidentiality of medical records:** Study subject confidentiality is strictly held in trust by the investigators and their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relevant to the study subjects.

Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the generated data will be released to any unauthorized third party a without prior written approval by the appropriate authorities.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB may inspect all study documents, generated data, and records required to be maintained by the investigators, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records related to the subjects involved in this study. The research study site will permit access to such records.

The study subject's contact information will be securely stored at each research site for internal used during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the IRB and/or the institutional regulations. Study subject's research data, which is for the purpose of statistical analysis and scientific reporting, will be transmitted to and stored at the Data Collection Center at Mayo Clinic Rochester. This will not include the study subject's contact or identifying information. Rather, individual study subjects and their research data will be identified by a unique study identification number. Throughout this study, blood samples will be collected from study subjects and stored at the study sites for potential future analysis. These will also be labeled using only unique study identification numbers.

Names and identifiers of study subjects will be coded, and the principal study documents will be kept secured in a password-protected database and in locked cabinets in locked offices. The information obtained from this study may be published in scientific journals or presented at scientific meetings; however, a subject's identity will be kept strictly confidential.

III. Potential Benefits of the Proposed Research to Human Subjects and Others

PSC is currently an untreatable condition. The only known effective therapeutic intervention is liver transplantation, a risky and expensive procedure. This study will be extremely valuable as a means to test the feasibility of VC as a safe and effective therapeutic agent for PSC. Any efficacy demonstrated will not only benefit those subjects, but will help pave the way for more comprehensive studies of VC and PSC and move towards a potential new and effective therapy for this disease. Considering the known safety data, the implemented risk minimization measures, the expected benefits in the target population, and the medical need for effective therapies for PSC, the risk-benefit evaluation is considered favorable

IV. Importance of the Knowledge to be Gained

This impact of VC on the progression of PSC is unknown. This small pilot study will help determine whether VC is likely to be used as a treatment option for PSC and whether more comprehensive clinical studies are warranted. Further, it will provide evidence for whether the proinflammatory cytokines IL-17 and IFNγ may be used as early markers of improvements in serum ALP in patients with PSC. This will be the first clinical trial to investigate this novel chemical entity as a treatment for PSC.

DATA AND SAFETY MONITORING PLAN

This Human Subjects Research meets the definition of a clinical trial. It involves intervention with a drug in individuals with an established diagnosis of PSC. The study will require data and safety monitoring.

A Data and Safety Monitoring Board (DSMB) will include independent clinicians and a statistician will be established to review study data, safety, and study design according to the DSMB Charter.

The Principal Investigator (PI) will periodically assess study progress including; subject accrual and retention; compliance with inclusion/exclusion criteria; status of all enrolled subjects; and subject protocol adherence.

The PI and other study staff will periodically review study forms to monitor for data quality and integrity. The PI and the study coordinators will ensure IRB compliance throughout the duration of the project.

Annual reports will be provided to the IRB and the sponsor. These reports include; a summary of recruitment and retention and reasons for dropouts; a list and summary of AEs; whether AEs are consistent with pre-study assumptions; and whether the study is on track to be completed and accomplish the stated aims.

The protections against risks related to subject confidentiality are outlined in the *Protection of Human Subjects* section. Briefly, the study protocol, documentation, data, and all other information generated will be held in strict confidence, with only authorized personnel permitted access. Study data and records will be stored securely in a password-protected database or in locked cabinets in locked offices. Study subjects, their samples, and their data will be labeled using only unique study identification numbers. Names and identifiers of study subjects will be coded and if data from the study are published or presented publicly, a subject's identity will be kept strictly confidential.

To ensure the safety of the participants and the validity and the integrity of the data, the clinical trial will be monitored by an independent DSMB with experience in clinical trials in liver diseases, including cholestatic liver diseases. They will act as the designated safety officers for the trial, reviewing clinical trial reports every 6 months and addressing all reports of AEs. Acting independently of the PI, they will monitor study progress, protocol adherence, IRB compliance, and adherence to the proposed protections against risks related to subject confidentiality.

A safety monitoring system will be used to ensure appropriate identification, tracking and reporting of adverse events (AEs). We will use the Common Terminology Criteria for Adverse Events (CTCAE) grading system to monitor the clinical trial's potential AEs and to grade the severity of any AEs that may occur. The CTCAE is a descriptive terminology that can be utilized for adverse event reporting. A grading scale based on severity is provided for each AE term. The CTCAE displays grades 1 through 5 with unique clinical descriptions of severity for each AE based on the general guideline shown in Table 6.

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	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Table 6. CTCAE grading system developed by the National Cancer Institute

The expected AEs related to this study and strategies to track, minimize, and manage these AEs have been discussed in detail in the *Protection of Human Subjects* section, but are summarized in Table 7 below.

Expected Adverse Event	Risk Mitigation and Monitoring Strategies
Hematuria	* Exclude patients with elevated uric acid levels * Advise subjects to drink sufficient liquids during trial * Lead-in dosing * Monitor hematuria by urine dipstick testing
Complications of Gilbert Syndrome	* Exclude patients with Gilbert syndrome * Exclude patients with elevated unconjugated bilirubin
Acute Ascending Cholangitis	* Monitor for symptoms during clinic visits and phone calls * Instruct patients to report any symptoms of acute ascending cholangitis or biliary tree stricture immediately
Cholangiocarcinoma	* Standard of care screening by liver ultrasound or MRI every 6-12 months
IBD Flare	* Monitor for symptoms during clinic visits and phone calls * Instruct patients to report any symptoms of IBD flare
Clostridium difficile Colitis	* Monitor for symptoms during clinic visits and phone calls * Instruct patients to report any symptoms of *Clostridium difficile* colitis
Colon Cancer	* Standard of care screening by colonoscopy annually

Table 7. Expected adverse events

The occurrence of an AE, serious AE (SAE), or unanticipated problem may come to the attention of study personnel during study visits, interviews of a study subject presenting for medical care unrelated to this study, telephone calls, laboratory test review, and/or upon review of study reports by the study monitor. An SAE is defined by the FDA as "an adverse event that, in view of the investigator, study coordinator, or the sponsor, results in any of the following: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect". All AEs will be captured and documented. Information collected about the AEs will include time of onset, nature, severity, relationship to the study drug, related to the natural history of PSC/IBD, subject hospitalized or not, intervention(s), time of resolution of resolution/stabilization, and outcomes.

Safety-related information (of any level) will be entered into the clinical database and reviewed routinely. All AEs, SAEs, and unanticipated problems will be managed in a manner consistent with local IRB guidelines. We will use the following strategy when reporting all AEs (regardless of severity and relationship to study drug or natural course of PSC/IBD):

1. If the AE(s) meet the criteria for study termination the clinical trial will be stopped immediately until further discussion with the sponsor.
2. For all other AEs and unanticipated problems, reports will be made within 72 hours to the clinical trial monitor and/or local IRB. The clinical trial monitor/IRB will assume the responsibility for reviewing the clinical trial data for safety concerns and reporting to any appropriate regulatory bodies including the sponsor.

The trial shall be stopped until further discussion with the sponsor if any of the following occur:

- 3 subjects develop the same grade 3 CTCAE
- 2 subjects develop any grade 4 CTCAE
- 1 subject develops a grade 5 CTCAE

Participation of individual study subjects shall be terminated if any of the following occur:

- Elevated ALP ≥ 3 x baseline that does not return to within 10% of baseline within two weeks
- Elevated AST and/or ALT ≥ 3 x baseline that does not return to within 10% of baseline within two weeks
- Elevated total bilirubin ≥ 2 x baseline that does not return to within 10% of baseline within two weeks
- Serious allergic reaction
- Pregnancy or breastfeeding

Non-adherence to the study protocol by a study subject may, at the discretion of the principal investigator, be grounds for termination.

Statistical Analysis Plan: The proportion of patients with normalization of serum ALP at 3 months and 6 months and their corresponding confidence intervals will be estimated using exact binomial method. The primary endpoint is ALP normalization(within the normal limits of the laboratory test) rate at 6 months. We will define success as a 95% CI that dose not overlap 0 at 6 months. Further, we will further investigate if the absolute ALP decreases over time using mixed model with repeated measures to account for within patient correlation over time.

All the liver biochemistries and proinflammatory cytokine profile over time will be analyzed using mixed model with repeated measures to investigate how VC treatment plays a role over the 6 months duration. The liver biochemistries and proinflammatory cytokine will be dichotomized into abnormal and normal groups if possible and further the abnormal proportions at different time points will be estimated to see the trend over time.

The incidence, type and relatedness of serious adverse events and adverse events will be monitored and reported for all the subjects.

Adherence to Regulatory Standards: The investigators will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR part 56, and the ICH; Good Clinical Practice E6.

ClinicalTrials.gov Requirements: The principal investigator will ensure that this clinical trial is properly registered at www.clinicatrials.gov, per the Public Law 110-85 (also known as the FDA Amendments Act (FDAAA) of 2007), no later than 21 days after the first subject is enrolled. The principal investigator will also ensure that the results (including adverse events) of this clinical trial are reported no later than 1 year after completion of the clinical trial.

Inclusion of Women and Minorities: Women and minorities will be included in this study although the disease is primarily one of males and has been largely described in Caucasian populations to date, usually in patients who originate from northern Europe, northern North America, or New Zealand. It is expected that the study population will roughly reflect the natural distribution among PSC patients in the United States with respect to gender and race/ethnicity.