Washington University

A PHASE I OPEN LABEL STUDY OF THE EFFICACY AND SAFETY OF TUDCA IN ULCERATIVE COLITIS TREATMENT

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Study Product: Tauroursosodeoxycholic acid, brand name Tudcabil™ These capsules will be supplied directly from Bruschettini Srl, Genoa, Italy which will be administered through our investigational pharmacy.

Protocol Number:

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Study Summary

Title	*A PHASE I OPEN LABEL STUDY OF THE EFFICACY AND SAFETY STUDY OF TUDCA IN ULCERATIVE COLITIS TREATMENT*
Short Title	
Protocol Number	
Phase	*Phase I*
Methodology	*Open label*
Study Duration	*6 Weeks*
Study Center(s)	*Single-center*
Objectives	*Primary: Determine the effect of TUDCA treatment on ER stress in colon biopsy tissues from subjects with symptomatic ulcerative colitis. Secondary: Measure the safety, tolerance and efficacy of TUDCA treatment in symptomatic ulcerative colitis.*
Number of Subjects	*12 (Twelve)*
Diagnosis and Main Inclusion Criteria	*Confirmed ulcerative colitis disease through radiographic, endoscopic and/or histologic criteria; Confirmed with moderate to severe ulcerative colitis (defined as a complete Mayo score ≥ 5 with endoscopic subscore of ≥ 1); On a stable dose of medications for IBD (i.e. no change in medication within 4 weeks of study enrollment).*
Study Product, Dose, Route, Regimen	*Tauroursoursodeoxycholic acid, brand name Tudcabil, Dosed in capsules containing 250 or 500mg of TUDCA for a total dose of 1.75-2g/day*
Duration of administration	*6 Weeks*
Reference therapy	*N/A*
Statistical Methodology	*We will be specifically interested in the statistical contrasts that compare before and after TUDCA treatment. Correlation between the primary end point and secondary end point will also be examined. The appropriateness of all analysis of variance and covariance models will be evaluated using regression residuals. If normality or homoscedasticity requirements are violated, data transformations will be explored and, if appropriate transformations cannot be found, analyses may be done semi-parametrically using the ranks of the data.*

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

IBD, which includes both Crohn's disease and ulcerative colitis, is caused by a dysregulated inflammatory response to intestinal microbiota and affects approximately 1.5 million people in the United States. No medical cure currently exists and patients require a lifetime treatment with immune modulators, such as infliximab, which have significant risks and adverse effects. Strategies for developing IBD therapies face an extraordinary challenge due to our poor understanding of the disease pathophysiology. Recently, emerging evidence has demonstrated that endoplasmic reticulum stress (ER stress) and the unfolded protein response (UPR) play fundamental roles in IBD development[1-5].

The ER is the organelle in eukaryotic cells responsible for intracellular Ca^{2+} homeostasis, lipid biosynthesis and ER protein folding, transport and quality control. Alterations in the ER environment, such as altered Ca^{2+}, redox state, nutrient status, increases in the rate of protein synthesis, pathogens or inflammatory stimuli, disrupt ER protein folding causing accumulation of unfolded or misfolded proteins, a condition termed ER stress, which activates the UPR. The UPR comprises three parallel signaling branches: PKR-like ER kinase (PERK)-mediated phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), Inositol requiring enzyme 1α (IRE1α)-mediated X-box binding protein 1 (XBP1) mRNA splicing and activating transcription factor 6α (ATF6α) processing. The outcome of UPR activation involves transient attenuation of protein synthesis and transcriptional activation to increase the capacity for ER protein folding, trafficking and secretion, and to increase protein degradative pathways, including ER-associated degradation (ERAD) and macroautophagy. If these adaptive mechanisms cannot resolve the protein-folding defect, cells enter apoptosis[6].

Recent studies link ER stress to the pathogenesis of IBD. Patients with active Crohn's disease and ulcerative colitis exhibit signs of ER stress in their ileal and/or colonic epithelium[7-9]. In addition, human genetic studies of IBD have identified primary genetic abnormalities in several genes including *XBP1*, *AGR2*, and *ORMDL3* that encode proteins associated with ER stress[1, 10-13]. Previous studies indicate that cells with a high load of protein folding and secretion are sensitive to altered ER homeostasis and this can induce inflammatory response gene expression[1, 2, 5, 14, 15]. Intestinal microbiota and their molecules stimulate IECs to increase secretion of mucins and antimicrobial peptides that can overwhelm the protein secretory capacity of IECs. Conversely, exposure to inflammatory stimuli can cause ER stress[15]. Upon exposure to high levels of exogenous antigens and inflammatory cytokines in the intestinal lumen, IECs may require efficient UPR-mediated ER chaperone induction to survive the heavy burden of protein folding and secretion.

1.2 Investigational Agent

Substance: Tauroursoursodeoxycholic acid, brand name Tudcabil™. (Abbreviation: TUDCA)
Chemical Name: (2-(3α, 7β-Dihydroxy-5β-cholan-24oylamino)ethanesulfonic acid)
Synthesized by Prodotti Chimici e Alimentaria S.p.A. for Bruschettini Srl, Genoa, Italy.

Manufacturing Process: Ursodeoxycholic Acid (3α, 7β-dihydroxy-5β-cholan-24-oic acid) is considered the starting material together with Taurine (2-aminoethansulfonic acid) to be used in the production of Tauroursodeoxycholic Acid : the synthetic pathway is based on the formation of a carboamidic bond between the carboxyl group in position 24 of the bile acid and the amine group in position 2 of taurine.

TUDCA has been licensed for the treatment of cholesterol gallstones and for therapy of chronic cholestatic liver disease in Europe since 1991. From 1996-2001 at least 20,000 doses had been dispensed and no reports related to the toxicity of Tudcabil capsules had arrived during this time period (please see product profile of IND 101,296). Infrequent, but predominant side-effects in human studies (summarized in attached table) are diarrhea, headache and abdominal pain[16].

Human studies relevant to this application: A pilot study conducted by Dr. Samuel Klein, also at Washington University in Saint Louis, treated 10 obese, insulin-resistant adults with 1.75 g/day for 4 weeks with no adverse events[17]. TUDCA was given to 16 liver transplant recipients at 500 mg/day for 12 months with no drop-outs or toxicities observed. A cohort of subjects with primary biliary cirrhosis were treated with 500-1500 mg/day of TUDCA for 6 months, with diarrhea reported as the only side-effect. Several human studies have shown that use of TUDCA is well tolerated in many populations including amyloidosis[16], primary biliary cirrhosis[18-20] and liver transplant[21].

Chronic toxicity has been studied in dogs for up to 26 weeks with treatment of up to 400-600 mg/kg/day (30 x that of the proposed dose) with no observed toxicity. Rats treated with 2-20 times the proposed dose showed no evidence of carcinogenic effects after 24 months of treatment (see attached, “animal toxicity”).

In summary, TUDCA has been tolerated at similar dose in humans with a variety of chronic medical conditions, with the major side-effect being mild diarrhea. The balance of the clinical information suggests that this drug is well tolerated and could have important health benefits for this study population. There are no known interactions between bile acids and immunomodelators or other IBD therapies.

1.3 Preclinical Data

We tested whether expression of ER chaperones prevent IBD development in mouse models. We found that protein misfolding in the ER caused by deletion of the ER co-chaperone gene *P58^{IPK}/Dnajc3* exacerbates experimental colitis in mice. Furthermore, ATF6α null mice, with significant reduced expression of many ER chaperones (*Bip*, *Grp94* and *P58^{IPK}*[6, 22]) and hyperactivation of pro-apoptotic UPR signaling in colonic IECs, were more sensitive to experimental colitis than control mice. In contrast, we found that oral delivery of the chemical chaperone tauroursodeoxycholate (TUDCA) dramatically decreased the clinical, histological and biochemical signs of inflammation in both innate immunity- and T cell-dependent colitis through reducing ER stress signaling in colonic IECs[4]. TUDCA has been proven to facilitate protein folding and reduce ER stress both *in vitro* and *in vivo* by stabilizing protein-folding intermediates and preventing protein aggregation[17, 23-26]. These findings indicate that ER stress plays an indispensable role in IBD development, and TUDCA administration could be a very efficient therapy for IBD patients.

In this study, we would like to bring this significant research finding from bench to the bedside. We propose to test the efficacy and safety of TUDCA in a small number (12) of symptomatic ulcerative colitis patients. TUDCA has been demonstrated as safe and well tolerated in many human clinical trials for other disease conditions. Our study will extend these findings to evaluate TUDCA's tolerance/safety in patients with active UC. If our research findings in mouse IBD models can be successfully translated to humans, it will benefit IBD patients in the near future.

1.4 Clinical Data to Date

This is the first clinical trial to date examining the role of TUDCA in ulcerative colitis patients. However, several clinical trials in other disorders have been completed or are ongoing.
https://clinicaltrials.gov/ct2/results?cond=&term=tudca&cntry=&state=&city=&dist=

1.5 Dose Rationale and Risk/Benefits

A weight based regimen for TUDCA will be used, administering it in 3 divided doses which corresponds to an average dose of 20-25 mg/kg/day accordingly using 1750 mg daily for patients <75 kg and 2000 mg daily for patients ≥75 kg.

It has been shown that TUDCA at the dose of 25mg/Kg/day can achieve maximum composition in the serum and bile in patients with primary biliary cirrhosis[19]. Previous clinical studies also have shown that 1 to 6 months of TUDCA treatment with 1750mg/day is efficacious and safe in patients with different diseases, including primary biliary cirrhosis[19, 27, 28], liver cirrhosis[29], HCV-related chronic hepatitis[30] and insulin resistance[17]. Animal studies also suggest that TUDCA appears to be very well tolerated even at high doses.

Patients will not be charged for the drug. This will be supplied in original packaging by Bruschettini Srl, Genoa, Italy. TUDCA distribution will be carried out by our Investigational Pharmacy at Washington University. Patients will receive a 6 week supply at initiation visit.

2 Study Objectives

Intestine biopsy samples from inflamed and adjacent non-inflamed intestine tissues before and after the TUDCA treatment will be harvested to evaluate ER stress status by western-blotting for ER stress induced proteins, qRT-PCR and immunochemistry staining. The disease severity will be measured by the Geboes histology index and the complete Mayo score before and after the TUDCA treatment will be monitored. The **overall goal** of this project is to determine if the research findings in mouse IBD models can be successfully translated to humans and thereby provide an adjunctive to optimize therapeutic options in IBD patients focused on the epithelium and with a desirable safety profile. To address this, we propose the following **Specific Aims**:
SA 1. Determine the effect of TUDCA treatment on ER stress in colon biopsy tissues from subjects with symptomatic ulcerative colitis as a primary endpoint.

We expect that TUDCA treatment will reduce ER stress in colon biopsy tissues from subjects with symptomatic ulcerative colitis (UC), as judged by expression levels of ER stress markers. As a primary study endpoint, we will assess:

A. The primary endpoint will be the change in ER stress markers, including eIF2α phosphorylation, XBP1s and BIP/GRP78, in colon biopsy tissues before and after 6 weeks of TUDCA treatment.

SA 2. Measure the safety and tolerance as well as efficacy of TUDCA treatment in symptomatic ulcerative colitis as secondary end points.

We expect that TUDCA treatment will alleviate the UC mucosal inflammation and symptoms and be well tolerated. To assess these secondary endpoints we will:

A. Actively monitor UC study patients for TUDCA safety and tolerability.

B. Examine efficacy endpoints endoscopic mucosal healing, clinical response (Mayo Score) as well as histologic severity.

2.1 General Design

Type of Study: Open-label clinical trial where all patients receive TUDCA at 1.75 or 2g/day for 6 weeks, divided into three doses, after meals.

Schematic of Trial Design*:* A total of 12 individuals with active ulcerative colitis and endoscopic evidence of inflammation will be eligible to participate in this open-label clinical trial using TUDCA (adjunct or primary therapy) at 1.75 or 2g/day for 6 weeks, divided into three doses, after meals as summarized in Figure 1. A full schedule of visit assessments and procedures is defined in Table 1 in Section 5.

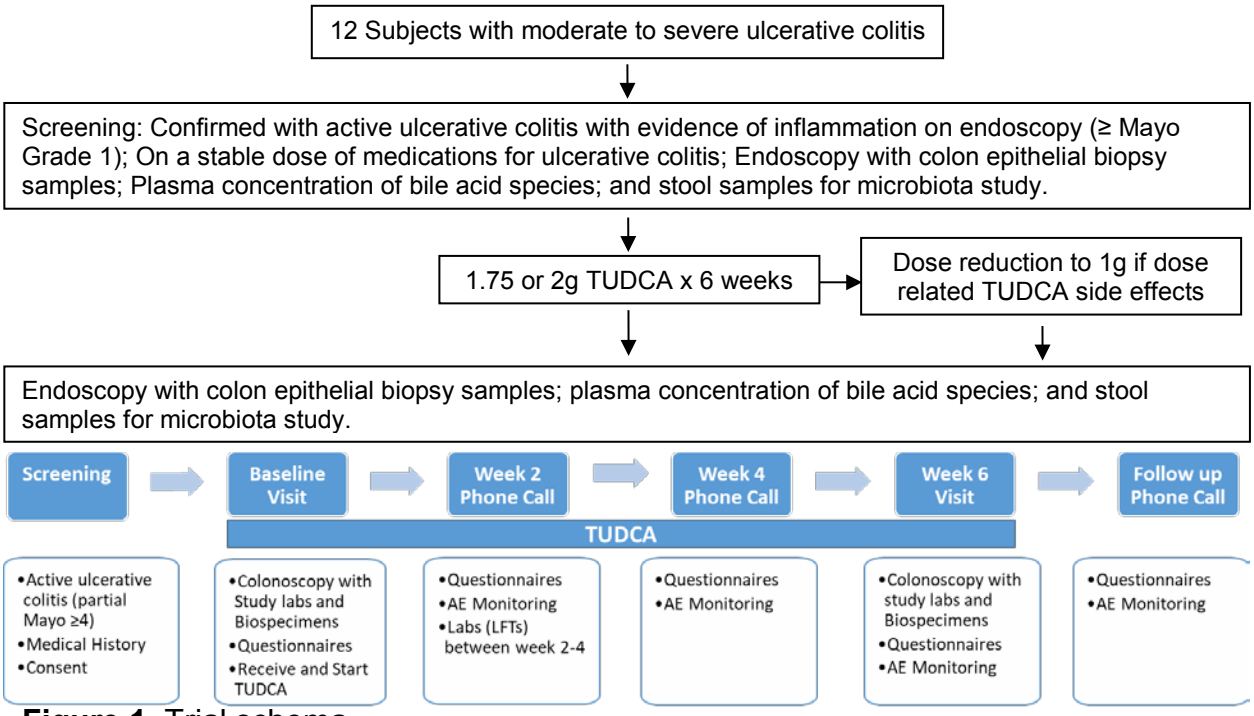

Figure 1. Trial schema

Duration of Subject Participation: 6 weeks on Trial + 2 weeks Follow up = 8 weeks.

2.2 Primary Study Endpoints

As the primary study endpoint, we will assess the change in ER stress markers, including eIF2α phosphorylation, XBP1s and BIP/GRP78, in colon biopsy tissues before and after 6 weeks of TUDCA treatment.

BIP, XBP1 splicing, phosphorylated eIF2α will be assessed by Western blot and RT-PCR and immunohistochemistry (IHC). Other ER stress markers, such as PERK, ATF4, CHOP, IRE1α and ATF6α will also be examined. In addition, we expect that cell death markers in colon biopsy tissues will also be downregulated, including cJUN, JNK, cleaved caspase 2 and cleaved caspase 3. We will also monitor expression of inflammatory cytokines, IL-1B, TNFα, IL-6 and IFNγ. Additional samples aliquots will be saved (flash frozen and RNAlater) for future studies evaluating adaptive and innate immune readouts.

2.3 Secondary Study Endpoints

1. Safety and Tolerance of TUDCA in Ulcerative Colitis patients
2. Efficacy of TUDCA treatment in symptomatic ulcerative colitis based on endoscopic mucosal healing, clinical response (Mayo Score) as well as histologic severity (Geboes histology index).

3 Subject Selection and Withdrawal

3.1 Inclusion Criteria

1) Ages Eligible for Study: 18 Years to 65 Years;

2) Confirmed ulcerative colitis disease through radiographic, endoscopic and/or histologic criteria;

3) Confirmed with active ulcerative colitis (defined as a complete Mayo score ≥ 5 with endoscopic subscore of ≥ 1) See Appendix for Mayo Score using recent adaptation to include any friability on endoscopy to be scored as “2”.

4) On a stable dose of medications for IBD (i.e. no change in medication within 4 weeks of study enrollment) and not planning to initiate new medication other than TUDCA.

3.2 Exclusion Criteria

1) Those that received other chemical chaperone therapies in the 3 months prior to screening;

2) Individuals accompanied by gallstones, other intestinal disorders or cancers, or any possible cholestatic pathologies that could alter the enterohepatic circulation of the bile acids, including previous cholecystectomy or short bowel syndrome;

3) Subjects with alcohol or drug abuse within the recent year;

4) Serious heart, lung, kidney, digestive, nervous, mental, or autoimmune diseases

5) Those with plans for abdominal surgery;

6) Those unable or unwilling to provide informed consent or failure to comply with the test requirements;

7) Pregnant, lactating women;

8) Those receiving or planning to receive medicines that inhibit the absorption of the bile acids in the intestine;

9) All female subjects must have birth control and not plan to become pregnant during the study. As TUDCA may interfere in the absorption of oral contraceptives, the acceptable methods of birth control should include abstinence or 2 of the following intrauterine device (IUD-with or without local hormone release), diaphragm, spermicides, cervical cap, contraceptive sponge, and /or condoms.

10) Subjects with baseline liver transamines (AST or ALT) > 1.5X the upper limit of normal.

11) Patients with complete biliary obstruction and known hypersensitivity or intolerance to TUDCA or any of the components of Tudcabil™ (or to other bile acids).

12) Patients with moderate-to-severe hepatic impairment.

13) Evidence of worsening liver function based on the 2 initial laboratory values used to establish the baseline. See Section 6.2.

3.3 Subject Recruitment and Screening

Recruitment*:* Division of Gastroenterology at Washington University: More than 2500 patients with IBD annually are cared for by the Division's IBD specializing gastroenterologists (n=10). The division actively participates in IBD clinical trials (8 in 2016 alone) and has an established infrastructure for recruitment and execution of new studies examining IBD specific medications. As a leading center for IBD patients sample Biobanking, the Washington University IBD Division also has a robust infrastructure for acquiring and rapidly processing research quality IBD specimens including serum samples, stool and biopsy material. Two of the Washington University site PIs (Drs. Gutierrez and Ciorba) have worked in the field of IBD for more than 10 years. We anticipate enrolling 2-3 patients per month.

Screening:

Medical examination. All subjects will be screened with a detailed history and physical examination, pregnancy test (*if female*) and laboratories including CBC, complete metabolic panel with a liver panel, C-reactive protein and fecal calprotectin.[31]

3.4 Early Withdrawal of Subjects

3.4.1 When and How to Withdraw Subjects

Subjects may be withdrawn from the study prior to the expected completion if that subject is deemed by the treating physician and study PI to have an adverse reaction to TUCDA (safety reasons and/or disease worsening), failure of the subject to adhere to

protocol requirements or if consent is withdrawn by the subject. The subjects will be informed orally and in writing that they are free to withdraw from the study at any time with no bias or prejudice.

3.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though subjects may be withdrawn prematurely from the study, we will collect all data available up until the point of withdrawal and at least confirm all survival data for two weeks after the final visit and attempts should be made to obtain permission to record at least survival data up to the protocol-described end of subject follow-up period. This will include at least 4 phone calls to subject, phone calls to next-of-kin if possible and sending of a certified letter. Of note, given the recognized safety of TUDCA, we do not in any way anticipate any risk of mortality, this is only for completeness of protocol.

4 Study Drug

4.1 Description

Tauroursoursodeoxycholic acid, brand name Tudcabil™ is a naturally occurring bile salt proven to reduce ER stress both *in vitro* and *in vivo,* dramatically decreases the clinical, histological and biochemical signs of inflammation in four different IBD mouse models through reducing ER stress in colonic IECs[4]. TUDCA has been tested in many clinical trials for other disease conditions and its safety in humans has been proven. Subjects will receive capsules containing 250 mg of TUDCA provided by Bruschettini Srl, Genoa, Italy which will be administered through our investigational pharmacy. The consituents other than TUDCA are microcystalline cellulose, lactose, corn starch and magnesium stearate

4.2 Treatment Regimen

A weight based regimen for TUDCA will be used, administering it in 3 divided doses which corresponds to an average dose of 20-25 mg/kg/day accordingly using 1750 mg daily for patients <75 kg and 2000 mg daily for patients ≥75 kg.

It has been shown that TUDCA at the dose of 25mg/Kg/day can achieve maximum composition in the serum and bile in patients with primary biliary cirrhosis[19]. Previous clinical studies also have shown that 1 to 6 months of TUDCA treatment with 1750mg/day is efficacious and safe in patients with different diseases, including primary biliary cirrhosis[19, 27, 28], liver cirrhosis[29], HCV-related chronic hepatitis[30] and insulin resistance[17]. Animal studies also suggest that TUDCA appears to be very well tolerated even at high doses.

Patients will not be charged for the drug. This will be supplied in original packaging by Bruschettini Srl, Genoa, Italy. TUDCA distribution will be carried out by our Investigational Pharmacy at Washington University. Patients will receive a 6 week supply at initiation visit.

4.3 Method for Assigning Subjects to Treatment Groups

Open label study. All patients will receive TUDCA.

4.4 Preparation and Administration of Study Drug

Please see TUDCA related manufacturing information from Bruschettini Srl.

No additional preparation of the study drug will be necessary. Bruschettini Srl, Genoa, Italy will supply our investigational pharmacy with 250 mg capsules in bottles of 120 capsules (15 day supply). The study drug will be distributed by our investigational pharmacy.

4.5 Subject Compliance Monitoring

Subjects will be addressed at the time points described for the study visits for compliance. A final outcome will be determined by study staff at the in-person visits. Subjects not achieving 90% compliance will be subject to withdrawal from the study.

4.6 Prior and Concomitant Therapy

Patients may be on any concomitant therapies as needed for their ulcerative colitis or other conditions. However, patients should not start any new medication that modifies ER stress. It is also not allowed to start any other new therapies for ulcerative colitis.

4.7 Packaging

Bruschettini Srl, Genoa, Italy will supply our investigational pharmacy with standard packaging bottles containing 120# of the 250 mg capsules (15 day supply).

4.8 Receiving, Storage, Dispensing and Return

4.8.1 Receipt of Drug Supplies

Bruschettini Srl, Genoa, Italy will be supply the drug in original packaging to our Investigational Pharmacy at Washington University who will distribute it.

4.8.2 Storage

Capsules are stored in a controlled environment at room temperature in the study pharmacy. Adherence will be made to the manufactures expiration date. No other special handling requirements during storage

4.8.3 Dispensing of Study Drug

Patients will receive a 6 week supply of the drug at initiation visit.

4.8.4 Study Drug discontinuation

All subjects who discontinue study drug should be considered withdrawn from the study and should complete the study completion visit assessments. If subjects fail to return for these visits, or are unable to do so, every effort will be made by the investigator to contact them or a knowledgeable informant by telephone or by

sending appropriate correspondence (i.e. certified letter) that will become part of the investigator's file to record that efforts were made to reach the subjects.

4.8.5 Subject Withdrawal Criteria

Subjects may voluntarily withdraw from or be withdrawn from the study at the discretion of the investigator (or sponsor) at any time. Patients may be withdrawn from the study if any of the following occur:

1. subject withdrew consent
2. Clinically significant new abnormal laboratory value that requires new therapy which may impact the study or suggests the patient is having an adverse response to TUDCA.
3. adverse event
4. clinically significant abnormal test procedure result
5. protocol violation
6. administration of prohibited concomitant medication

If such withdrawal occurs, subjects should complete the Study Completion visit assessments at the investigators' discretion. If subjects fail to return for these visits, or are unable to do so, every effort should be made by the investigator to contact them or a knowledgeable informant by telephone or by sending appropriate correspondence (i.e. certified letter) that will become part of the investigator's file to record that efforts were made to reach the subjects. If the subjects fail to return for visits, the investigator must determine the primary reason for a subject's premature withdrawal from the study, and record this information on the Study Completion CRF page.

4.8.6 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

5 Study Procedures

Table 1 summarize study procedures, complementing Fig 1 above. Also see Appendix 1

	Visit	1	2	3	4	5	5
	Week #	≤ 2 weeks prior to baseline	0	2	4	6	8
Event		Screening	Baseline	Week 2	Week 4	Final visit - 6 weeks	Phone Call follow up
Visit type	Clinic or Phone call based on clinic visit	x					
	Endoscopy		x			x	
	Phone Call			x	x		x
Coordinator Services	Informed consent	x					
	Inclusion/Exclusion Criteria	x					
	Demographics	x					
	Con Med review	x	x	x	x	x	
	Provide stool collection kit to patient	x					
	Stool Specimen Collection		x			x	
	Phlebotomy (Note LFT's will be drawn between week 2 to 4)		x	x		x	
	Lab Processing		x			x	
	Vital Signs		x			x	
	QoL Questionnaire (SIBDQ)		x	x	x	x	
	Medication Dispensing-CRC		x				
	Medication Accountability			x	x	x	
	Adverse Event Monitoring		x	x	x	x	x
	Telephone Call Assessment/Follow-up			x	x		x
Investigator Services	Partial Mayo Score Assessment			x	x		x
	Full Mayo Score with Endoscopic Score		x			x	
	Medical History for study	x					
Procedure	Sigmoidoscopy/Colonoscopy		x			x	

Endoscopic disease activity. The complete Mayo score will be completed at baseline and after 6 weeks of treatment, based on stool frequency, rectal bleeding, endoscopic findings and physician's global assessment[32]. In this trial, we are adapting the Mayo Scoring used in the OCTAVE clinical trial program.[33] Whereas standard Mayo scoring designates mild friability as an endoscopic subscore of 1, in the OCTAVE clinical trial program, any sign of friability led to an endoscopic subscore of 2. See Appendix 1.

Colonoscopic biopsies will be obtained under our current approved protocol **(#201111078,** DDRCC Biobank). Patients will be enrolled and consented by the Washington University site coordinators. Consent will be obtained in the outpatient clinic, the outpatient endoscopy suite or in the hospital with the permission of the treating physician. Up to 12 additional endoscopic biopsies will be obtained and used as a source for RNA, DNA or protein extraction, immunohistochemical analysis as described below.

Tissue Biopsy. Tissues will be obtained from inflamed and adjacent non-inflamed colon as available. The PI and Co-I's as well as clinical collaborators are highly skilled at colonoscopy with biopsy having performed thousands of these procedures. Moreover, investigators have routinely used colonoscopy for grading of endoscopic severity and tissue sampling for histology and study purposes. These procedures are generally tolerated well by patients.

Stool sample collection will be performed by protocol of the Washington University DDRCC Biobank Core https://ddrcc.wustl.edu/ with patient directed collection and courier- based delivery.

Geboes Histology Score*:* All biopsy specimens will be evaluated by the simplified Geboes Histology Score[34] based on the original Geboes Index.[35] This is provided in Appendix 1, Table 2.

Quality of Life Questionnaire*: Health Related Quality of Life will be assessed by the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) which has been validated in ulcerative colitis.[36, 37] Measures quality of life as measured in four domains, bowel symptoms, emotional health, systemic systems and social function.*

Additional Analysis on Samples and collection of samples for future studies.
Measurement of serum bile acid concentration*.* Blood will be collected before and after TUDCA treatment, along with safety labs. Plasma bile acids will be measured using high-performance liquid chromatography tandem mass spectrometry.[38] These studies will be carried out in the metabolomics core at Washington University School of Medicine and overseen by Dr. Davidson. We will measure total AND individual serum bile acids (including TUDCA). These measurements will be conducted on fasting serum obtained at the start of TUDCA and at timed intervals (at endoscopic evaluation and week 2).

Microbiota composition analysis. As indicated above, stool samples before and after TUDCA treatment will be collected for potential future analysis of microbiota. In the event that we proceed with metagenomic profiling (which would be based on the objective responses noted) microbial community composition will be determined by 16s metagenomic sequencing using the Illumina MiSeq platform. Libraries will be prepared in-house according to Illumina protocols and pooled libraries will be sent to the SBPMDI for deep sequencing. The resulting sequence data will be analyzed with the current version of Qiime [39].

We recognize that TUDCA treatment may not have a significant impact on gut microbiota composition, as our preliminary data from IBD mouse models showed and for this reason will collect stool samples but not undertake metagenomic profiling at this time.

Future analyses. We will biobank tissues, stool and serum for deeper immunologic analyses that may be pursued with additional funding from alternative sources. This study will provide a rich resource for expanded immunological studies and will bank biospecimens (snap frozen, RNA later tissue, serum, stool) for future studies including full cytokine profiles and innate/adaptive immune readouts. These will be processed and stored in our NIDDK sponsored Digestive Diseases Research Center Biobank Core. Stool will also be stored for future microbial/metabolome analyses.

6 Statistical Plan and Outcome Analyses

ER stress and cell death markers. We expect that TUDCA treatment will reduce ER stress in colon biopsy tissues from subjects with symptomatic ulcerative colitis (UC), as judged by expression levels of ER stress markers as we have observed in IBD mouse models. Colon biopsy samples from inflamed and (if available) adjacent non-inflamed colon before and after 6 weeks of TUDCA treatment will be harvested for analysis listed below.

As the primary study endpoint, we will assess the change in ER stress markers, including eIF2α phosphorylation, XBP1s and BIP/GRP78, in colon biopsy tissues before and after 6 weeks of TUDCA treatment.

BIP, XBP1 splicing, phosphorylated eIF2α will be assessed by Western blot and RT-PCR and immunohistochemistry (IHC). Other ER stress markers, such as PERK, ATF4, CHOP, IRE1α and ATF6α will also be examined. In addition, we expect that cell death markers in colon biopsy tissues will also be downregulated, including cJUN, JNK, cleaved caspase 2 and cleaved caspase 3. We will also monitor expression of inflammatory cytokines, IL-1B, TNFα, IL-6 and IFNγ. Additional samples aliquots will be saved (flash frozen and RNAlater) for future studies evaluating adaptive and innate immune readouts.

Dr. Randal J. Kaufman, the co-PI of this study, has a broad background in ER stress and cell death, and discovered the therapeutic role of TUDCA in IBD mouse models with support from CCFA. His laboratory has extensive experience in evaluation of ER stress, inflammation and cell death markers by western-blotting, PCR and IHC in both mouse models and human samples. All the colon biopsy samples will be sent under dry ice for analysis by Dr. Kaufman's laboratory. Dr. Ruishu Deng, a Research Associate in Dr. Kaufman's laboratory will be responsible for analyzing and processing data from the clinical samples.

***Significance of primary outcomes**:* Changes in ER stress markers will clarify the mechanisms through which TUDCA may act to alleviate inflammation in colon tissue of subjects with UC.

6.1 Sample Size Determination for Primary Endpoint

Estimates of change in the primary outcomes: We expect that TUDCA treatment will lead to similar changes on ER stress markers in UC patients as that in IBD mouse models. As judged by quantification results of western blot, the expression levels of ER stress markers in IBD mouse models were significantly reduced by 25% to 45% after TUDCA treatment.[4] A previous publication, as well as our own data, has shown that standard deviations of the ER stress markers are less than ¼ of the means in intestinal biopsy samples of IBD patients[40]. Considering there might be greater variation after the TUDCA treatment, we set the standard deviations at half of the means of all the ER stress markers (Table 2).

Table 2 Sample size calculation

ER stress markers	Difference between means	Estimated standard deviation	Sample size at power=0.8	Sample size at power=0.9
p-eIF2α	1.16-0.64=0.52	0.26	5.09	6.39
XBP1s	1.21-0.73=0.48	0.24	5.09	6.39
BIP	1.13-0.84=0.30	0.15	5.09	6.39

Power Estimates for each primary endpoint: We used the quantification results of western blot on ER stress markers to calculate the sample size needed. Since the impact of TUDCA on intestine tissues in humans has not been examined, we used the difference between the mean values of ER stress markers in groups treated with or without TUDCA that we obtained in murine studies.[4] Power analysis using the R software shows that 7 patients are sufficient to detect the difference before and after the treatment, when $P<0.05$ is considered significant. Due to the relatively variability in IBD patient disease activity and high dropout rate in IBD clinical trials, we will enroll a total of 12 patients will be recruited for the study with an expected

Statistical analysis: We will be specifically interested in the statistical contrasts that compare before and after TUDCA treatment. Correlation between the primary end point and secondary end point will also be examined. The appropriateness of all analysis of variance and covariance models will be evaluated using regression residuals. If normality or homoscedasticity requirements are violated, data transformations will be explored and, if appropriate transformations cannot be found, analyses may be done semi-parametrically using the ranks of the data.

6.2 Secondary Endpoint Analyses

As this is an open label, Phase I study, secondary endpoints of safety and tolerability will be reported qualitatively. Efficacy will be measured and reported qualitatively by change in Mayo Score, Goebes Histology Index and SIBDQ. As appropriate Student's t test, Mann Whitney U or ANOVA will be used to compare between time points.

1. **Active monitoring of UC study patients for TUDCA safety and tolerability**

TUDCA has a strong record of safety in several patient populations[19, 20, 30] (See Detailed Safety Data Review below). However, TUDCA has not been examined specifically in a UC population. Therefore, we aim to define its safety/tolerability in this population through frequent monitoring and symptom assessment as detailed in the study procedures. Adverse events will be categorized according to CTCAE v5.0 criteria and grading.

Infrequent side effects of TUDCA therapy include diarrhea and headache. Our study nurse coordinator will contact all study patients biweekly to assess for any experienced

adverse events. Additionally, in line with WUSM Center for Clinical Trials protocols, patients will have 24 hour access to report suspected Adverse Events. All adverse events or serious adverse events will be recorded and reviewed with treating physician as well as PI. In the event that patients experience an adverse event potentially related to TUDCA, the patient will be offered the option of dose de-escalation or study withdrawal.

TUDCA dose de-escalation strategy: In the setting of a toxicity that the treating physician determines most likely related to TUDCA, we will reduce the dose to 1000 mg (42-50% of initial dose). Common side effects are diarrhea and headache. Since diarrhea is already a symptom implicit to inclusion, we will consider an increase of ≥3 bowel movements/day to be a likely drug toxicity.

Early stopping rules and Dosage Reduction: In the event that ≥50% of patients experience a TUDCA associated side effect or intolerance, we will initiate early study termination or enroll all future patients at the 1000 mg dose. This decision will be made with the guidance of the appointed Data Safety and Monitoring Committee. Full Stopping rules are described in Section 7.4. Note, individual and study stoppage criteria should be applied to these patients as well.

2. **Active Monitoring and Management of new elevations in liver transaminases:**

The following is our plan to a plan to evaluate subjects for other causes of liver injury and for the potential of drug-induced liver injury (DILI) and include protocol responses to persistent or new deterioration of hepatic function.

For new elevations in transaminases greater than 2x ULN in subjects with normal baseline values or 2x baseline in subjects with abnormal baseline values, repeat measurement should be performed within 48 hours. If elevations persist, subjects should be evaluated for other causes of transaminase elevations and with tests of hepatic function. This may include imaging and/or labs at the treating physicians discretion and, if appropriate, in consultation with the study PI. If no other cause is found then the subjects need to be "Monitored Closely" and the drug should be discontinued as per the recommendations in the Guidance for subjects with normal baseline liver function. For patients with abnormal baseline indices, we will follow the recommendation below.

Recommendation: For patients with normal baseline liver biochemistries, the drug should be discontinued as per: The Guidance for Industry - Drug Induced Liver Injury (Guidance for Industry-Drug Induced Liver Injury: Premarketing Clinical Evaluation at: **http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf**)

The guidance provides recommendations for monitoring and decision-making for drug discontinuation in trials that enroll subjects who have normal liver transaminases and bilirubin at baseline. Drug should be discontinued, and the subject followed until resolution of symptoms or signs in the following situations:

- ALT or AST >8x ULN

- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TB >2x ULN or INR >1.5)
- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%)

For patients with abnormal baseline indices, see the recommendations below.

- Baseline values should be established (by at least two samples obtained at least 4 weeks and no more than 8-12 weeks apart or up to 16 weeks apart if the potential subject has ≥3 LFT labs in the clinical chart) to account for disease related changes in liver enzymes and bilirubin while on study that may otherwise be inappropriately attributed to study drug. The differences in repeat measurements of baseline serum AST, ALT, ALP and total bilirubin (TBL) should be small (<20%) to be eligible for study entry. You should not enroll subjects with evidence of worsening liver function based on the 2 initial laboratory values used to establish the baseline.
- If subjects with abnormal baseline liver indices develop elevations of AST or ALT >2x baseline or total bilirubin >1.5x baseline values during the study, repeat testing should be performed within 48 -72 hours. If there are persistent elevations (ALT or AST >2x baseline or TBL >1.5x baseline values) upon repeat testing, then close observation (testing and physical examination 2-3 times per week) should be implemented and discontinuation of drug should be considered (see c below).

A decision to discontinue or temporarily interrupt the study drug should be considered based on factors that include how much higher than baseline ALT and AST were relative to the upper limit of normal (ULN) and how much the on study ALT and AST levels have increased relative to baseline, in addition to whether there is concomitant elevation of bilirubin or INR. You need to discontinue or temporarily interrupt the study drug:

- If baseline measurements (BLM) were <2x ULN, discontinue if ALT or AST increases to >5x BLM.
- If BLM ≥2x ULN but <5x ULN, discontinue if ALT or AST increases to >3x BLM
- If BLM ≥ 5x ULN, discontinue if ALT or AST increases to >2x BLM
- Discontinue if ALT or AST increase > 2x BLM AND the increase is accompanied by a concomitant increase in TBL to >2x BLM OR the INR concomitantly increases by >0.2.

In any subjects with signs and symptoms of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

3. Examine efficacy endpoints endoscopic mucosal healing, clinical response (Mayo Score) as well as histologic severity (Geboes histology index).

We anticipate that TUDCA therapy will reduce ER stress and promote mucosal healing. As TUDCA has the potential to increase stool frequency, it is important to measure efficacy through objective measures (endoscopy and histology) as well as clinical symptoms. Thus, we will obtain a complete Mayo UC score at weeks 0 and 6 and a partial (no endoscopic subscore) at weeks 2 and 4.[32] Biopsies will be obtained at both

endoscopic procedures and objectively scored by the Goebes index. Thus, secondary endpoints will include the following using FDA recommended UC Clinical trial guidelines[41]:

1) **Mucosal Healing:** A decrease of at least 1 point on the Mayo endoscopic subscore with a final score of 0 or 1 will be used as a marker of mucosal healing.
2) **Clinical Response**: A decrease in Mayo Score of ≥ 3 points and a decrease in Mayo Score of ≥ 30% from baseline and a decrease in the rectal bleeding score of ≥ 1 or an absolute rectal bleeding score of 0-1.
    a. **Clinical Remission** will also be recorded if achieved as defined by a total Mayo Score of ≤2 with no subscore >1 and improvement of endoscopic appearance of the mucosa (Mayo 0 or 1).
3) **Histologic activity:** *Histologic disease activity.* Colon biopsy samples will be harvested and fixed as described. H&E stained sections will be evaluated for tissue damage and inflammation by a gastrointestinal pathologist in a double-blinded manner, using the simplified Geboes histology index[34, 35]

We expect that TUDCA treatment will significantly alleviate inflammation in colon tissues, which will be correlated with increased plasma concentration of TUDCA or the deconjugated form UDCA. We will collect stool samples both before and after TUDCA treatment for potential (longer range) studies of gut microbiota composition.

Data analysis, interpretation and alternative plans: We expect that there will be minimal adverse effects due to TUDCA treatment, as its safety in humans has been proven in clinical trials for other disease conditions. We expect that TUDCA treatment will improve endoscopic and histologic parameters of inflammation. We also anticipate that it will improve overall Mayo scores and lead to clinical response, if not remission. It is possible though that TUDCA will exacerbate diarrhea in UC patients and clinical symptoms may worsen even if mucosal healing occurs. It is possible that we will not achieve significant improvement in the severity of inflammation, as mucosal healing can take as long as several months (Lialda induction trial) and our TUDCA treatment duration is only 6 weeks. In this case, we will analyze whether expression of ER stress, inflammation and cell death markers in colon biopsy tissues is correlated with the plasma concentration of TUDCA and its metabolites, which will provide a reliable indication for long-term mucosal healing.

7 Safety and Adverse Events

As Safety and tolerability are secondary endpoints, they are further described above in section 6.

7.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries will be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

What are *serious adverse events* or *unanticipated problems*?

Any incident, experience, or outcome that meets **all** of the following criteria:

(1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

(2) related or possibly related to participation in the research (in this guidance document, *possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);

(3) and suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 14 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 Recording of Adverse Events

At each contact with the subject, the investigative team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events

7.3.1 IRB Notification by Investigator

Reports of all serious adverse events or unanticipated problems (including follow-up information) must be submitted to the IRB within 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

7.3.2 FDA Notification by Sponsor

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

7.4 Stopping Rules

Individual stoppage criteria-

Based on extensive clinical trial data, we anticipate that TUDCA will be well tolerated. However, patients will be monitored via study visits and phone calls and will permanently discontinue study drug for any adverse event possibly or probably related to study drug that is considered of *moderate or severe intensity*. To define intensity, we will utilize a standard severity rating scale to ensure this safety monitoring plan is enforced in a standardized fashion. Individual subjects should be discontinued for any adverse event rated a Grade 3 or greater in severity on the Common Terminology Criteria for Adverse Events (CTCAE_v5.0 November 2017) scale v, unless it is clearly related to the underlying disease.
For disease specific symptoms (e.g. diarrhea, rectal bleeding) we will consider disease worsening as an increase in >3 points on the Mayo Score to be potentially related to TUDCA and will lower the dose to 1000 mg as described. If symptoms persist or worsen over the subsequent 5 days, the patient will be permanently discontinued from the study.

Study stoppage criteria-
Enrollment of new subjects should be interrupted should 2 or more patients experience CTCAE Grade 3 event, or 1 or more patients experience CTCAE Grade 4 events not clearly related to the underlying disease. Study enrollment may be resumed following review of all available safety data by the investigator and study team and a concurrent opinion that the totality of the data suggests this event is unlikely to be related to TUDCA. The study should be terminated if, following the review of the safety data, it has not been possible to demonstrate that the adverse event has a clear relationship to the underlying disease or associated co-morbidity.

We will initiate early study termination or enroll all future patients at the 1000 mg dose. This decision will be made with the guidance of the appointed Data Safety and Monitoring Committee. Individual and study stoppage criteria should be applied to these patients as well.

7.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

7.5.1 Internal Data and Safety Monitoring Board

Adverse Events and Serious Adverse Events will be assessed and recorded with oversight by an institutional DSMC (Data Safety and Monitoring committee) that will meet after the 7th patient has completed the study. At that point, the Data and Safety Monitoring Committee (DSMC) will meet to determine whether enrollment may continue to the randomized portion of the study.

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the DSMC will meet to review toxicity data at least every 6 months. The report will be prepared by the statistician with assistance from the study team and will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by arm
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by arm
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or the Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

Additionally, if two or more serious adverse events of a similar nature occur and a causal relationship to the investigational product cannot be excluded, accrual to the randomized portion will not occur.

A DSMC will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Like investigators, DSMC members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMC will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMC must also be disclosed.

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC at https://siteman.wustl.edu/research/clinical-research-resources/

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

8.4 Records Retention

The investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have

elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

The Principal Investigator at Washington University School of Medicine is Dr. Matthew Ciorba. The Data Monitoring Plan for the Study is as follows:

Study recruitment, progress and safety issues are reviewed by the investigators, research nurse and research coordinators at a weekly meeting. During the meeting the following will be reviewed:

- Subject enrollment
- Preliminary finds that might affect the risk/benefit ratio or alter the procedures described in the protocol
- Any out of range lab values including the resulting course of action
- Stopping rules
- Any adverse events or serious adverse events that may result from the research procedures

PI will monitor the study for any adverse and/or serious adverse events. All serious adverse events will be reported to the IRB as follows:

1) Death – Immediately
2) Life-Threatening – Within seven (7) calendar days
3) IDE – Within seven (7) working days
4) All other SAEs - Within 7 calendar days

An event that meets the three criteria above generally will warrant consideration of substantive changes in the research protocol or informed consent or other corrective actions in order to protect subjects.

The same will be reported to the sponsor, and subsequently the FDA (if applicable).

Should there be a serious adverse event that occurs that increases the risks to the participants, the study will be stopped, an investigation will be conducted, and a findings report will be generated before the study is resumed. These findings will be shared with the IRB, the Research Subject Advocate, and the General Clinical Research Center (if applicable). If necessary, the research procedure will be modified and appropriate revisions to the consent document and consenting process will be made.

9.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. A copy of the Subject Informed Consent Form is included. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Publication Plan

Results will be submitted for publication or data disseminated via meeting abstracts after approval by the PI and all authors/involved investigators.

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