Official Title: Double-blind Randomized Controlled Trial of Anakinra, Pentoxifylline, and Zinc Compared to Methylprednisolone in Severe Acute Alcoholic Hepatitis

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The University of Texas Southwestern Medical Center at Dallas Institutional Review Board

Novel Therapies in Severe Acute Alcoholic Hepatitis

1. Study Specific Aims:

Aim 1: Evaluate the effects of corticosteroids versus a combination of interleukin-1 inhibitor plus pentoxifylline plus zinc supplements in patients with MELD≥20. Patients will be randomized to receive 28 days of methylprednisolone 32 mg daily OR therapy that includes a combination of anakinra (interleukin-1 receptor antagonist, IL-1 RA) 100mg by subcutaneous injection daily for 14 days PLUS pentoxifylline 400 mg p.o. three times daily for 28 days PLUS zinc supplements (220 mg of zinc sulfate) given orally for 6 months. This combination strategy will treat the acute inflammatory component of the disease (anakinra) and protect against development of hepatorenal syndrome (pentoxifylline), one of the most frequent causes of death in severe AAH. Gut mucosal integrity will be restored (zinc supplements), a critical component in preventing repeated episodes of AAH. The primary outcome will be 6 month mortality rate. Secondary outcomes will be measured at 30, 90 and 180 days. They include change in gut mucosal permeability; changes in the levels of serum endotoxin, IL-1, tumor necrosis factor-1 (TNF1) and other cytokines from baseline; mortality; and change in MELD score.

Aim 2: Document the natural history of severe alcoholic hepatitis. Patients who decline randomization will be offered the option of inclusion in the study prospectively for data collection purposes and research study procedures.

Aim 3: Create a data and tissue biorepository. This biorepository will serve as a national resource for all studies related to acute alcoholic hepatitis. Our research group will use the samples and share with others for future studies on biomarkers for AAH severity/outcome, cost analysis, rationales for new therapies, etc.

2. Background:

Acute alcoholic hepatitis (AAH) is an important cause of morbidity, mortality and healthcare costs in the United States and most Western countries. The 2007 nationwide inpatient sample from the Healthcare Cost and Utilization Project reported that 56,809 patients (0.71% of the total) were hospitalized with AH[(1)]. Average hospital costs were $37,769, more than twice the cost of myocardial infarction. Length of stay was significantly higher in those who died compared to those who survived (9.3 vs. 6.2 days). Total charges (~$85,000) were 2.5-fold higher in those who died than those who survived. The 28-day mortality of AAH in Denmark rose from 12 to 15%, and the 84-day mortality increased from 14 to 24% between 1999-2008[(2)] with 5-year mortality of 56%. These data from Denmark are similar to the 1991 VA Cooperative data: 4-year mortality of 42% with AH alone, and 65% with AH plus cirrhosis[(3).] Aggregated mortality of 661 placebo-treated patients from 19 trials was 20% at 30 days and 34% at a median of 160 days follow-up[(4)].

Statistics illustrate the grim outcome of AAH despite major advances in critical care and other medical interventions over the last 3 decades. While hepatic steatosis develops in the majority of heavy drinkers, alcoholic hepatitis and cirrhosis occur in only 20-25% (reviewed in [(5)]). All patients with AAH have a background of hepatic steatosis that represents a consequence of metabolic and oxidative stress. The inflammatory nature of alcoholic hepatitis is highlighted clinically by elevation in white blood cell count (WBC) and occasionally fever and pathologically by infiltration of the liver with neutrophils. A number of studies have implicated the gut flora, in particular gut-derived toxins, as a “second hit” in the pathogenesis of acute alcoholic hepatitis and cirrhosis. Endotoxin (lipopolysaccharide, LPS) binds to toll-like receptor 4 (TLR4), a receptor that plays a key role in initiating release of tumor necrosis factor (TNF1) and other cytokines by activated Kupffer cells in experimental models of alcoholic liver injury[(6-8)].

High serum levels of TNF 1 are associated with the clinical severity of alcoholic hepatitis[9] and there is increased production of TNF 1 in monocytes isolated from patients with alcoholic hepatitis[10]. Other studies have suggested that alcohol increases gut permeability in animal models and in heavy drinkers[11], a potential contributing factor in how endotoxin or other bacterial products reach the liver. Endotoxemia occurs frequently in patients with cirrhosis and in those with chronic hepatitis[12, 13] probably due to increased gut bacterial translocation. Furthermore, recent studies reported alterations in the gut microbiome in alcohol-fed animals[14], a finding that could also promote increased permeability of the gut mucosa. **We hypothesize that impairment of gut mucosal integrity is the unifying "second hit" with potential contributing factors including the microbiome, nutritional deficiencies and direct effects of alcohol.**

Published data show that several agents are beneficial in short-term treatment of severe AAH, but none has proven to impact 6 month mortality when used as monotherapy[15]. Combining therapeutic agents that target different pathways while avoiding the risks of infection and hepatorenal syndrome may improve mortality particularly in the most severely ill patients with AAH. Furthermore, no specific interventions or medications benefit patients with mild to moderate AAH[14]. The proposed study will use specific agents to target both impairment in gut mucosal integrity and pathways involved in inflammation in patients with the most severe form of AAH. The potential benefit is to not only improve treatment but also to provide evidence of the roles of gut mucosal integrity and inflammation in the pathogenesis of AAH.

The proposed study represents the culmination of a planning process involving the members of the proposed Executive Committee and other co-investigators. The criteria for selecting the proposed treatments included current FDA approval for other indications and a known safety profile in other disease states so that a beneficial treatment(s) could be brought forward for a phase III clinical trial and/or transform treatment for AAH, a condition for which the current treatment paradigm is unsatisfactory. **The selection of the medications was based on the hypothesis that the syndrome of AAH results from inflammation and release of IL-1, TNF 1, and other pro-inflammatory cytokines, triggered by gut derived endotoxins and other bacterial products as a consequence of increased permeability of the gut mucosa.**

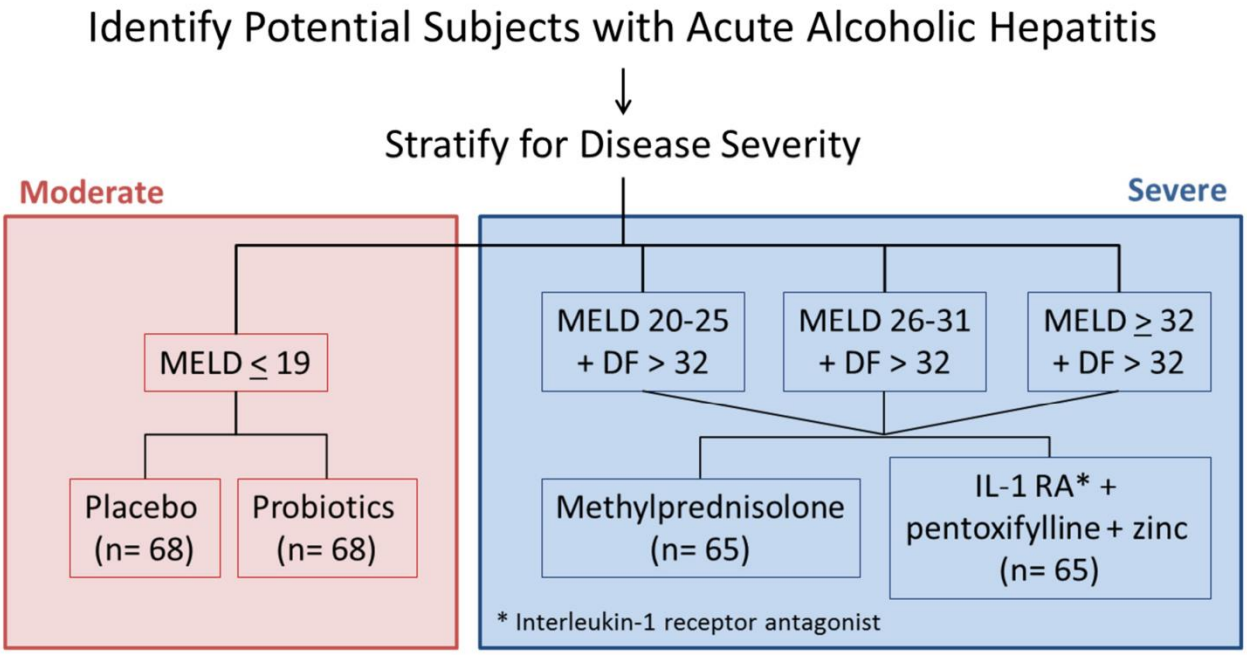
The standard of care (corticosteroids) is recommended by both the American Association for the Study of Liver Diseases and the American College of Gastroenterology in published practice guidelines[5, 16]. A second agent, pentoxifylline, provides protection from development of acute kidney injury[17]. Both agents improve short-term survival (28 day) but 6 month mortality remains high, 30% – 50%[18]. In this clinical trial, corticosteroids (methylprednisolone 32 mg/d for 28 days) will be compared with a drug regimen designed to decrease inflammation (interleukin-1 receptor antagonist for 28 days) and improve gut permeability (oral zinc for 6 months). In addition the study drug regimen will include pentoxifylline to protect against acute kidney injury (AKI, hepatorenal syndrome).

3. Concise Summary of the Project:

Our study will test the hypothesis that the syndrome of acute alcoholic hepatitis (AAH) results from severe inflammation and dysregulated cytokines, providing the "second hit" that causes an acute deterioration. We further hypothesize that gut derived endotoxins and other bacterial products trigger inflammation and are a consequence of increased permeability with altered gut barrier function. Steroid monotherapy is not effective in all patients with AAH. Consequently, our studies will utilize compounds that have the potential to improve gut barrier function (both in moderate and severe disease) AND to reduce the associated inflammation (severe disease) AND to prevent the development of hepatorenal syndrome and other organ failure (severe disease).

We will utilize published models for predicting mortality in patients with AAH to stratify patients

based on severity. Although previous studies of treatment for severe AAH utilized 30-day mortality as an endpoint, 6-month mortality is a more relevant outcome, in part because most transplantation programs require 6 months of abstinence before active listing. Furthermore, the number of deaths between 6 and12 months in abstinent patients is low compared to the number in the first 6 months. Patients will be stratified into those with Model of End-stage Liver Disease (MELD) score ≤20 and MELD≥20. Those stratified into the moderate group are described in a separate application. The primary difference between the Maddrey discriminant function (DF) and the MELD is that the latter includes creatinine. We propose to use the MELD score to stratify for severity but require that patients in the severe group also have a DF > 32, to avoid including patients with chronic kidney disease, not hepatorenal syndrome.



Specific Aim 1 = Randomized controlled trial for severe acute alcoholic hepatitis: MELD ≥20

: Patients with severe AAH will be randomized equally into two arms. Patients with MELD 20-25, patients with MELD 26-31, and patients with MELD ≥ 32 will be randomized separately into two equal arms to ensure comparable severity in the three groups. The trial will also be double-blinded by re-packaging all oral medications into capsules to ensure that each group receives an equal number of capsules each day for the entire duration of the study.

Patients will be treated with pentoxifylline 400 mg three times daily for 28 days PLUS zinc supplements for a total of 6 months (Group A) or methylprednisolone 32 mg daily for 28 days PLUS placebo capsules daily for the remaining 5 months (Group B). An injection of either anakinra (100 mg), Group A) or a placebo (Group B) will be given subcutaneously each day for 14 days. Patients, investigators and physicians involved in the care of the patients will be blinded as to the treatment.

The primary outcome will be 6-month mortality. Secondary outcomes will include 30 and 90 day mortality; changes in MELD score at 30, 90 and 180 days and changes in gut mucosal permeability measured by serum tests. Serum endotoxin levels and serum cytokine profiles (IL-1, TNF1, etc.) will also be measured in both groups at baseline, 2, 7, 30, 90 and 180 days. The attached table indicates the timing of samples performed both for research purposes and for insuring standard of care for the subjects.

Specific Aim 2 = Natural history of severe acute alcoholic hepatitis: MELD ≥ 20:

Patients with severe AAH who decline to be randomized but consent to prospective data collection and minimal risk non-invasive studies (blood drawing and urine collection). We will also look to enroll subjects with alcoholic hepatitis/alcoholic liver disease who are not eligible for the Drug Trial but who are willing to provide specimens and data, valuable information regarding the natural history of alcoholic hepatitis and the aims noted above. Specimens including blood, urine, stool, and liver tissue if a liver biopsy is done as part of subject's standard treatment, will be collected from subjects participating in this sub study. This will be a one-time collection of specimens to follow the procedure of the Study Day 0 for all enrolling participants.

Specific Aim 3 = Subjects will be asked for a DNA blood draw during the first day of the study.

All human subjects will be patients with a clinical presentation consistent with acute alcoholic hepatitis (AAH). The trial is designed to test the hypothesis that AAH results from inflammation triggered by gut derived endotoxins and other bacterial products as a consequence of increased permeability of the gut mucosa. Patients with AAH will be stratified by disease severity as determined by the Model for End-Stage Liver Disease (MELD) score and enrolled in one of two randomized controlled trials.

Subject population: The total subject population will be 260 patients with alcoholic hepatitis, 130 in each disease severity group (moderate disease is defined as MELD $\leq$ 20 and severe disease as MELD 20-25, MELD 26-31, and MELD $>$ 32). The age range will be 21 – 70 years old. The health status of the population will be that of a group with significant acute clinical presentation from alcoholic liver disease.

Sampling plan: The sample size was calculated based on the analysis of patients with alcoholic liver disease in the (SRTR) database, accounting for drop-out, lost-to-follow-up and non-evaluable results and including a factor for possible non-representativeness of the original database. In addition, preliminary data for 114 patients meeting criteria were used in the calculations and stratification decisions.

4. Criteria for Inclusion of Subjects:

a. Ability to provide informed consent by subject or appropriate family member
b. Age between 21-70 years
c. Recent alcohol consumption > 50 g/d for > 6 months, continuing within two months before enrollment
d. At least 2 of the following symptoms or signs of acute alcoholic hepatitis: Anorexia, nausea, RUQ pain, jaundice, leukocytes, hepatomegaly, AND
e. Elevation of AST ≥ 50 U/L, but < 500 U/L at the time of admission or within 3 days of baseline visit; AST > ALT and ALT < 200 U/L; total bilirubin >3 mg/dL ANDf. Liver biopsy showing alcoholic hepatitis (steatohepatitis) OR ultrasound of liver showing increased echogenicity OR CT scan showing decreased attenuation of liver compared to spleen or MRI showing fatty liver (decreased signaling intensity on T1 weighted images). If the liver biopsy (done within 60 days of inclusion) confirms diagnosis of AAH then inclusion e will be waived.
g. Model for End-Stage Liver Disease (MELD) ≥ 20 and Maddrey $\geq$ 32
h. Willingness to utilize two reliable forms of contraception (both males and females of childbearing potential) from screening through the first six weeks of the study

5. Criteria for Exclusion of Subjects:

a. Hypotension with BP < 80/50 after volume repletion
b. Pregnancy; incarceration; inability to provide consent or lack of appropriate family member

c. Signs of uncontrolled systemic infection: Fever > 38℃ and positive blood or ascites cultures on appropriate antibiotic therapy for ≥ 3 days within 3 days of inclusion
d. Acute gastrointestinal bleeding requiring > 2 units of blood transfusion within the previous 4 days
e. Undue risk from immunosuppression: Positive HBsAg; a positive skin PPD skin test, a positive quantiferon, or history of treatment for tuberculosis; history of any malignancy except skin cancer but including hepatocellular carcinoma; known HIV infection
f. Treatment with corticosteroids or other immunosuppressive medications including specific anti-TNF therapy (not including pentoxifylline), calcineurin inhibitors for >3 days within 3 days of inclusion.
g. Evidence of acute pancreatitis: CT evidence or amylase or lipase > 5 X ULN
h. Serious cardiac, respiratory or neurologic disease or evidence of autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, hemochromatosis, secondary iron overload due to chronic hemolysis, alpha-1-antitrypsin deficiency
i. Acute or chronic kidney injury with serum creatinine > 3.0 mg/dl.

6. Study Procedures:

Aim 1

Patients will be screened, stratified as moderate or severe acute alcoholic hepatitis and randomized to one of two arms for the clinical trial. Randomization will be in blocks of 6 using a sealed, opaque envelope. The study procedures are tabulated and divided into standard of care ("Standard") and research-specific ("Research") studies. Between days 2 and 7, between days 8 and 27, at 2 months, 4 months and 5 months follow-up, clinically indicated standard studies will be obtained. Study procedures are described in the table.

Study Procedures by Study Day:
A summary of evaluations is located in the schedule of events table provided at the end of this section. The procedures and evaluations are outlined below.

Screening:
Complete screening for eligibility will occur once the informed consent has been obtained as described above. Patients will be evaluated on the basis of clinical and laboratory data during pre-screening and stratified into moderate or severe alcoholic hepatitis. Patients will be evaluated for entry into the study according to the stated inclusion and exclusion criteria. Individuals who are identified during screening as not eligible for the study need not complete all screening procedures but will be provided the option to enroll in the natural history study. The reason for ineligible status will be documented on the Screen Failure Log.

The following procedures will be obtained or performed to evaluate each patient's general health and qualifications for participation in the study:

- Signed, written informed consent
- Demographic information including gender, date of birth, race and ethnicity.
- Medication history over the past 30 days , including prescription and non-prescription over-the-counter medications, herbal medications, vitamins and minerals.
- Alcohol usage in the past 60 days prior to assessment specifically and the pattern of chronic usage.
- Review inclusion and exclusion criteria
- Collection of blood for hematology, coagulation, clinical chemistry to include amylase and lipase, ammonia, and in female patients, a pregnancy test.

- Physical examination, including height, weight (body mass index will be calculated based on these variables), and vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate and body temperature)
- Ultrasound or cross –sectional imaging of the abdomen. This is standard of care in patients hospitalized with alcoholic hepatitis.

Study treatment:

Patients will be randomized to receive 28 days of methylprednisolone 32 mg daily OR therapy that includes a combination of anakinra (interleukin-1 receptor antagonist, IL-1 RA) 100mg by subcutaneous injection daily for 14 days plus pentoxifylline 400 mg orally three times daily for one month PLUS zinc supplements (220 mg of zinc sulfate) given orally for 6 months.

Assessments at baseline and during study treatment:

Study day 0 – baseline (within 48 hours of study enrollment)
- Complete collection of all baseline history. Review of alcohol use history.
- Complete collection of all indicated standard of care laboratory evaluations (chemistry, hematology, coagulation, urine, microbiology, viral, and TB testing) .
- Ascites evaluation, if present.
- Collection of baseline research testing to include urine, blood, DNA and stool testing.
- Collect prior and concomitant medications.
- Clinical assessment to include vital signs and physical exam details.
- Nutritional assessment with a hand grip dynamometer

Treatment day 1 – time zero (alternatively note this as treatment day one with no time and above as study day 1 baseline)
- Begin continuous AE review and reporting
- Begin subcutaneous injected study medication or placebo injection
- Begin dosing of oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation

Treatment day 2:
- Inpatient assessment to include:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
  - Collection of research laboratory studies including urine and blood.
  - Complete 24 hour urine collection.
- Continue subcutaneous injection of study drug or placebo injection.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous adverse event (AE) review and reporting.

Treatment days 3-6:
- If patient remains inpatient or has clinically indicated outpatient visit:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
- Continue subcutaneous injection of study drugs or placebo injection.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

Treatment day 7 ± 2 days:

- Inpatient assessment or **required** outpatient clinic visit to include:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
  - Collection of research laboratory studies including urine and blood.
  - Nutritional assessment with a hand grip dynamometer
- Continue subcutaneous injection of study drugs or placebo injection.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

Treatment days 8-14:

- If patient remains inpatient or has clinically indicated outpatient visit:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
- Continue subcutaneous injection of study drugs or placebo injection.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

Treatment day 15:

- If patient remains inpatient or has clinically indicated outpatient visit:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
- **STOP** subcutaneous injection of study drugs or placebo injection.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

Treatment days 16-27:

- If patient remains inpatient or has a clinically indicated outpatient visit:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

Treatment day 28 ± 2 days:

- Inpatient assessment or **required** outpatient clinic visit to include:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
  - Collection of research laboratory studies including urine and blood.
- Continue oral study medications (pentoxifylline 400 mg 3 times daily PLUS zinc sulfate 220 mg once daily)/placebo or oral methylprednisolone/placebo preparation.
- Continuous AE review and reporting.

- Nutritional assessment with a hand grip dynamometer

Treatment day 29:

- **STOP** dosing of oral study drug or oral methylprednisolone preparation
- **Continue** once daily dosing of oral zinc supplement or oral placebo preparation
- Continuous AE review and reporting.

Treatment days 29 to 2 months ± 2 weeks:

- Continue once daily dosing of oral zinc supplement or oral placebo preparation
- Continuous AE review and reporting.
- If patient remains inpatient at treatment month 2 or completes clinically indicated outpatient visit at treatment month 2:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.

Treatment 3 months ± 2 weeks:

- Inpatient assessment or **required** outpatient clinic visit to include:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
  - Collection of research laboratory studies including urine, blood and stool.
- Continue once daily dosing of oral zinc supplement or oral placebo preparation
- Continuous AE review and reporting.

Treatment 4 and 5 months ± 2 weeks:

- Continue once daily dosing of oral zinc supplement or oral placebo preparation
- Continuous AE review and reporting.
- If patient remains inpatient or completes clinically indicated outpatient visit at treatment months 4 or 5:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.

Treatment 6 months:

- **STOP** all study and placebo medications.
- Complete abdominal ultrasound.
- Inpatient assessment or **required** outpatient clinic visit to include:
  - Review baseline history for changes. Review alcohol intake history.
  - Collection of prior and concomitant medications
  - Clinical assessment including vital signs and physical exam details
  - Collection of blood for clinically indicated laboratory studies.
  - Collection of research laboratory studies including urine, blood and stool.
- Continuous AE review and reporting.
- Nutritional assessment with a hand grip dynamometer

Follow up of early termination:

A safety follow up visit will be performed approximately 30 days after discontinuation of study drug for all patients that discontinued without withdrawing informed consent. Evaluation will include:

- Collection of blood for standard of care labs to include chemistry, hematology and coagulation studies.
- Clinical assessment to include vital signs and physical exam details.

- Review baseline history for changes. Review alcohol intake history.
- Collection of concomitant medications.
- AE review and reporting.
- Review and recording of reason for early termination.

Purpose	Test	Day 0	Day 2 – 6*	Day 7	Day 8 – 27*	Day 28	2 mos *†	3 mos †	4 mos *†	5 mos *†	6 mos †
Standard	Clinical assessment	x	x	x	x	x	x	x	x	x	x
	Alcohol intake	x	x	x	x	x	x	x	x	x	x
	Liver panel (bilirubin, alkaline phosphatase, AST, ALT) and GGT	x	x	x	x	x	x	x	x	x	x
	Serum electrolytes, creatinine, BUN	x	x	x	x	x	x	x	x	x	x
	Serum albumin, total protein	x	x	x	x	x	x	x	x	x	x
	Prothrombin time, INR	x	x	x	x	x	x	x	x	x	x
	Complete blood count	x	x	x	x	x	x	x	x	x	x
	Haptoglobin	x									x
	Lipid panel	x									x
	Amylase, lipase	x									
	HIV	x									
	HAV Ab, HBsAg, HBcore Ab (total)	x									
	HCV Ab	x									
	PPD	x									
	Blood cultures x2	x									
	Urine culture & urinalysis	x									
	24 hour urine (Na, K, creatinine, protein)	x									
	Urine toxicology	x									
	Ascites for albumin, total protein, cell count and differential (if present)	x									
	Ascites culture	x									
	Stool studies (if diarrhea): C. difficile, culture	x									
	Complete abdominal ultrasound or cross-sectional radiology	x									x
Research	Gut permeability studies	x ‡				x		x			x
	Serum zinc, urine zinc	x				x		x			x
	Stool (DNA microbiome)	x						x			x
	Urine (KIM-1)	x	x day2	x		x		x			x
	Biomarkers = Cytokines	x	x day2	x		x		x			x

	DNA = Pharmacogenomics	x									
	Serum micro RNA	x		x		x		x			X
	Monocyte prep (U Mass, U Louisville)	x		x							x
Blood	Research total volume = 179 mL	45		30		37		30			37
	SOC total volume = 208 mL	82	14	14	14	14	14	14	14	14	14
	Combined = 387 mL = 26 tbspns	117	14	44	14	51	14	44	14	14	51

- * clinically indicated; † ± 2 weeks ‡ within first 28 days for non-ICU patients

Sub-Study Procedures

1. Optional Natural History (minimal risk with urine and blood collection): All screened subjects meeting inclusion and exclusion criteria below or declining consent to randomization will be invited to join this study of the natural history of severe acute alcoholic hepatitis. The natural history of patients with severe alcoholic hepatitis is not well documented in the current era. Prospective collection of data in parallel with the randomized study subjects has great potential value. Every patient who agrees and gives consent will be seen in follow-up as clinically indicated. At each visit that coincides with collection of samples for research, or as close as possible to Baseline, Day 7, Day 28, Month 3 and Month 6, the subjects will be offered the opportunity to participate. Patients may also
be asked to participate in the natural history study as a one-time draw at the day of consent.

4. Criteria for Inclusion of Subjects for Natural History Specimen Collection:

a. Ability to provide informed consent by subject or appropriate family member
b. Age between 21-70
c. Suspected or proven alcoholic liver disease

5. Criteria for Exclusion of Subjects for Natural History Specimen Collection:

a. Pregnancy; incarceration; inability to provide consent or lack of appropriate family member
b. Signs of uncontrolled systemic infection: Fever > 38o C; positive blood or ascites cultures on appropriate antibiotic therapy for >3 days within 3 days of inclusion
c. Evidence of acute pancreatitis: CT evidence or amylase or lipase >5 X ULN
d. Evidence of other liver diseases such as autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, biopsy proven hemochromatosis, alpha-1-antitrypsin deficiency

Over the entire 6 months study period 387 mL of blood will be drawn, comprising 208 mL for standard of care tests and 179 mL for research studies.

Optional DNA Blood Sample Collection: A biorepository can serve as a national resource for all studies related to acute alcoholic hepatitis. A major goal of the U-01 consortium is to share clinical samples. Our research group will use the samples and share with others for future studies on biomarkers for AAH severity/outcome, cost analysis, rationales for new therapies, etc. Blood and tissue samples will be collected as indicated in the table. Samples for DNA preparation will be shipped to the Cleveland Clinic and specimens will be shipped to those translational sites that are studying biomarkers or pharmacogenomics (U Mass, U Louisville). Specimens will only be identified by the unique study number. De-identified data obtained from the various pharmacogenomics, miRNA and transcriptosome screens will be placed on the website of the U-01 consortium and be available to the U-01 investigators throughout the study.

The amount of blood that would be collected is 8 mL (2 tbspns) and it will be stored at the Cleveland Clinic Foundation as part of the NIH Tissue and Biorepository for Alcoholic Hepatitis Studies.

7. Sources of Research Material: Specimens: Blood, urine, stool

Blood for research purposes will be collected at the same time as blood for clinical care. Urine specimens will be collected either with clinical care samples or at special research visits during follow-up after discharge. All samples will only be identified using a unique study number and processed as necessary for local storage or shipping to central testing sites.

Stool samples will be collected by giving a collection kit to the subjects.

A unique study number will be assigned to each subject. The unique study number will be assigned

centrally at the time of randomization and used to code all samples. The study site will be identified by the first digit (1 – 4), the subject by the last 3 digits and the disease stratification by the total number of digits (4 for moderate alcoholic hepatitis). This procedure allows any site to exceed 100 subjects enrolled in either study.

Data: Any past medical history, family history, social history (including dietary and occupational history), medications and other ingested supplements potentially related to etiology or complications of liver disease and alcohol consumption not provided in the electronic medical records.

Electronic medical record: Data pertaining to current hospitalization, past medical history, family history, social history (including dietary and occupational history), medications and other ingested supplements potentially related to etiology or complications of liver disease and alcohol consumption; physical examination.

Standard of care laboratory investigations: Results of routine blood chemistry tests = blood electrolytes, renal and liver function tests, pancreas enzymes, lipid panel, urine and fluid chemistry; hematology = complete blood count, coagulation and hemolysis studies, fluid cell counts; infectious diseases = viral hepatitis serologies, tests for HIV and tuberculosis; blood, urine and fluid cultures; stool culture and toxins. Results of routine radiology = chest x-ray, complete abdominal ultrasound.

Data from the results of research studies: gut permeability, blood and urine zinc, blood DNA (genomics) and stool DNA (microbiome), blood micro RNA, blood and urine inflammation biomarkers, blood mononuclear cells responses.

A measure of nutritional status willbe obtained via a hand grip measurement with the JAMAR dynamometer.

A list of study patients will be maintained in the electronic medical record at each site and in no other place. The list will be assigned to one investigator at each site. Access to the list, within the electronic medical record, will be granted to the other investigators and research nurses at the study site. The security of the electronic medical record will provide full protection to any individually identifiable private information.

8. Recruitment Methods and Consenting Process:

Potential patients will be identified from daily results of routine chemistry tests (bilirubin > 3 mg/dL, AST > ALT and > 1.5x upper limit of normal but < 500 U/L) and screened for inclusion and exclusion criteria. The primary physicians will be approached for permission to meet the patient and will introduce the researchers.

The potential subject will be introduced to the researcher(s) by provider(s) on the healthcare team responsible for the hospitalization (inpatients) or encounter (Emergency Department or outpatient). The study will be discussed in detail, with a translator as needed, and the consent form explained. Subjects will be given a copy of the consent form to keep, with a Spanish fully translated version as needed. When they have made a decision, the consent will be signed and witnessed if they are agreeable to participating. The time frame is 7 days from initial identification as eligible until study enrollment.

Circumstances for consent: Subjects will be approached by one of the physician investigators, in a confidential environment with family members present if desired. The information provided will be all that data in the IRB-approved consent form, using interpretation service as needed via hospital language lines. The consent document, in English or Spanish, will be given to the subject as described above. After agreement to participate, the form will be signed and witnessed; a complete copy of the entire document will be provided to the subject in English or Spanish as required.

If the subject’s condition is considered mentally unable to provide informed consent by the primary management team then a legal representative will have the option to sign the consent form until such time as the subject regains capacity. One of the complications of severe alcoholic hepatitis is hepatic encephalopathy. If this complication is diagnosed clinically, then the legal representative will be

required to sign the consent form until the encephalopathy has resolved.

9. Potential Risks:

Physical risks:

Methylprednisolone is the standard medical care for severe acute alcoholic hepatitis. Methylprednisolone has anti-inflammatory effects that can alter the body's reaction to other injuries. Methylprednisolone may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

Side effects	Frequent>20% of subjects	Occasional2 - 20% of subjects	RareLess than 2% of subjects
Serious	Infection (1.5 x risk in controls)	Psychosis (5%)	Peptic ulcer Bone necrosis
Less Serious	Weight gain	High blood sugar High blood pressure	Muscle weakness
Minor	Facial swelling Fluid retention Indigestion Increased appetite	Difficulty sleeping Mood changes Euphoria Headache	

Anakinra (study drug) is another anti-inflammatory treatment. It can alter the body's reaction to other injuries. Anakinra (study drug) may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

	Frequent >20% of subjects	Occasional 2 - 20% of subjects	Rare Less than 2% of subjects
Serious		Serious and life-threatening Infection (2 – 3%)	
Less Serious		Low blood count	
Minor	Injection site redness		

Pentoxifylline (study drug) may protect your kidneys from damage following changes in blood flow. It is used to treat patients with blood flow diseases. Pentoxifylline (study drug) may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

	Frequent >20% of subjects	Occasional 2 - 20% of subjects	Rare Less than 2% of subjects
Serious			Confusion Depression Seizure Hepatitis (rare)
Less Serious		Nausea, vomiting	
Minor	Headache Dizziness	Indigestion	
	Frequent >20% of subjects	Occasional 2 - 20% of subjects	Rare Less than 2% of subjects

Zinc is an essential nutrient found in highest quantities in oysters, shellfish, meat, chicken and fish. It is essential for the body's cells to function. When given in tablets, zinc may cause some, all or none of the side-effects listed below at the dose and duration used in the study.

	Frequent >20% of subjects	Occasional 2 - 20% of subjects	Rare Less than 2% of subjects

Serious			
Less Serious	Nausea & vomiting		
Minor	Indigestion		

10. Subject Safety and Data Monitoring:

Data Safety Monitoring Board (DSMB): The DSMB will consist of three experienced clinicians to exercise oversight of the safety of trial participants. It will be chaired by Dr. Willis Maddrey (Professor of Internal Medicine, UT Southwestern Medical Center), review periodic safety reports prepared by the trial biostatistician (Dr. Bruce Barton), request additional data/information (if necessary), and advise the trial Executive Committee regarding continuation/discontinuation of the study.

The External Medical Safety Monitor (EMSM, Dr. Maddrey) will closely monitor the incidence rates of all adverse events reported, whether serious or not, throughout the study, and will alert the DSMB if a trend is observed. A summary of all AEs and SAEs, a summary of all reports, and a coded list of all subjects who were terminated from the study due to study-related adverse events, will be included in reports submitted by the DMSB to the IRBs. An annual report will be submitted to the IRBs at all participating sites.

Clinical performance sites will report all fatal events, unanticipated problems and other serious adverse events and suspected adverse reactions to the Data Safety Monitoring Board (DSMB) and IRB by secure email **within 24 hours of first knowledge of the event**. Additionally, all current study data for that particular subject will be entered to allow for timely review by the EMSM, Dr. Willis Maddrey.

11. Procedures to Maintain Confidentiality:

Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep information confidential; however, this cannot be guaranteed.

Privacy of individuals: The privacy of individuals will be protected by using confidential surroundings for all communications, eliciting and honoring requests for the inclusion and exclusion of family members and partners and following the institutions' codes for ethical and professional conduct of research and patient-centered care.

Confidentiality of data: No personally identifiable information will be recorded outside the electronic medical record. Only those individuals with rights to access the patient's electronic medical record will know the identity of the study subjects. While no efforts at security are completely free of risk, this approach is such that the risk of accidental exposure is negligible.

12. Potential Benefits:

The potential benefits to every subject participating in the clinical trial are related to receiving optimal patient-centered care for their condition. This includes ensuring the mobilization of available resources for rehabilitation programs and the education of all involved patients and their families on the possible consequences of alcohol consumption, particularly in high risk sub- populations. While ideally occurring in the care of all patients with alcoholic hepatitis, the study team will provide patients with the personal healthcare navigation assistance that is not available to every individual.

There may be immediate additional benefits to those in the active study arms if the outcomes are favorable. If the outcomes are favorable, all patients with severe alcoholic hepatitis may benefit from extrapolation of the findings. If the outcomes are negative, other subjects will be protected from the risks of treatment that has no additional benefit to convey.

There is a potential societal benefit in the general outcomes of the clinical trial with respect to alcohol consumption, alcoholism and alcoholic liver disease. In addition, the potential financial benefits of a successful program are substantial.

13. Biostatistics:

Sample size for study of patients with moderate acute alcoholic hepatitis

To compare change from baseline in the two groups of moderate AAH patients, we find that a two-sided two sample t-test of change in MELD score from baseline (mean=17.0) to 30 days with an overall sample size of 108 patients (54 in each treatment group) achieves 80% power with an alpha level of 0.05 to detect a difference in mean change of MELD score of 5.0 in the control group compared to a mean change of 8.0 in the intervention (probiotic) group with a common estimated group MELD score standard deviation of 5.559.

Adjusting for a total of 20% withdrawal, lost to follow-up or non-evaluable, we have n* = 108/ (1- 0.20) = 108/.80 = 136 patients total or 68 patients in each treatment group. We expect that, in a multivariate model, we will have the power to detect smaller treatment group differences due to the partition of the variance among the predictors.

Statistical analysis plan: Our primary analysis of change in MELD score from baseline to 30 days will be an unadjusted comparison of change (Day 30 – Baseline) using a standard two- group t-test at a two-sided alpha level of 0.05 with appropriate adjustment for unequal variances. We will also conduct secondary analyses to further investigate factors that influence the change as well as adjust for any imbalances in patient characteristics and covariates between treatment groups. Our initial model will be an Analysis of Covariance (ANCOVA) model with change in MELD score as the outcome and with treatment group (0=control, 1=probiotic) and baseline MELD score as predictors. Additional models will include patient characteristics such as age, gender, time since diagnosis as well as other covariates such as ALT, AST, and other laboratory measures at baseline. Finally, as it is likely that MELD score can be calculated throughout the duration of the study, we will use mixed models to analyze the longitudinal MELD score data. Mixed (random effects) models are used to adjust for the inherent correlation among the laboratory (and, thus, MELD score) measures on the same patient. We will consider treatment as a fixed effect, but most other factors as random effects. The model will also include a time metric, so that, rather than the change from baseline, we can use the coefficients of the times as estimates of the change from baseline (time=0), adjusted for the other factors in the model. So, for example, if MELD score is calculated at Days 0, 7, 14, 21, and 30, the model might look like: $MELDt = \beta0 + \beta1*tx + \beta2*gender + \beta3*age + \beta4*Day7 + \beta5*Day14 + \beta6*Day21 + \beta7*Day30$ where MELDt = MELD score at time t (t=0,7,14,21,30), β0...β7 = regression coefficients, tx = treatment (0=control, 1=probiotic), gender = patient's gender (0=female, 1=male), age=patient's age at last birthday, Day7 = MELD score at Day 7 (0=No, 1=Yes), ..., Day30 = MELD score at Day 30 (0=No, 1=Yes). This implies that the baseline MELD score is represented by Day7=Day14=Day21=Day30=0. Interpreting this model, which makes maximum use of all available MELD scores, the coefficient β4 is the estimate of the change in MELD score from baseline to Day 7, β5 is the estimate of the change in MELD score from baseline to Day 14, etc. By combining all of the information into a single model, we can adjust the MELD score changes for other factors as well as test for a difference in the slopes of the changes between the two treatment groups. The model can, of course, be extended beyond 30 days if measures are available. The fit of the model will be assessed primarily through the likelihood ratio test of the fitted model to the intercept only model. All coefficients will be tested for significance at a critical level of p=0.05.

Sample size for study of patients with severe acute alcoholic hepatitis

Using the Lakatos approach(19) to estimating the sample size for a log-rank test, we find that a two-sided logrank test with an overall sample size of 130 patients (65 patients each of the control and treatment groups) achieves 80.0% power at a 0.05 significance level to detect a hazard ratio of 0.4447 when the proportion surviving at 6 months in the control group is 0.64 and 0.82 in the treatment group. The total study duration is 2.5 years of which subject accrual (entry) occurs in the first 2 years. We assume that the accrual pattern is uniform throughout the accrual period and that the proportion drop-out in each group is 0.10 during the 6 months with no treatment cross-overs. As a sensitivity analysis of these estimates, we used the Lachin- Foulkes approach[(20)], a slightly less flexible approach and, with the same assumptions as above, we estimate 53 patients in each group, reasonably close to the Lakatos estimates. Thus, to be conservative in our estimates, we will set our

sample size as 130 patients total or 65 patients in each treatment group. PASS II (NCSS, Kaysville, Utah) was used for all sample size calculations[21].

Statistical analysis plan: Our primary analysis of this outcome will be the unadjusted log-rank test of the Kaplan-Meier survival estimates for the two treatment groups at a two-sided alpha level of 0.05 with survival censored at 6 months. Secondary analysis will use a Cox proportional hazards regression model (with survival censored at 6 months) to adjust the treatment difference in survival for patient characteristics (e.g., age, gender, time since diagnosis) and other covariates (e.g., ALT, AST, other lab measures). Predictors will be added to the model using reference cell coding, as with the mixed models above. The Cox model generates estimates of the hazard ratio for each factor compared to the reference level; e.g., for male patients vs. female patients, assuming gender = 1 for male and 0 for females, a hazard ratio of 1.25 indicates that males have a 25% higher probability of death overall by 6 months compared to females. We can also add time-dependent covariates to the model, such as ALT levels at 2 and 4 months, to estimate their predictive value for mortality. To assess the fit of the model, we will use Nagelkerke's R2 constructed from the ratio of the likelihoods for the fitted model to the intercept only model. As further secondary analysis, we can eliminate the survival censoring

at 6 months and test the overall survival throughout the duration of follow-up.

General analysis plan for experiments with fold change as the outcome: Initial analyses will be descriptive in nature, allowing for investigation of appropriate normalization approaches. Initial comparative analyses of fold-change between treatment groups (or between other groups of interest) will be through a standard two-group t-test at the p=0.05 level with appropriate adjustment for unequal variances. If the fold change data are longitudinal in nature, we will use mixed (random effects) models to estimate the change in fold-change over time. In these models, fold-change at each time point will be the outcome with treatment (or other group indicator) and time as predictors. Additional patient characteristics can be added to the model to create adjusted estimates of treatment (or other group) differences. Mixed models can adjust for the correlation due to measures within patients and due to repeated microarrays at each time point.

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