ALCHEPNET CONSORTIUM CLINICAL STUDY PROTOCOL

Protocol Title

A multicenter, randomized, double blinded, placebo-controlled clinical trial of Anakinra (plus zinc), or prednisone in patients with severe alcoholic hepatitis by the AlcHepNet Consortium

Protocol Number: AlcHepNet – 02 IRB: 20190963

IND: 144031

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Primary Investigators at each site: Indiana University in Indianapolis, Indiana, (PI: Dr. Samer Gawrieh (IND holder) and Dr.Kavish Patidar), **University of Louisville** in Louisville, Kentucky (PI: Dr. Craig McClain and Dr.Vatsalya Vatsalya), **Beth Israel Deaconess Medical Center** in Boston Massachusetts (PI: Dr. Gyongyi Szabo), **Mayo Clinic** in Rochester, Minnesota (PI: Dr. Vijay Shah and Dr. Patrick Kamath), **Cleveland Clinic Foundation** in Cleveland, Ohio (PI: Dr. Srinivasan Dasarathy), **University of Pittsburgh Medical Center** in Pittsburgh, Pennsylvania (PI: Dr. Ramon Bataller), **University of Texas Southwestern Medical Center** in Dallas, Texas (PI: Dr. Mack Mitchell), and **Virginia Commonwealth University** in Richmond, Virginia (PI: Dr. Arun Sanyal).

Disclosure

This study is conducted by the AlcHepNet consortium which is funded by the National Institute of Alcohol Abuse and Alcoholism (NIAAA) to pursue translational investigations in Alcoholic hepatitis (AH). The AlcHepNet Consortium is made up of investigators from Indiana University (Indianapolis, IN), Mayo Clinic (Rochester, MN), Virginia Commonwealth University (Richmond, VA), University of Pittsburgh (Pittsburgh, PA), University of Louisville (Louisville, KY), Beth Israel Deaconess Medical Center (Boston MA), Cleveland Clinic Foundation (Cleveland, OH), and University of Texas Southwestern (Dallas, TX).

SPONSOR'S approval of the protocol reviewed and approved by:

1/31/2022

Samer Gawrieh, MD
Primary Investigator (Indiana University)

Date

Institutional Signing Official (If Applicable)

Date

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1. ETHICAL CONDUCT OF THE STUDY AND REGULATORY REQUIREMENTS

1.1 Institutional Review Board (IRB)

The study protocol and any amendments will be reviewed by the Western Institutional Review Board (WIRB). The IRB will review the participant safety, informed consent form (ICF), their updates (if any), and any written materials given to the participants.

1.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, Good Clinical Practice (GCP) and applicable regulatory requirements.

1.3 Participant Information and Consent

The investigator will obtain a freely given written consent from each participant after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the study that are relevant to the participant's decision to participate. In appropriate language, the consent form must be signed and dated by the participant before he/she is exposed to any protocol-specific procedure.

The investigator will explain that the participants are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify.

The participant will receive a copy of the signed informed consent.

The participant will be told if information becomes available that may be relevant to his/her willingness to continue participation in the study.

Each participant will be informed that a monitor or a health authority inspector, in accordance with applicable regulatory requirements, may review the portions of their source records and source data related to the study. Data protection and confidentiality will be handled in compliance with federal, state, local and university guidelines.

2. EXECUTIVE SUMMARY

Title of Trial

A multicenter, randomized, double blinded, placebo-controlled clinical trial of Anakinra (plus zinc) or prednisone in participants with severe alcoholic hepatitis by the AlcHepNet Consortium.

Name of Active Ingredient(s)

a. Anakinra
b. Prednisone
c. Zinc-Sulfate

Pharmacological Class of the Drugs

a. Anakinra is immunological agent, an inhibitor of the Interleukin-1 receptor (IL-1).
b. Prednisone is an adrenal glucocorticoid.
c. Zinc-sulfate is a nutritional supplement.

Indication

Severe alcoholic hepatitis.

Study Type

Phase 2B multicenter randomized, 2-arm, double blinded, placebo controlled clinical trial.

Investigational Sites

Indiana University, Mayo Clinic, Virginia Commonwealth University, Cleveland Clinic Foundation, University of Louisville, Beth Israel Deaconess Medical Center, University of Pittsburgh Medical Center, and University of Texas Southwestern Medical Center

Planned Number of Participants

Approximately 258 participants will be randomized in a 1:1 ratio into 1 of 2 treatment arms (129 participants per arm): (1) Prednisone + Placebo for Anakinra/Zinc (2) Anakinra + Zinc + Placebo for Prednisone.

Objectives

This multicenter, randomized, double blinded, placebo-controlled clinical trial is focused on novel treatments for severe alcoholic hepatitis (AH), a life-threatening stage of alcoholic liver injury that has a short-term mortality rate much higher than that of other liver diseases.

The primary objective of the study is to determine the clinical efficacy and safety of Anakinra (plus zinc) compared to the current standard medical treatment consisting of prednisone in participants with clinically severe AH. Key secondary objectives broadly are as follows: (a) to evaluate the use of biomarkers to assess disease severity and treatment response; and (b) to develop novel endpoints to overcome the limitations of current assessment strategies for severe AH.

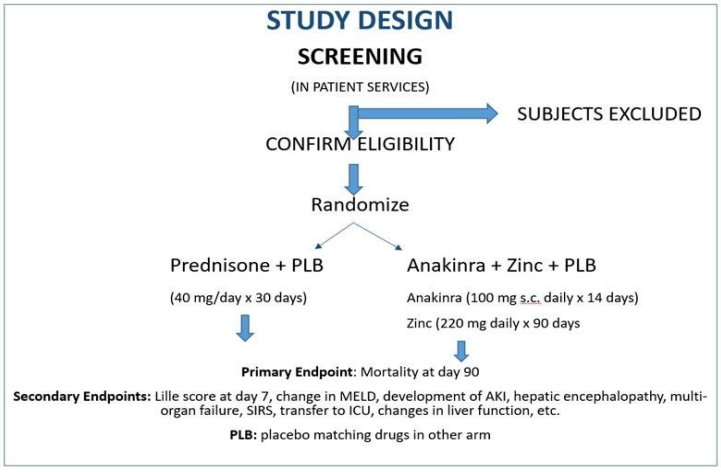
Overview of Study Design and Conduct

The proposed study is a Phase 2b, multicenter, prospective, randomized, 2-arm, double blinded, placebo-controlled clinical trial to assess the efficacy of Anakinra, an interleukin (IL)-1 receptor antagonist plus zinc compared to prednisone for the treatment of severe AH (Model for End Stage Liver Disease (MELD) score $\geq$ 20). All participants will receive standard care for treatment of severe AH as described in recent guidelines endorsed by American Association for the Study of Liver Diseases (AASLD) and American Gastroenterological Association (AGA). The study will be conducted at 8 clinical sites across the United Stated selected by the National Institute of Alcoholic Abuse and Alcoholism (NIAAA) and will be supported by a Data Coordinating Center at Indiana University (DCC-IU). The biorepository will be managed by University of Massachusetts.

The study will be conducted according to Good Clinical Practice (GCP) and in compliance with local, state, and federal regulatory requirements. Adverse events during the course of the trial will be identified, recorded, assessed for causality, and reported in accordance with FDA guidance. In addition to general assessment, we will specifically focus on (1) rates and types of infection as well as their severity, (2) potential development of drug-induced liver injury, (3) injection site reactions, and (4) hematological adverse events.

This study will be approved by an appropriately convened single IRB, Western Institutional Review Board (WIRB), and will be monitored by an NIAAA appointed Data and Safety Monitoring Board (DSMB). We will conduct this study under an Investigational New Drug (IND) application from the United States Food and Drug Administration (FDA).

It is anticipated that a centrally located investigational pharmacy located at Indiana University will dispense the study medications to all participating sites under close coordination from the DCC-IU. Anakinra plus zinc, prednisone and matching placebos will be provided by the study to the participants.



Interventions to be tested

Concomitant treatments in this trial include standard care for severe AH for all participants as recommended in recent guidelines[1]. The standard of care for severe AH includes attention to early diagnosis and treatment of bacterial and fungal infections, portal hypertension and its complications including acute kidney injury, gastrointestinal bleeding, fluid overload (ascites and edema) and hepatic encephalopathy.

In addition, all participants will be offered as part of the standard of care, non- pharmacologic intervention for treatment of alcohol use disorders, such as cognitive behavioral therapy and 12-step programs intervention as aligned with the best practices at the participating study site.

Participants with severe AH are very sick and often have multiple active medical problems requiring intervention. The use of specific interventions during this time has the potential to modify treatment response. Ideally, these interventions would be completely standardized. However, the standard of care varies by region, institution and the specialties involved in the care of the participant. The decision regarding treatments for these complications will be delegated to the primary physicians caring for the participants during hospitalizations and to the investigators during the ambulatory follow-up of the participants. Other than the general guidelines noted, no specific treatments for specific complications such as those noted are required. However, such treatments might include, but are not limited to, the administration of IV fluids, intravenous human serum albumin, antibiotics including antifungal medications, renal replacement therapy, supplemental oxygen, assisted ventilation and intravenous pressors. Diagnostic procedures such as paracentesis, upper endoscopy, colonoscopy, endoscopic ultrasound, and liver biopsy will be permitted as required to provide standard care.

During the initial six months of trial, the investigators will develop a "manual of clinical operations" that will include best practices on the use of vasopressors, albumin, ICU care, mechanical ventilation and renal replacement therapy using Surviving Sepsis Campaign, International Guidelines for Management of Severe Sepsis and Septic Shock. The investigators will also provide best practices for management of nutrition and alcohol counseling and minimization of recidivism. Although the management of these complications will not be mandated by protocol, the care provided will be recorded to develop novel designs for future trials and for practice guidance and quality improvement programs.

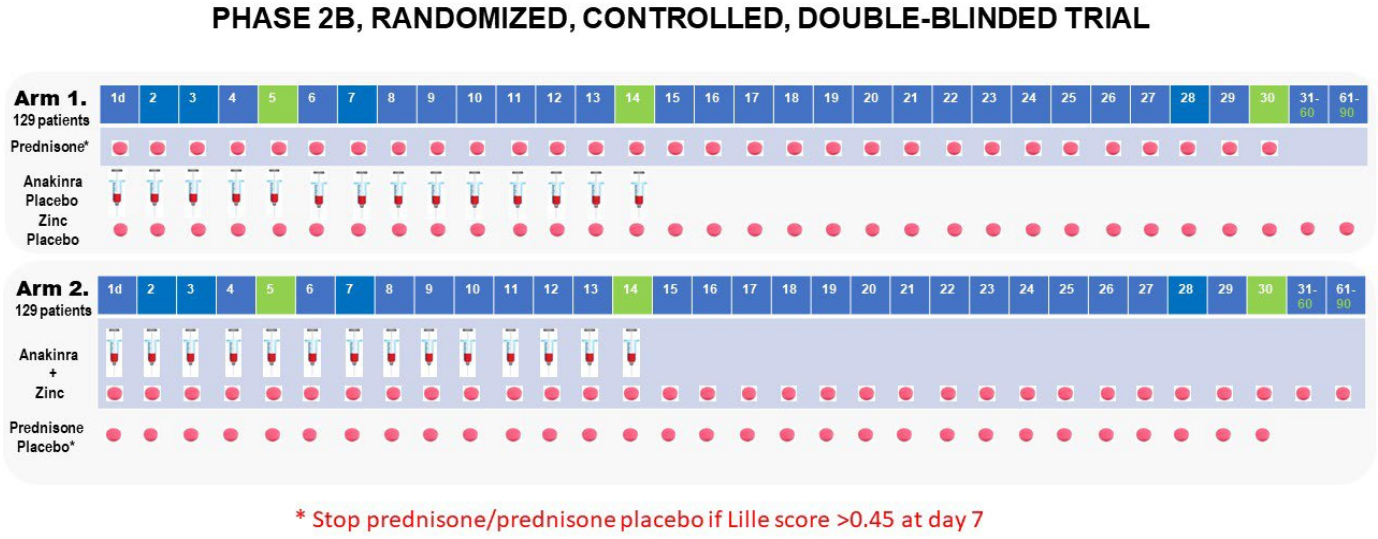
After informed consent has been obtained and all of the inclusion are met and none of the exclusion, participants will be randomized to receive one of the following interventions in addition to standard care.

Group 1: Standard of care **plus** prednisone 40 mg orally once daily on Days 1-30 and matching placebos for Anakinra (1 syringe s.c. once daily on Days 1-14), and zinc (matched pill once daily on Days 1-90).

Group 2: Standard of care **plus** Anakinra (100 mg s.c.) once daily on Days 1-14 zinc sulfate 220 mg once daily on Days 1-90, and placebo for prednisone (matched pill once daily on Days 1-30).

Lille score will be calculated from day 7 labs, participants will stop prednisone or prednisone placebo if Lille score >0.45.

Figure 1. Distribution of study drug and placebo



Participants or guardian will be instructed in subcutaneous injection technique to continue self-administration of these experimental treatments after discharge from the hospital. Study personnel will ensure participants and guardian are proficient in the technique before the end of study visit and an instructional document will be provided.

Primary and Important Secondary Endpoints

Primary Endpoints Survival at 90 days

Secondary Endpoints

- **Changes in score(s):** Changes in Lille score, change in MELD score, development of AKI, multi-organ failure, SIRS, transfer to ICU, changes in liver function will all be evaluated at 7, 30 and 90 days.
- **Organ dysfunction:** Measure of changes in Sequential Organ Failure Assessment (SOFA) scores and proportions requiring hemodynamic support for MAP < 65 mm Hg and lactate > 2 mmol/l, renal replacement therapy or mechanical ventilation. The SOFA score will be modified and re-evaluated without platelet counts given that these are usually low in AH.
- **Infections and Sepsis:** The endpoints and case definitions related to sepsis may vary depending on what will be used (clinical care, research, surveillance or quality improvement). Given the central role of sepsis as a driver of outcomes, measuring the types of infection will occur but also identify the proportions of those with sepsis, septic shock or quick-SOFA (qSOFA) criteria based on SEPSIS-3 guidance[2,3]. Key parameters will be captured and needed for various sepsis related endpoint construction aligned by the recent guidance from the European Drug Development Hub (http://eddh-cro.wixsite.com/fdtsfv).
- **Renal dysfunction:** In alignment with guidance from the acute disease quality initiative, the AKI development will be quantified and its progression through persistent AKI, acute kidney disease to chronic kidney disease.
- **Need for care escalation:** Proportion of participants requiring transfer to ICU for care, intubation for airway control, need for ventilator support or RRT.
- **Indicators of gut permeability**: (endotoxin and bacterial 18S DNA) and pro-inflammatory cytokine/chemokines (TNFa, MCP1, IL-6, IL-1ß) will be assessed in serum/plasma samples.
- **Survival at 30 days and 180 days**

Limitations of mortality as an endpoint in AH and plans to overcome these

All-cause mortality is most relevant when mortality is substantial and attributable to a single mechanism in a homogeneous population. Participants with severe AH are highly heterogeneous with respect to disease severity, end organ involvement and the care they receive. Moreover, as in sepsis and heart failure, death involves failure of multiple organs (liver, heart, kidney, lungs) making it difficult to ascertain which one is the primary cause of death. This has led to development and regulatory acceptance of composite endpoints including mortality and key parameters driving mortality for acute heart failure trials. These guiding principles to construct a novel and innovative key secondary composite endpoint which will include death or worsening of the modified SOFA score[4,5] by ≥2 points and worsening of MELD score by ≥2 points. These are associated with mortality and capture the organ recruitment that marks progression of AH towards death. This endpoint will also be validated with respect to reliability, construct-, criterion- and content validity to establish this as a primary endpoint in future trials. Model changes in SOFA scores and MELD scores against mortality to further optimize this endpoint and cross-validate it in the observation cohort will occur.

Novel (experimental) endpoints to overcome limitations of conventional endpoints Efficacy endpoints must capture "clinically meaningful benefit". While traditional endpoints such as mortality are meaningful, they also have multiple shortcomings in the context of AH as noted below. Innovation will be used to construct, measure, and validate novel additional endpoints to overcome the limitations of currently used endpoints and cross-validate them in the observational cohort. The combined expertise in endpoint development and the use of harmonized measurements will make this feasible. These will provide novel assessments in AH and provide the scientific evidence-base to use these as primary endpoints in future trials.

Inclusion Criteria

1. AH, as defined by the NIAAA pan-consortia for AH[6]:

a) Onset of jaundice *(defined as serum total bilirubin >3mg/dL)* within the prior 8 weeks to screening visit

b) Regular consumption of alcohol with an intake of > 40 gm daily or >280gm weekly on average for women and > 60 gm daily or >420gm weekly on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice

c) AST > 50 IU/l

d) AST: ALT > 1.5 and both values < 400 IU/l

e) and/or histological evidence of AH*

2. MELD 20-35 on day of randomization.

3. Ages ≥ 21

** In patients with possible AH or AH with confounding factors such as possible ischemic hepatitis, possible DILI, uncertain history of alcohol use (e.g., patient denies excessive alcohol use), and atypical/abnormal laboratory tests (e.g., AST < 50 IU/L or > 400 IU/L, AST/ALT ratio < 1.5), antinuclear antibody > 1:160 or SMA > 1:80, a standard of care liver biopsy may be performed during current hospital admission to confirm AH and exclude competing etiologies [17]*

Exclusion Criteria

1. MELD SCORE <20 or > 35
2. Active sepsis (positive blood or ascitic cultures) with Systemic Inflammatory Response Syndrome (SIRS) or hemodynamic compromise requiring intravenous pressors to maintain tissue perfusion
3. Pneumonia as evidenced by radiological exam
4. Multi-organ failure
5. Renal failure defined by GFR <35 mL/min by CKD- EPI.
6. Clinically active C. diff infection
7. History of imaging of the liver (ultrasound, computerized tomography, or magnetic resonance) showing other causes of jaundice
8. History of other liver diseases including hepatitis B (positive HBsAg or HBV DNA), hepatitis C (positive HCV RNA), autoimmune hepatitis, Wilson disease, genetic \hemochromatosis, alpha1-antitrypsin deficiency, or strong suspicion of Drug Induced Liver Injury (DILI). Previously treated hepatitis C that was cured (sustained virological response with negative RNA ≥24 weeks following treatment) is not an exclusion.
9. History of HIV infection (positive HIV RNA or on treatment for HIV infection)
10. History or presence of cancer (including hepatocellular carcinoma) other than non-melanoma skin cancer
11. History of other significant medical problems such as autoimmune diseases, severe asthma, psoriasis, Inflammatory Bowel Disease (IBD), etc. that might require immunosuppressive treatments
12. Pregnancy or breastfeeding
13. Prior exposure to experimental therapies in last 3 months
14. Prior exposure to systemic corticosteroid (glucocorticoid) or immunosuppressive therapy for more than 4 days within previous 30 days
15. Need for inotropic pressor support to maintain perfusion to critical organs within prior 48 hours before randomization and initiation of experimental treatment
16. Clinically significant pancreatitis- abdominal pain, elevated lipase (> 3 X ULN) and at least edema of pancreas with fat-stranding on CT scan
17. Total WBC count > 30,000/mm3
18. Known allergy or intolerance to therapeutic agents to be tested
19. Inability to voluntarily obtain informed consent from participant or guardian
20. Perceived inability to follow study procedures and comply with protocol
21. Platelet count < 40,000 k/cumm.
22. Positive PCR test for COVID -19 within 7 days prior to the baseline day 0 visit*
23. Active gastrointestinal bleeding defined as hematemesis or melena with a decrease in hemoglobin more than 2 g/dl in 24 hrs. Due to gastrointestinal bleeding, or with a decrease in mean arterial BP to < 65 mmHg

Positive PCR test for COVID-19 is exclusionary only during screening period. If a patient tests positive any time after baseline randomization, a positive PCR test for COVID-19 will be considered as a SAE.

The participants for this study will be recruited from a hospitalized population of participants meeting the eligibility criteria outlined above who live within one day travel from one of the

participating clinical centers and who have provided informed consent to participate in this clinical trial and who are willing to continue their participation for the anticipated follow-up period of the trial. Although the primary endpoint is survival at 90 days, follow-up visits will be continued up to 6 months.

Study Visits and Data Collection Schedule***

	Screening	Treatment Phase							Follow-up phase
		D0	**D3**	**D7**	**D14**	**D28**	**D60**	**D90**	**D180**
Window (days)		±2	±2	±2	±2	±7	±7	±7	±7
Informed consent	x								
History and physical	x	x	x	x	x	x	x	x	x
Vital signs, weight ***	x	x	x	x	x	x	x	x	x
Alcohol consumption history	x	x			x	x	x	x	x
Randomization		x							
Concomitant medicines	x	x	x	x	x	x	x	x	x
Dispense Study Drug		x		*					
Adverse events			x	x	x	x	x	x	x
Electrocardiogram*****	x								
Evaluation of compliance			x	x	x	x	x	x	
CBC, PT/INR, Hepatic Function Panel, BMP	x	x	x	x	x	x	x	x	x
Test for COVID-19*****	x								
Sepsis studies, as indicated	x	x	x	x	x	x	x	x	x
Pregnancy test, serum**	x								
Pregnancy test, urine**		x			x	x	x	x	x
Blood collection for trough Anakinra levels		x	x	x	x				
Specimen Banking¶		x		x	x	x	x	x	x
Saliva Banking+		x						x	
Questionnaires δ	x					x	x	x	x

¶Specimen banking includes blood and stool (when possible) D0, D7, D14, D28, D60, D90, D180, and, when available, urine and liver biopsy/tissue. Blood will be used to extract germline DNA at baseline and at Day 180 as well as to extract serum/plasma/PBMC at all visits. *Type of specimen collection is site specific.*

+ Saliva collection at D0 and D90. Collected until an adequate number of samples are collected.

δ Questionnaires include Alcohol Use Disorders Identification Test (AUDIT), Alcohol Timeline Follow Back (TLFB), and Chronic Liver Disease Questionnaire (CLDQ). The timeline follow back questionnaire will be the only questionnaire administered at days 28, 60, 90, and 180. All are given at screening.

*****Day 7 study drug dispensation will be unscheduled and only on an as-needed basis, based on safety lab evaluations **(outpatient only.)**

****** if applicable

******* height at screening only.

******** Test for COVID-19 PCR only if not done as SOC with 7 days prior to the baseline Day 0 visit.

********* In response to COVID-19, we are lessening restrictions if applicable or necessary such as: allowing wider windows D14, D28, D60 to +/- 10 days. Allowing replacement of protocol mandated in-person study visits with one or more of the following, phone calls, telemedicine virtual visits, implement digital technology to record responses to questionnaires. Lastly, allowing blood draws at remote or commercial laboratories

********ECG can be done as standard of care within 7 days of screening**

Abbreviations: CBC: Complete blood count, BMP: Basic metabolic, INR: International normalized ratio and PT: Prothrombin Time.

Data Collection

Clin= clinical (relevant elements of history, physical exam), Biosamples: whole blood, plasma, serum, PBMC, DNA, and stool (when possible) at all time-points, liver biopsy/tissue and urine (20ml) if available, Questionnaires= AUDIT, alcohol timeline follow back, CLDQ, SOC (standard of care) labs= CBC, Hepatic Function Panel, BMP: Basic Metabolic Panel, PT/INR, Sepsis studies at baseline and during trial duration, as indicated (blood cultures, chest X ray, stool for WBC and C. Diff as indicated, ascites tap, UA with culture, liver ultrasound or other imaging, endoscopy if available within 6 months).

Relevant clinical care parameters will be recorded on days specified. As noted above, attention will be paid to recording concomitant care provided to treat complications related to AH and/or portal hypertension including those treatments not specified by protocol, including but not limited to antibiotics, IV fluids, albumin, pressors, renal replacement, etc.

Standardizing study conduct and good clinical practice (GCP)

Variations in protocol implementation and clinical care can have a major impact on trial outcomes. This will be optimized by: (1) GCP training and certification of all study personnel, (2) investigator/coordinator training and certification prior to site activation, (3) monthly performance reports, (4) development of manual of operations that provide best practices, and (5) annual investigator meeting to review developments and clinical and trial practice patterns. (6) DSMB will convene every 6 months to ensure relevant study procedures are followed.

Study Population

The study population will be comprised of men and women of all races, ethnic groups and socioeconomic status of the ages of 21 and greater admitted to the hospital with a clinical diagnosis of AH. A full description of the statistical analysis and calculation of sample size is recorded below. The definition of AH is established by the NIAAA pan-consortia for AH. A conscious decision not to require a liver biopsy for inclusion was made based on the current low rate of liver biopsy used to establish this diagnosis in the US and the ability to diagnose the condition with relative accuracy based on the experience in the first cycle of funding. Eligible participants will be identified based on inclusion/exclusion criteria as defined above.

Enrollment sites

Enrollment sites will be limited to the 8 clinical sites selected during the competitive renewal of the U01 study for treatment of acute AH. These enrollment sites include: Indiana University School of Medicine affiliated hospitals in Indianapolis, Indiana (PI: Dr. Kavish Patidar), University of Louisville affiliated hospitals in Louisville, Kentucky (PI: Dr. Craig McClain), Beth Israel Deaconess Medical Center in Boston, Massachusetts (PI: Dr. Gyongyi Szabo), Mayo Clinic in Rochester, Minnesota (PI: Dr. Vijay Shah and Patrick Kamath), Cleveland Clinic Foundation in Cleveland, Ohio (PI: Dr. Srinivasan Dasarathy), University of Pittsburgh Medical Center in Pittsburgh, Pennsylvania (PI: Dr. Ramon Bataller), University of Texas Southwestern Medical Center in Dallas, Texas (PI: Dr. Mack Mitchell), and Virginia Commonwealth University in Richmond, Virginia (PI: Dr. Arun Sanyal). Each site will be permitted to use more than one hospital or other healthcare facility to recruit participants based on the inclusion/exclusion criteria.

Expected enrollment per site**:** 32-33 severe AH participants

Central IRB: The DCC-IU with WIRB as the central IRB for this study. This will require that IRBs from all 8 participating centers establish the contractual agreements with WIRB.

Statistical Design and Power

The primary analysis will be comparisons of 90-day mortality of Anakinra plus zinc vs Prednisone using survival analysis. Mortality event times greater than 90 days will be censored at 90 days or at time of liver transplant, whichever comes first. The experiment-wise significance level will be 5%. The most recent data from the AlcHepNet consortium suggest a 90-day mortality rate for prednisone is approximately 20%. We estimate that with a total of 258 participants (129 per treatment arm), we will be able to detect a clinically significant hazards ratio of 0.325, which translates to a 13% reduction in 90-day mortality, i.e., 80% survival in prednisone vs 93% in anakinra plus zinc groups with 85% power by using two-sided logrank tests. We use O'Brien and Fleming α-spending function to determine the efficacy stopping boundaries for the sequential tests. Specifically, if the test statistics of the log-rank test is greater than 2.963 or less than -2.963, we will claim a significant difference between the two treatments and stop the trial. Otherwise, we will continue enrolling more patients until the full sample size is reached and then the final analysis will be performed. For the final analysis, if the test statistic value is greater than 1.969 or less than -1.969, we will claim a significant difference between the treatments. Otherwise, we will declare that no statistically significant difference is detected.

Besides the group sequential tests, we have in place an early stopping rule for futility based on conditional power. At the time of interim analysis, we will calculate the conditional power, i.e., the probability of claiming difference at the end of the trial given the data at the interim analysis. We will stop the trial for futility when the conditional power is less than 0.1. In other words, based on data that become available at the interim analysis, assuming that the current trend continues, we should

early stop the trial if the probability of declaring anakinra + zinc superior in the final analysis is low (e.g., less than 10%). We use this early stopping rule to limit patient exposure to a potentially inefficacious treatment.

Group Assignment

Participants will be randomized to the two treatment groups with matching placebos for drugs in the other arm, in equal proportions. Randomization will be stratified by site and MELD score. Random blocks of size 4 will be generated to ensure treatment assignments are balanced between the two treatment arms within each block. Both participants, coordinators, and investigators will be blinded to the randomization plans.

Statistical Analyses

The primary analyses of 90-day mortality will be performed in an Intention-to-Treat (ITT) framework using survival analysis. Specifically,

(1) we will use all-cause mortality in the first 90-days as the primary outcome event of interest. We will compare the distributions of time to mortality between the two treatment arms. In the primary analysis, mortality event times greater than 90 days will be censored at the 90 days or at the time of liver transplant, whichever comes first. We will use the Logrank test to compare the survival functions of the two treatment groups. Cox regression analysis can be performed if it is necessary to control for the influences of unbalanced patient characteristics, such as MELD score, between the treatment groups. If evidence suggests a violation of the hazard function proportionality, we will conduct an analysis of the restricted mean survival time (RMST) of the first 90 days.
(2) as a secondary analysis, we will compare transplant-free survival in the first 90 days of treatment initiation as between the two treatment arms, as done in other clinical trials. We will perform logrank test, Cox regression, and RMST analyses as appropriate.
(3) we plan to conduct one interim analysis and one final analysis for this trial. The interim analysis will be conducted when **129** patients, half of the enrollment number, complete the 90-day follow-up. We use the group sequential method to control the type 1 error rate of the trial.
(4) as another secondary analysis, we will examine the treatment effects in patients with baseline MELD scores ≤25 and >25 by assessing the equality of treatment effects of the anakinra in high and low MELD groups, we will test the interaction between the intervention indicator and baseline MELD score in the Cox regression model.

Subject Participation Duration

180 days

Recruitment and Retention Plan

Sources of participants:

Identification of participants with severe AH requires collaboration with those physicians who manage these participants within the hospital and the emergency department. Almost 100% of participants are admitted to hospital based on the severity of their liver disease. Occasionally participants are seen in free-standing urgent care centers, free-standing emergency rooms or in office/clinic practices. Since the standard of care is to admit these participants for further evaluation to exclude infections as a precipitating cause of decompensated liver disease, most will ultimately be identified in a hospital setting. Letters will also be sent to referring MDs and referring hospitals.

Some sites in the current U01 have found that screening laboratory results for elevated AST and total bilirubin is a useful way to identify potential participants. In accordance with Institutional Review Board Policies, once identified the primary team caring for these participants must be contacted by the investigators and agree to ask the participant about their interest in participating in a clinical trial as part of the treatment of the underlying AH. Once the participant expresses willingness to be contacted the investigators may discuss details of the study and obtain informed consent prior to beginning treatment.

Management of barriers for recruitment:

Special conferences with medicine, ICU and ER staff will increase awareness of the trial~~,~~ and its significance. Contact information for study personnel will be made available so that they can meet with participants and their family to educate them about AH and the trial. For those who live at a distance, travel costs will be budgeted in alignment with local practices for other trials.

Contingency plans:

Recruitment will be tracked with monthly review of expected versus actual enrollment. Screen fail rates will also be assessed. There will be direct communication between DCC-IU, NIAAA and study leadership with site-PI and staff to better understand the barriers for recruitment and develop/implement solutions. Best practices from high-performing sites will be shared with the under-performing sites. For continued failure, the site budgets will be cut, and funds redirected towards supporting enrollment at high performing sites after discussion with NIAAA project officer.

Retention plan:

It is anticipated that some participants will drop out after the initial hospitalization. This will be minimized by participant education, frequent communication by study coordinator and provision of ways for participants and their family to communicate with the study team. Travel support will be provided when needed.

Competition from other trials:

The participating clinical centers will not engage in other trials for AH that have similar eligibility criteria for the duration of the trial.

Safeguards**:**

Each clinical center will establish safeguards for vulnerable populations, especially institutional employees, and their families, in close collaboration with their institutional review boards.

Investigator's Affirmation

I have received and read the current version of the Investigator's Brochure (IB) for Anakinra, zinc, and prednisone and this protocol AlcHepNet - 02. Having fully considered all the information available, I agree that it is ethically justifiable to give Anakinra, zinc, and prednisone to selected participants/participants according to this protocol.

I understand that all information concerning Anakinra, zinc, and prednisone supplied to me by the AlcHepNet Consortium and from SOBI Pharmaceuticals and not previously published is confidential. This includes the IB, Clinical Trial Protocol, Case Report Forms (CRF) and any other preclinical and clinical data.

I understand that no data are to be made public or published without prior knowledge and written approval by the AlcHepNet Steering Committee.

By my signature below, I hereby attest that I have read, understood and agreed to abide by all the conditions, instructions and restrictions contained in protocol AlcHepNet - 02 and in accordance with Good Clinical Practice (CPMP/ICH/135/95), 21CFR Part 312 and all applicable regulatory requirements.

I acknowledge that the Steering Committee of the AlcHepNet Consortium has the right to discontinue this trial at any time.

_____

Investigator's Name (Printed)

_____ _____

Investigator's Signature Date

Abbreviations

Abbreviation	Definition
6-ECDCA	6α-ethyl chenodeoxycholic acid
AASLD	American Association for the Study of Liver Disease
AAH	acute alcoholic hepatitis
AE(s)	adverse event(s)
AGA	American Gastroenterological Association
AH	alcoholic hepatitis
AKI	acute kidney injury
ALT	alanine aminotransferase
ALP	alkaline phosphatase
ANR	anakinra
AST	aspartate aminotransferase
AUDIT	alcohol use disorders identification test
BL	baseline
BP	blood pressure
BAS	bile acid sequestrants
BUN	blood urea nitrogen
CBC	complete blood count
CDCA	chenodeoxycholic acid
C.Diff	clostridium difficile
CFR	code of federal regulations
CLDQ	chronic liver disease questionnaire
CMP	complete metabolic panel
C_{max}	maximum concentration
CRA	clinical research associate
COVID-19	Coronavirus disease 2019
CRF	case report form
CT	computerized tomography
DCC	data coordinating center
DCA	deoxycholic acid
DCC-IU	data coordinating center-Indiana University
DILI	drug induced liver injury
dL	deciliter(s)
DSMB	data and safety monitoring board

ECG	electrocardiogram
ER	emergency room
FDA	Food and Drug Administration
FGF-19	fibroblast growth factor-19
FXR	farnesoid X receptor
GGT	gamma-glutamyltransferase
GMP	good manufacturing practice
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HBV DNA	hepatitis B Virus deoxyribonucleic acid
HCV RNA	hepatitis C virus ribonucleic acid
HDPE	high-density polyethylene
HIV	human immunodeficiency virus
HIV RNA	human immunodeficiency virus ribonucleic acid
IB	investigator's brochure
IBD	inflammatory bowel disease
ICF	informed consent form
ICH	International Conference on Harmonization
ICU	intensive care unit
IgM	immunoglobulin M
IL-1	interleukin-1
IL-1β	interleukin-1beta
IL-6	interleukin-6
IND	investigational new drug
INR	international normalized ratio
IRB	institutional review board
ISF	investigator site file
ITT	intention to treat
IU/L	international units per liter
IV	intravenous
LTSE	long term safety extension
MAP	mean arterial pressure
MCP1	monocyte chemoattractant protein-1
MELD	model for end stage liver disease
mg	milligram(s)

mL	milliliter(s)
mm Hg	millimeter of mercury
mmol/l	millimoles per liter
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NDB	nutrition database
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIH	National Institute of Health
PBC	primary biliary cirrhosis
PI	principal investigator
PLB	placebo
PK	pharmacokinetic(s)
PROMIS	patient reported outcomes measurement system
qSOFA	quick-sequential organ failure assessment
SAE	serious adverse event
SIRS	systemic inflammatory response syndrome
SOC	standard of care
SOFA	sequential organ failure assessment
SUSAR	suspected, unexpected serious adverse reaction
SAP	statistical analysis plan
TEAE(s)	treatment-emergent adverse event(s)
TIPS	transjugular intrahepatic portosystemic shunt
TNF-α	tumor necrosis factor-alpha
TNF-β	tumor necrosis factor-beta
UA	urinary analysis
UDCA	ursodeoxycholic acid
U/L	units per liter
ULN	upper limit(s) of normal
US	United States (of America)
vs	versus
WBC	white blood cell
WIRB	Western Institutional Review Board

2.1 Introduction and Rationale- Anakinra, Prednisone and Zinc in Severe AH

2.1.1 Clinical Experience

Anakinra, prednisone and zinc used in this trial have FDA-approved labeling for other indications. FDA-approved labeling is included in the Investigator's Brochure.

1. Anakinra is indicated for reduction in signs and symptoms and slowing the progression of structural damage in moderately to severely active rheumatoid arthritis. It has been previously studied in AH[7].
2. Zinc-sulfate is a nutritional supplement. Zinc supplementation reverses the clinical signs of zinc deficiency in participants with alcoholic liver disease.
3. Prednisone is indicated for numerous conditions including inflammatory disease. Corticosteroids, such as prednisolone, are considered standard of care in alcoholic liver disease.

2.1.2 Status of Drugs in Other Countries

Anakinra, zinc sulfate, and prednisone are licensed in numerous other countries and have not been withdrawn.

2.1.3 Pharmacology and Toxicology Information

Drug Interactions:

There are no drug interactions listed in MicroMedex or PubMed with the other agents given concurrently in the protocol –Anakinra + zinc sulfate (current as of 23 Jan 2019). Prednisone is given as the single study agent in arm 1. The investigator at each site will check for drug interactions between study drugs and other concurrent medications for each participant.

2.2 Rationale for Trial Design and Dose for Study Medication

2.2.1 Study Rationale:

Severe AH continues to be associated with a high mortality and represents a significant public health burden[8-13]. Prednisone is the standard of care but is associated with a modest and transient survival benefit at best and increased risk of severe bacterial and fungal infections[14-16]. A recent large study indicated that pentoxifylline is not significantly superior to placebo[15]. While several new targets are being evaluated, they are not sufficiently powered to provide definitive data. There is therefore a need for well-designed, appropriately powered efficacy (phase 2B or 3) trials to define the utility of newer therapies for severe AH against prednisone.

2.2.2 Rationale for Prednisone in Severe AH, current standard of care for severe Acute Alcoholic Hepatitis (AAH):

Corticosteroid therapy is recommended by the American Association for the Study of Liver Diseases as primary treatment for participants with severe AAH, defined by the Maddrey DF > 32 based on Class I, level A evidence[1]. Participants with DF < 32 have six-month survival of > 85% whereas those with DF > 32 have a survival of only 60%. Almost all trials of corticosteroids have treated participants for 28 days. Although short-term mortality is reduced by 28 days of corticosteroids, 6-month survival is similar to standard care. Many reasons account for late deterioration in steroid treated participants. Infections develop after the first 30 days in some participants treated with steroids. Tapering the dose of steroids (median of 15 days) after 50% improvement in total bilirubin and prothrombin time combined with enteral nutrition reduced the risk of late infections[17]. The investigators postulated that the benefit of enteral nutrition was mediated through improvement in integrity of the gut mucosal barrier and reduced gut bacterial translocation and endotoxemia.

Early improvement in bilirubin within the first week is predictive of a good response to corticosteroids at 28 days[18]. Although corticosteroids improve survival in a subset of participants with severe AAH, some participants do not respond, either because of steroid "resistance" or due to the underlying severity of the disease. Steroids are not well tolerated in participants with active GI bleeding or systemic infections.

Corticosteroids represent a clinically tested treatment for severe AH and will be offered to a third of the participants in this randomized clinical trial.

2.2.3 Rationale for testing anakinra plus zinc as novel drugs for severe AH against the standard treatment (Prednisone in Arm 2):

There are currently no new drugs formally approved by the FDA for severe AH. The rationale to repurpose already available agents for AH treatment is to generate evidence to support moving to pivotal trials by the drug manufacturers or support the off-label use of these compounds and thus provide much needed benefit to those with severe AH.

2.2.4 Clinical applications of anakinra:

Preliminary data from the interim analysis of the ongoing clinical trial from the Defeat Alcoholic Steatohepatitis (DASH) UO1 consortium suggests that the treatment arm with anakinra plus pentoxiphylline plus zinc may provide better survival at day 90 and 180 compared to the steroid treated group. Side effects including infection rates were comparable between the anakinra and steroid arms.

Anakinra blocks the effects of IL-1 by inhibiting the binding of IL-1ß and IL-1α to the IL-1 receptor. It is FDA-approved for treatment of moderate to severe rheumatoid arthritis (RA) since 2001, and is used also in patients with Still's disease, and in familial cold autoinflammatory and Muckle-Wells syndromes[20-22].

Anakinra has an excellent safety profile compared to other biological therapies. The rate of serious infections was 3.2 per 100 participant-years in participants exposed to Anakinra[23]. A meta-analysis of trials evaluating anakinra in RA reported a 1.4% incidence of serious infections with anakinra, compared with 0.5% with placebo, although this difference was not significant after adjustment for underlying comorbidities[24]. This is important given that participants with severe AH are prone to bacterial infections. No adverse reactions or microbial superinfections were attributable to anakinra treatment in participants with severe sepsis[25]. Other FDA approved drug candidates for inhibiting IL- 1 are canakinumab and rilonacept[26,27], that are human monoclonal antibodies and both target IL-1ß (and not IL-1α). The advantage of anakinra is that it prevents both IL-1ß and IL-1α effects. An additional advantage of the anakinra is its short half-life for participants with alcoholic liver disease given the concern of susceptibility to infections in this participant population.

Anakinra, a human recombinant IL-1 receptor antagonist, prevents IL-1 receptor signaling [28-30]. IL-1ß, that activates the IL-1receptor, is a unique therapeutic target in AH because unlike other pro-inflammatory cytokines, IL-1ß secretion is under control of two cytokines, IL-1ß secretion is under control of two signals[31]. This dual regulation suggests that accumulation of multiple danger signals will trigger IL-1ß secretion[28]. We have recently identified that gut-derived LPS and sterile danger signals such as ATP and uric acid from alcohol-damaged hepatocytes act in concert to activate the multiprotein complex, NLRP3 inflammasome, that results in activation for IL-1ß secretion via caspase-1/pro-IL-1ß cleavage[32,33]. The biological significance of increased IL-1ß production extends beyond the direct pro-inflammatory effects of IL-1ß[29]. IL1ß, after engaging the IL-1 receptor, amplifies pro-inflammatory cytokine production including IL-1, TNF, MCP-1, and IL-6[34]. In addition, IL-1ß mediates biological effects that contribute to salient pathological features of ALD and AH. Specifically, IL-1ß promotes steatosis, hepatocyte death, liver fibrosis and inhibits liver regeneration[19,30,35]. All of these biological effects of IL-1ß and IL-1receptor activation are blocked by the naturally occurring IL1receptor antagonist (IL-1ra). Testing of the IL-1 receptor antagonist, anakinra, in mice fed with the Lieber-DeCarli alcohol or control diet for 4 weeks revealed that alcohol-induced increase in serum ALT, liver steatosis and upregulated inflammatory cytokine production (TNFα, pro-IL-1β, mature IL-1β) were all significantly reduced by IL-1ra administration.

(**Fig 1**)[30].

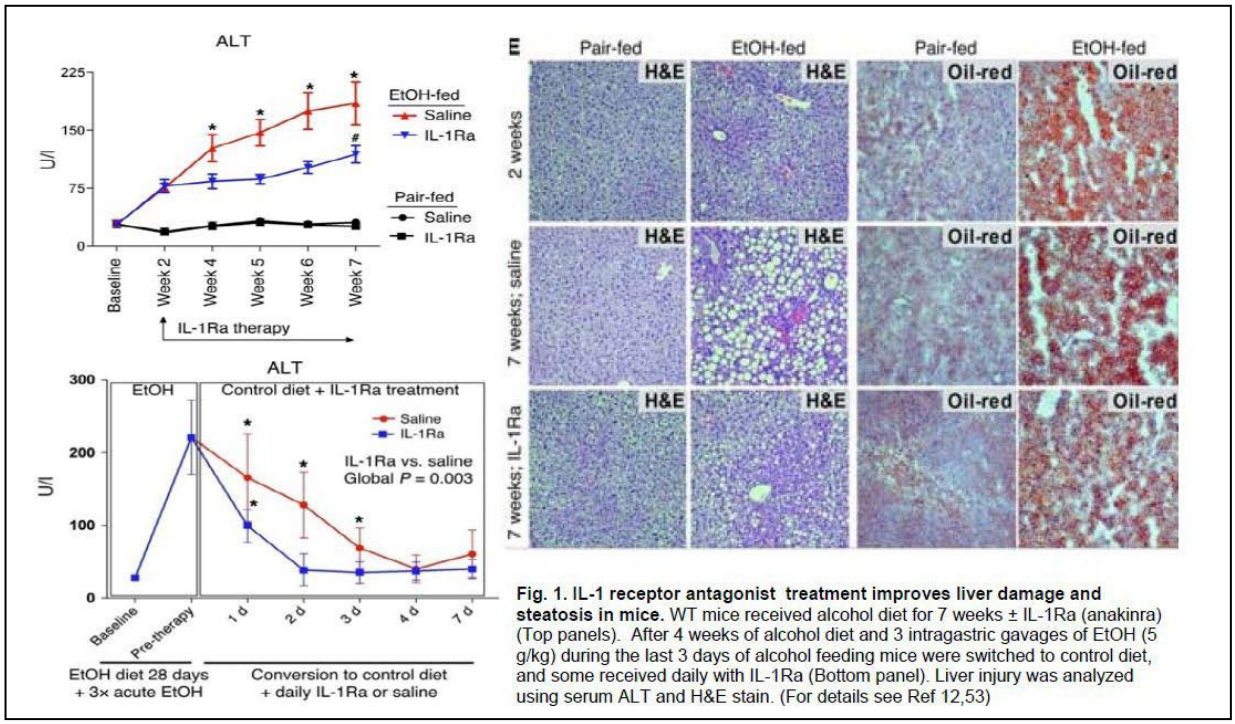


Fig. 1. IL-1 receptor antagonist treatment improves liver damage and steatosis in mice. WT mice received alcohol diet for 7 weeks ± IL-1Ra (anakinra) (Top panels). After 4 weeks of alcohol diet and 3 intragastric gavages of EtOH (5 g/kg) during the last 3 days of alcohol feeding mice were switched to control diet, and some received daily with IL-1Ra (Bottom panel). Liver injury was analyzed using serum ALT and H&E stain. (For details see Ref 12,53)

2.2.5 Clinical applications of Zinc supplementation:

Zinc is an essential trace element required for normal cell growth, development, and differentiation[36]. It is a critical component of many proteins/enzymes, including zinc- dependent transcription factors. Importantly, oxidative stress (e.g., resulting from ethanol metabolism) may cause release of zinc from critical zinc-finger proteins and subsequent loss of DNA-binding activity and function[36]. Zinc deficiency/altered zinc metabolism is regularly observed in people with AH and may result from decreased dietary intake, increased urinary excretion, abnormal activation of certain zinc transporters, and induction of hepatic metallothionein[37]. Zinc deficiency has been shown to cause/exacerbate gut barrier dysfunction, endotoxemia, increased proinflammatory cytokine production, oxidative stress, decreased zinc finger function, and hepatocyte steatosis/injury/death in experimental ALD[36,38-51]. Similarly, zinc supplementation improved experimental ALD, in part, by stabilizing tight junctions, reducing endotoxemia, lowering levels of metabolic toxins such as ammonia, decreasing production of pro-inflammatory cytokines, reducing oxidative stress, and attenuating hepatocyte steatosis/injury/death. **(Figure 2)** [36,38-51].

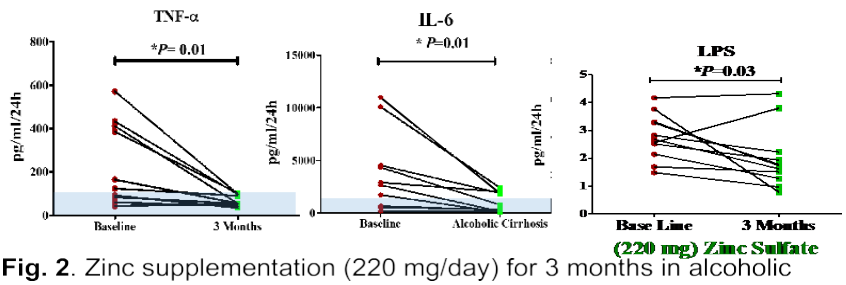


Fig. 2. Zinc supplementation (220 mg/day) for 3 months in alcoholic cirrhotics reduced LPS-stimulated ex vivo cytokine production and serum endotoxin levels. Placebo treatment (data not shown) produced no beneficial effects.

Moreover, limited studies in human ALD have generally shown similar mechanistic beneficial effects of zinc (Figure 2-unpublished data). The dose of zinc used for treatment of ALD usually is 50 mg of elemental zinc taken with a meal to decrease the potential side effect of nausea. This clinical trial will use zinc supplementation to inhibit multiple mechanisms involved in the pathogenesis/progression of AAH and possibly other organ failure, beginning with intestinal barrier dysfunction **(Figure 2).**

2.2.5 Impact Statement:

The proposed trial will provide definitive evidence of the safety and efficacy of Anakinra plus zinc vs prednisone in severe AH; if positive, it will change the standard of care for severe AH and, if negative, it will discourage their off label use thereby saving cost and potential exposure to an ineffective drug in afflicted participants. In either case, the proposed study will alter how participants with severe AH are managed.

2.3 Summary of Known Potential Risks with Study Medication

<u>Corticosteroids</u> (including prednisone) are the standard medical care for severe acute AH and have anti-inflammatory effects that can alter the body's reaction to other injuries. Prednisone 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 participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious	Infection	Psychosis, Pancreatitis, Ulcerative esophagitis, Cushing's syndrome	Anaphylaxis, Hypersensitivity reaction, Peptic ulcer, Bone necrosis, Blood clot in a deep vein
Less Serious	Weight gain	High blood sugar, High blood pressure, Wound healing impairment, Increased intraocular pressure, Increased liver enzymes	Muscle weakness
Minor	Facial swelling Fluid retention Indigestion Increased appetite	Difficulty sleeping, Mood changes, Euphoria, Headache, Bruising	

Anakinra (study drug) is another anti-inflammatory treatment. It can alter the body's reaction to other injuries. Anakinra 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 participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious		Infection, including cellulitis, pneumonia and bone and joint infections)	Anaphylaxis, hypersensitivity reactions, Angioedema, Malignancy
Less Serious		Low blood count, (Neutropenia), Joint pain	Low blood count (Eosinophilia, Platelets), Nausea, Diarrhea
Minor	Injection site redness	Fever, Nasopharyngitis, Skin rash	

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.

Side effects	Frequent >20% of participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious			
Less Serious	Nausea, Vomiting, Dizziness, Headache, Abdominal cramps, Diarrhea		
Minor	Indigestion		

3. TRIAL OBJECTIVES

The primary purpose of this study is to determine whether Anakinra plus zinc is superior to standard treatment with Prednisone in the treatment of severe AH.

Primary Endpoint

- Survival at 90 days.

Secondary Endpoints

- Change in Lille score, change in MELD score, development of AKI, multi-organ failure, SIRS, transfer to ICU, changes in liver function will all be evaluated at 7, 30 and 90 days.

- **Organ dysfunction:** Changes in Sequential Organ Failure Assessment (SOFA) scores and proportions requiring hemodynamic support for MAP < 65 mm Hg and lactate > 2mmol/l, renal replacement therapy or mechanical ventilation. We will also modify and re-evaluate the SOFA score without platelet counts given that these are usually low in AH.

- **Infections and Sepsis:** It is now recognized that the endpoints and case definitions related to sepsis may vary depending on what these will be used for (clinical care, research, surveillance, or quality improvement). Given the central role of sepsis as a driver of outcomes, we will not only measure the types of infection but also identify the proportions of those with sepsis, septic shock or quick-SOFA (qSOFA) criteria based on SEPSIS-3 guidance[2,3]. Capturing the key parameters needed for various sepsis related endpoint construction aligned by the recent guidance from the European Drug Development Hub (http://eddh-cro.wixsite.com/fdtsfv).

- **Renal dysfunction:** In alignment with guidance from the acute disease quality initiative, we will quantify AKI development and its progression through persistent AKI, acute kidney disease to chronic kidney disease.

- **Need for care escalation:** Proportion of participants requiring transfer to ICU for care.

- **Indicators of gut:** Permeability (endotoxin and bacterial 18S DNA) and pro- inflammatory cytokine/chemokines (TNFa, MCP1, IL-6, IL-1ß) will be assessed in serum/plasma samples.

- **Survival at 30 and 180 days.**

Limitations of mortality as an endpoint in AH and plans to overcome these:

All-cause mortality is most relevant when mortality is substantial and attributable to a single mechanism in a homogeneous population. Participants with severe AH are highly heterogeneous with respect to disease severity, end organ involvement and the care they receive. Moreover, as in sepsis and heart failure, death involves failure of multiple organs (liver, heart, kidney, lungs) making it difficult to ascertain which one is the primary cause of death. This has led to development and regulatory acceptance of composite endpoints including mortality and key parameters driving mortality for acute heart failure trials. We will leverage these guiding principles to construct a novel and innovative key secondary composite endpoint which will include death or worsening of the SOFA score[4,5] by ≥2 points and worsening of MELD score by ≥2 points. These are associated with mortality and capture the organ recruitment that marks progression of AH towards death. This endpoint will also be validated with respect to reliability, construct-, criterion- and content validity to

establish this as a primary endpoint in future trials. We will also model changes in SOFA scores and MELD scores against mortality to further optimize this endpoint and cross-validate it in the observation cohort.

Novel (experimental) endpoints to overcome limitations of conventional endpoints Efficacy endpoints must capture "clinically meaningful benefit". While traditional endpoints such as mortality are meaningful, they also have multiple shortcomings in the context of AH as noted below. We will therefore innovate to construct, measure and validate novel additional endpoints to overcome the limitations of currently used endpoints and cross-validate them in the observational cohort. Our combined expertise in endpoint development and the use of harmonized measurements will make this feasible. These will provide novel assessments in AH and provide the scientific evidence-base to use these as primary endpoints in future trials.

4. INVESTIGATIONAL PLAN

4.1 Overall Trial Design and Plan

Overview of Study Design and Conduct

The proposed study is a Phase 2b, multicenter, prospective, randomized, 2-arm, double blinded, placebo-controlled clinical trial to assess the efficacy of Anakinra, an IL-1 receptor antagonist plus zinc compared to prednisone for the treatment of severe AH (Model for End Stage Liver Disease (MELD) score ≥ 20). All participants will receive standard care for treatment of severe AH as described in recent guidelines endorsed by AASLD and AGA[1]. The study will be conducted at **8 clinical sites** across the United Stated selected by the NIAAA and will be spearheaded by a **Data Coordinating Center (DCC-IU) at Indiana University**.

The study will be conducted according to good clinical practice (GCP) and in compliance with local, state, and federal regulatory requirements. Adverse events during the course of the trial will be identified, recorded, assessed for causality, and reported in accordance with FDA guidance. In addition to general assessment, we will specifically focus on (1) rates and types of infection as well as their severity, (2) potential development of drug-induced liver injury, (3) injection site reactions, and (4) hematological adverse events. These are related to the mechanisms of action of the drugs to be tested.

It is anticipated that this study will be approved by an appropriately convened single IRB and will be monitored by an NIAAA appointed data and safety monitoring board. We will conduct this study under an Investigational New Drug (IND) application from the United States Food and Drug Administration (FDA).

It is anticipated that a centrally located investigational pharmacy located at Indiana University will dispense the study medications to all participating sites under close coordination from the DCC- IU. Anakinra plus zinc and prednisone and matching placebos will be provided by the study to the participants.

Interventions to be tested

Concomitant treatments in this trial include standard care for severe AH for all participants as recommended in recent guidelines. The standard of care for severe AH includes attention to early diagnosis and treatment of bacterial and fungal infections, portal hypertension and its complications including acute kidney injury, gastrointestinal bleeding, fluid overload (ascites and edema) and hepatic encephalopathy. As part of the standard of care, all participants will be offered non-

pharmacologic intervention for treatment of alcohol use disorders, such as cognitive behavioral therapy and 12-step programs intervention as aligned with the best practices at the participating study site.

Participants with severe AH are very sick and often have multiple active medical problems requiring intervention. The use of specific interventions during this time has the potential to modify treatment response. Ideally, these interventions would be completely standardized. However, the standard of care varies by region, institution and the specialties involved in the care of the participant. The decision regarding treatments for these complications will be delegated to the primary physicians caring for the participants during hospitalizations and to the investigators during the ambulatory follow-up of the participants. Other than the general guidelines noted, no specific treatments for specific complications such as those noted are required. However, such treatments might include, but are not limited to the administration of IV fluids, intravenous human serum albumin, antibiotics including antifungal medications, renal replacement therapy, supplemental oxygen, assisted ventilation and intravenous pressors. Diagnostic procedures such as paracentesis, upper endoscopy, colonoscopy, endoscopic ultrasound, and liver biopsy will be permitted as required to provide standard care.

During the initial six months of trial, the investigators will develop a "manual of clinical operations" that will include best practices on the use of vasopressors, albumin, ICU care, mechanical ventilation and renal replacement therapy using Surviving Sepsis Campaign, International Guidelines for Management of Severe Sepsis and Septic Shock. The investigators will also provide best practices for management of nutrition and alcohol counseling and minimization of recidivism. Although the management of these complications will not be mandated by protocol, the care provided will be recorded to develop novel designs for future trials and for practice guidance and quality improvement programs.

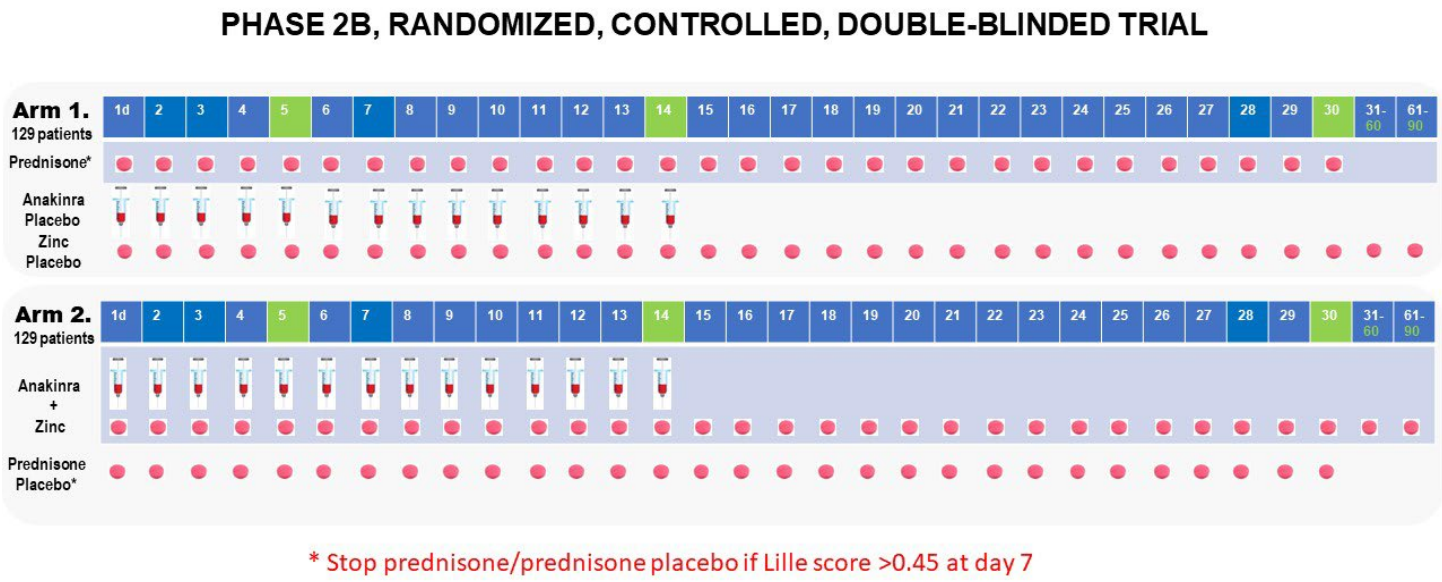
After ensuring that participants meet all of the inclusion criteria and none of the exclusion criteria, and after the informed consent has been obtained participants will be randomized to receive one of the following interventions in addition to standard care.

Group 1: Standard of care **plus** prednisone (40 mg orally) once daily on Days 1-30 and matching placebos for Anakinra (1 syringe s.c. once daily on Days 1-14) and zinc (matched pill once daily on Days 1-90).

Group 2: Standard of care **plus** Anakinra (100 mg s.c.) once daily on Days 1-14, zinc sulfate 220 mg once daily on Days 1-90, and placebo for prednisone (matched pill once daily on Days 1-30).

Lille score will be calculated from day 7 labs, participants will stop prednisone or prednisone placebo if Lille score >0.45.

Figure 1. Distribution of study drugs and placebo



Participants or guardian will be instructed in subcutaneous injection technique to continue self-administration of these experimental treatments after discharge from the hospital. Study personnel will ensure participants and guardian are proficient in the technique before the end of study visit.

Relevant clinical care parameters will be recorded on days specified. As noted above, attention will be paid to recording concomitant care provided to treat complications related to AH or portal hypertension including those treatments not specified by protocol, including, but not limited to, antibiotics, IV fluids, albumin, pressors, renal replacement, etc.

Standardizing study conduct and good clinical practice (GCP)

Variations in protocol implementation and clinical care can have a major impact on trial outcomes. This will be optimized by: (1) GCP training and certification of all study personnel, (2) investigator/coordinator training and certification prior to site activation, (3) monthly performance reports, (4) development of manual of operations that provide best practices and study procedures to follow, and (5) annual investigator meeting to review developments and clinical and trial practice patterns.

4.2 Schedule of Trial Procedures

Table 1. Study Visits and Data Collection Schedule***

	Screening	Treatment Phase							Follow-up phase
		D0	**D3**	**D7**	**D14**	**D28**	**D60**	**D90**	**D180**
Window (days)		±2	±2	±2	±2	±7	±7	±7	±7
Informed consent	x								
History and physical	x	x	x	x	x	x	x	x	x
Vital signs, weight ***	x	x	x	x	x	x	x	x	x
Alcohol consumption history	x	x			x	x	x	x	x
Randomization		x							
Concomitant medicines	x	x	x	x	x	x	x	x	x
Dispense Study Drug		x		*					
Adverse events			x	x	x	x	x	x	x
Electrocardiogram* *****	x								
Evaluation of compliance			x	x	x	x	x	x	
CBC, PT/INR, Hepatic Function Panel, BMP	x	x	x	x	x	x	x	x	x
Test for COVID-19****	x								
Sepsis studies, as indicated	x	x	x	x	x	x	x	x	x
Pregnancy test, serum**	x								
Pregnancy test, urine**		x			x	x	x	x	x
Blood collection for trough Anakinra levels		x	x	x	x				
Specimen Banking¶		x		x	x	x	x	x	x
Saliva Banking+		x						x	
Questionnaires δ	x					x	x	x	x

¶Specimen banking includes blood and stool (when possible) D0, D7, D14, D28, D60, D90, D180, and, when available, urine and liver biopsy/tissue. Blood will be used to extract germline DNA at baseline and at Day 180 as well as to extract serum/plasma/PBMC at all visits. *Type of specimen collection is site specific.*
+ Saliva banking at D0 and D90. Collected until an adequate number of samples are collected.

δ Questionnaires include Alcohol Use Disorders Identification Test (AUDIT), Alcohol Timeline Follow Back (TLFB), and Chronic Liver Disease Questionnaire (CLDQ). The timeline follow back questionnaire will be the only questionnaire administered at days 28, 60, 90, and 180. All are given at screening.

***** Day 7 study drug dispensation will be unscheduled and only on an as-needed basis, based on safety lab evaluations **(outpatient only.)**

****** If applicable

******* Height at screening only.

******** Test for COVID-19 PCR only if not done as SOC with 7 days prior to the baseline Day 0 visit.
********* In response to COVID-19, we are lessening restrictions if applicable or necessary such as: allowing wider windows D14, D28, D60 to +/- 10 days. Allowing replacement of protocol mandated in-person study visits with one or more of the following, phone calls, telemedicine virtual visits, implement digital technology to record responses to questionnaires. Lastly, allowing blood draws at remote or commercial laboratories.
********ECG can be done as standard of care within 7 days of screening**

Abbreviations: CBC: Complete blood count, BMP: Basic metabolic panel, INR: International normalized ratio and PT: Prothrombin Time.

Data Collection

Clin= clinical (relevant elements of history, physical exam), Biosamples: whole blood, plasma, serum, PBMC, DNA, (and stool (when possible) at all time-points, Liver biopsy/tissue and urine (20ml) if available, Questionnaires= AUDIT, alcohol timeline follow back, CLDQ. SOC (standard of care) labs= CBC, Hepatic Function Panel, BMP, PT/ INR, Sepsis studies at baseline and during trial duration, as indicated (blood cultures, chest X ray, stool for WBC and C. Diff, as indicated, ascites tap, UA with culture, liver ultrasound or other imaging, endoscopy if available within 6 months).

4.3 Duration of Trial by Phase

Time expected for all participants to be enrolled:	5 years
Duration of individual participant participation:	90 days during the treatment phase 90 days during the follow up phase 180 days total participation
Total duration of trial (excluding data collection and analysis):	5 years

5. PARTICIPANT SELECTION

5.1 Participant Population

The study population will be comprised of men and women of all races, ethnic groups and socioeconomic status of the ages of 21 and greater admitted to the hospital with a clinical diagnosis of AH. The definition of AH is based on criteria established by NIAAA. A conscious decision not to require a liver biopsy for inclusion was made based on the current low rate of liver biopsy used to establish this diagnosis in the US and the ability to diagnose the condition with relative accuracy based on the experience in the first cycle of funding. Eligible participants will be identified based on inclusion/exclusion criteria as defined below.

Inclusion Criteria/ Exclusion Criteria

Inclusion Criteria

1. AH, as defined by the NIAAA pan-consortia for AH:
    a) Onset of jaundice *(defined as serum total bilirubin >3mg/dL)* within the prior 8 weeks
    b) Regular consumption of alcohol with an intake of > 40 gm daily or >280gm weekly on average for women and > 60 gm daily or >420gm weekly on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice
    c) AST > 50 IU/l
    d) AST: ALT > 1.5 and both values < 400 IU/l
    e) and/or histological evidence of AH*

2. MELD 20-35 on day of randomization.

3. Ages ≥ 21

** In patients with possible AH or AH with confounding factors such as possible ischemic hepatitis, possible DILI, uncertain history of alcohol use (e.g., patient denies excessive alcohol use), and atypical/abnormal laboratory tests (e.g., AST < 50 IU/IU/L or > 400 IU/IU/L, AST/ALT ratio < 1.5), antinuclear antibody > 1:160 or SMA > 1:80, standard of care liver biopsy may be performed during current hospital admission to confirm AH and exclude competing etiologies [17].*

Exclusion Criteria

1. MELD SCORE <20 or > 35
2. Active sepsis (positive blood or ascitic cultures) with Systemic Inflammatory Response Syndrome (SIRS) or hemodynamic compromise requiring intravenous pressors to maintain tissue perfusion
3. Pneumonia as evidenced by radiological exam
4. Multi-organ failure
5. Renal failure defined by GFR <35 mL/min by CKD EPI.
6. Clinically active C. diff infection
7. History of imaging of the liver (ultrasound, computerized tomography, or magnetic resonance) showing other causes of jaundice
8. History of other liver diseases including hepatitis B (positive HBsAg or HBV DNA), hepatitis C (positive HCV RNA), autoimmune hepatitis, Wilson disease, genetic \hemochromatosis, alpha1-antitrypsin deficiency, or strong suspicion of Drug Induced Liver Injury (DILI). Previously treated hepatitis C that was cured (sustained virological response with negative RNA ≥24 weeks following treatment) is not an exclusion.
9. History of HIV infection (positive HIV RNA or on treatment for HIV infection)
10. History or presence of cancer (including hepatocellular carcinoma) other than non-melanoma skin cancer
11. History of other significant medical problems such as autoimmune diseases, severe asthma, psoriasis, Inflammatory Bowel Disease (IBD), etc. that might require immunosuppressive treatments
12. Pregnancy or breastfeeding
13. Prior exposure to experimental therapies in last 3 months
14. Prior exposure to systemic corticosteroid (glucocorticoid) or immunosuppressive therapy for more than 4 days within previous 30 days
15. Need for inotropic pressor support to maintain perfusion to critical organs within prior 48 hours before randomization and initiation of experimental treatment
16. Clinically significant pancreatitis- abdominal pain, elevated lipase (> 3 X ULN) and at least edema of pancreas with fat-stranding on CT scan
17. Total WBC count > 30,000/mm3
18. Known allergy or intolerance to therapeutic agents to be tested
19. Inability to voluntarily obtain informed consent from participant or guardian
20. Perceived inability to follow study procedures and comply with protocol
21. Platelet count < 40,000 k/cumm.
22. Positive PCR test for COVID -19 within 7 days prior to the baseline day 0 visit*
23. Active gastrointestinal bleeding defined as hematemesis or melena with a decrease in hemoglobin more than 2 g/dl in 24 hrs. Due to gastrointestinal bleeding, or with a decrease in mean arterial BP to < 65 mmHg.

**Positive PCR test for COVID-19 is exclusionary only during screening period. If a patient tests positive any time after baseline randomization, a positive PCR test for COVID-19 will be considered as a SAE.*

The participants for this study will be recruited from a hospitalized population of participants meeting the eligibility criteria outlined above who live within one day travel from one of the

participating clinical centers and who have provided informed consent to participate in this clinical trial and who are willing to continue their participation for the anticipated follow-up period of the trial. Although the primary endpoint is survival at 90 days, follow-up visits will be continued up to 6 months.

5.2 Participant Compensation and Recruitment

5.3.1 Participant Compensation

Participant compensation is site specific.

5.3.2 Participant Recruitment

Sources of participants:

Identification of participants with severe AH requires collaboration with those physicians who manage these participants within the hospital and the emergency department. Almost 100% of participants are admitted to hospital based on the severity of their liver disease. Occasionally participants are seen in free-standing urgent care centers, free-standing emergency rooms or in office/clinic practices. Since the standard of care is to admit these participants for further evaluation to exclude infections as a precipitating cause of decompensated liver disease, most will ultimately be identified in a hospital setting. Letters will also be sent to referring MDs and referring hospitals.

Some sites in the current U01 have found that screening laboratory results for elevated AST and total bilirubin is a useful way to identify potential participants. In accordance with Institutional Review Board Policies, once identified the primary team caring for these participants must be contacted by the investigators and agree to ask the participant about their interest in participating in a clinical trial as part of the treatment of the underlying AH. Once the participant expresses willingness to be contacted the investigators may discuss details of the study and obtain informed consent prior to beginning treatment.

5.4.1 Individual Drug Discontinuation Criteria Reasons for Mandatory Trial Hold – Infection

If one of the following criteria is met, the study drugs **should be held for 3 days:**

1. **Documented Infection**
    - Pneumonia defined as new infiltrate by CXR or chest CT scan
    - Positive cultures (blood, ascites) for bacteria or fungus
    - Positive fungal cultures in urine (> 50,000 colonies/ml)
    - CNS infection defined as positive cultures or WBC > 5 in CSF
    - Severe soft tissue or bone infections including clinical diagnosis of cellulitis

2. **SIRS (defined as two or more abnormalities in temperature, increased heart rate, respiration, or white blood cell count) with increase in SOFA score ≥2 points**

Participants will be reassessed on the third day of the study drugs hold: If infection clears and participant stabilizes, study drugs will be resumed.

For participants with documented infection: If infection is responsive to antibiotics and participant is stabilized or improved, study drugs may be resumed at the discretion of the clinical care team. If there is no improvement after 3 days, study drugs will be stopped

For participants meeting only SOFA+SIRS (#2 above) criteria for stopping: if cultures of urine, blood, ascites, stool (including C. diff) are negative and CXR is unchanged, study drugs may be resumed at the discretion of the clinical care team.

If the participant has a second hold of study drugs due to infection/SIRS+SOFA criteria, study drugs will be stopped.

5.4.2 Reasons for Mandatory Trial Discontinuation

1. **Persistent infection:** a) Participants with documented infection will be reassessed on 3rd day of the study drugs hold. If infection is responsive to antibiotics and participant is stabilized or improved, study drugs may be resumed at the discretion of the clinical care team. If there is no improvement after 3 days, study drugs will be stopped. b) For participants meeting only SOFA+SIRS criteria for stopping: if cultures of urine, blood, ascites, stool (including C. diff) are negative and CXR is unchanged, study drugs may be resumed at the discretion of the clinical care team. c) If the participant has a 2nd hold of study drugs due to infection/SOFA +SIRS criteria, study drugs will be stopped.

2. **Pregnancy: If a female participant becomes pregnant, she must stop taking study medication and must be withdrawn from the trial.** The participant must be followed by the investigator through the end of her pregnancy. The mother (and infant) will be followed as considered appropriate by the investigator and the study designated physician (Dr. Gawrieh or his designee). For reporting purposes, pregnancy is not considered a serious adverse event (SAE).

3. **Clinical deterioration:** During the trial, if Maddrey's Discriminant Factor (DF) increases by 5 points and also exceeds 32, or MELD scores increases by 5 points and also exceeds 20, the study medication should be stopped, however the participant will remain in the trial, completing procedures as detailed in the Schedule of Procedures (Table 1). The study drugs will also be stopped if severe leukocytosis (≥100,000/mm3) develops. Treating physicians may offer any other treatment that is considered as medically indicated and appropriate.

4. **Suspected drug induced liver injury (DILI):** Hold the study drug if MELD increases by 5 from baseline or Child's score increases by 3 points from baseline plus >3-fold increase in ALT from baseline on two consecutive occasions. These instances may represent either lack of therapeutic efficacy or DILI or some other etiology. If there is a competing etiology and DILI can be excluded, study drug can be resumed within 3-4 days. However, if DILI cannot be excluded, then the study drug will be permanently discontinued. Additional reasons for study drug discontinuation include unexplained elevation in ALT > 5 x baseline or > 500 U/L on two consecutive occasions or unexplained elevation in Alk P > 3 x baseline or >500 U/L on two consecutive occasions. It should be noted that cholestatic DILI may have a devastating impact on participant survival in individuals with advanced liver disease.

5. **Lille score**: Participants in either arm who have a Lille score of >0.45 on day 7 will stop bottle 1(prednisone/ placebo) and will continue with bottle 2 (zinc/zinc placebo.)

a) Other Reasons for Trial or Treatment Discontinuation

The following events are considered appropriate reasons for a participant to discontinue from the trial:

- The participant withdraws consent or requests to be withdrawn from the trial. It is fully understood that all participants volunteer for the trial and that they may withdraw their consent at anytime.
- The participant experiences an adverse event that in the opinion of the site investigator or the primary investigator of the trial (i.e., study designated physician, Dr. Gawrieh) is caused by or exacerbated by any of the trial procedures or study medication, of sufficient intensity to warrant discontinuation.
- The participant refuses to comply with the requirements for trial participation.
- Termination of the trial due to DILI concerns: The Data Safety and Monitoring Board (DSMB) should carefully monitor the number of discontinuations due to MELD/Child's Score worsening in three treatment groups. Since this trial is not powered for rare adverse events, one should not look for statistically significant imbalance among treatment groups, but the DSMB should consider numerical imbalances that its members deem clinically significant. While it may be straight forward in some instances (for example, 2 discontinuations in groups A but 5 discontinuations in group B) but in other instances it may not be as clear cut and one may continue the trial for a growing trend. In any case, the DSMB is advisory in nature, and it makes a recommendation to the Sponsor, NIAAA. In addition to seeking the AlcHepNet Steering Committee input, the NIAAA may seek external input to assist in its deliberation if the DSMB recommends that the clinical trial must be terminated due to safety concerns.

When possible, the investigator should discuss the potential discontinuation of a participant with the study designated physician (Dr. Gawrieh or his designee) in advance.

b) Participant Discontinuation Notification

The investigator must notify the study designated physician (Dr. Gawrieh or his designee) as soon as possible if any participant prematurely discontinues from the trial. The date when the participant is withdrawn and the primary reason for discontinuation must be recorded in the CRF; additional information may be requested to complete a discontinuation narrative. Participants will be considered "lost to follow up" only after reasonable, documented attempts to reach the participant prove unsuccessful. In all cases, a reasonable effort must be made to determine the reason(s) that a participant fails to return for required trial visits or discontinues from the trial.

If a participant stays in the study but discontinues study medication (either by participant's choice, or by the investigator's decision), then study measurements should be performed according to the Schedule of Trial Procedures, Table 1 (rather than expediting study visits, or measurements). Patients will be followed until the final evolution (stability, worsening, or resolution) of the event that invoked drug discontinuation and followed until the end of the trial, if they are willing to do so.

6. INVESTIGATIONAL PRODUCT (IP)/ STUDY MEDICATION

6.1 Description of IP/ Study Medication

For the purpose of this protocol, the term 'investigational product (IP)' is interchangeable with the term 'study medication'. Descriptions of the drugs, physical and chemical characteristics, and biological effects are included in the Labeling Information filed the Investigator's Brochure.

6.1.1 Study Medication

A. Prednisone, Zinc Sulfate and Placebo Oral Therapy

1. The University of Iowa Pharmaceuticals (UIP) will over-encapsulate the active prednisone and zinc sulfate tablets and manufacture placebo capsules.

    a. The UIP will purchase commercially available prednisone and zinc sulfate.

        1. Prednisone 20 mg tablets (Westward Pharmaceuticals)

        2. Zinc Sulfate 220 mg tablets

        3. The UIP will use stock excipients to manufacture placebo capsules.

2. Prednisone, Zinc Sulfate and Placebo capsules will be identical, encapsulated in a size AAA empty capsule shell

3. UIP will package study medications as

    a. Prednisone 40 mg capsules, 50 capsules per bottle

    b. Zinc Sulfate 220 mg capsules, 50 capsules per bottle

    c. Placebo for Prednisone 40 mg/Zinc Sulfate 220 mg Capsules, 100 capsules per bottle

B. Anakinra and Placebo Subcutaneous Therapy

1. Anakinra 100 mg/0.67 mL Prefilled Syringes will be procured by IU Health Investigational Drug Service through wholesaler Cardinal Health (NDC 66658-0234-07, SOBI Pharmaceuticals)

2. Placebo for Anakinra 100 mg) will be prefilled syringes purchased directly from SOBI Pharmaceuticals and distributed by IU Health Investigational Drug Service. Each syringe is labeled by Sobi Pharmaceuticals as "Placebo for Kineret." Each 1 mL syringe is filled to 0.67 mL.

6.1.2 Treatment Phase

Study medication will be provided to each site (pharmacy) as unblinded product, over labeled at each site and blinded based on the randomization scheduled, and then provided to participants in a blinded manner (i.e., pharmacy, investigator, site staff and participant will be unable to distinguish treatment assignment). Study medication will consist of either

1. **Group 1**: Standard of care **plus** prednisone (40 mg orally) once daily on Days 1-30 and matching placebos for Anakinra (1 syringe s.c. once daily on Days 1-14) and zinc (matched pill once daily on Days 1-90).
2. **Group 2:** Standard of care **plus** Anakinra (100 mg s.c.) once daily on Days 1-14, zinc sulfate 220 mg once daily on Days 1-90, and placebo for prednisone (matched pill once daily on Days 1-30).

6.2 Packaging, Labeling, and Storage

The packaging and labeling of study medication for provision to the clinical trial sites will be performed according to GMP standards by designated qualified vendors.

The designated IP distribution vendor will be responsible for the distribution of the study medication to the clinical trial sites. Prednisone, Zinc Sulfate and Placebo capsules should be stored at controlled room temperature (15°C to 30°C) and protected from excess humidity. Anakinra and Placebo prefilled syringes should be stored in the refrigerator (2°C to 8 °C) and protected from light. Avoid shaking and protect from freezing.

Study medication will be packaged for the trial as described below.

6.3 Labels for Study Drugs and Study Drug Dispensing Units:

Study medication will be packaged for the trial as described below.

Anakinra Prefilled Syringes, and the Placebo Prefilled Syringes provided by SOBI will have the manufacturers label on the study drug. Prednisone, Zinc Sulfate and Placebo Capsules will be labeled by the University of Iowa Pharmaceuticals.

Over-Encapsulation of Prednisone (2 x 20 mg Tablets) 40 mg C

Lot #: XXXXXXXXX

50 Capsules per Bottle

Store at Controlled Room Temperature

Caution: New Drug – Limited by Federal Law to Investigational Use Only.

The University of Iowa Pharmaceuticals – College of Pharmacy – The University of Iowa – Iowa City, Iowa 52242

Over-Encapsulation of Zinc Sulfate 220 mg Capsules

Lot #: XXXXXX

Store at Controlled Room Temperature

Caution: New Drug – Limited by Federal Law to Investigational Use Only.

The University of Iowa Pharmaceuticals – College of Pharmacy – The University of Iowa – Iowa City, Iowa 52242

Placebo Capsules for Prednisone and Zinc Sulfate

50 Capsules Lot #:

XXXXXX

Store at Controlled Room Temperature

Caution: New Drug – Limited by Federal Law to Investigational Use Only.

The University of Iowa Pharmaceuticals – College of Pharmacy – The University of Iowa – Iowa City, Iowa 52242

6.4 Dose, Administration and Blinding

Two treatment groups will be evaluated in the trial: 1) Prednisone, and 2) Anakinra plus zinc

Participants will be instructed to begin dosing on day 1. Oral study medication should be taken with water as instructed on study drug label. Patients will receive two oral medications. Bottle 1 will be Prednisone or Placebo and will be taken daily on Days 1-30 per protocol. Bottle 2 will be Zinc Sulfate or Placebo and will be taken daily on Day 1-90 per protocol.
Participants must be instructed to swallow the capsule whole; they must not open capsule.
Subcutaneous study medication should also begin on Day 1 and be administered daily on Days 1 to 14.

6.4.1 Dispensing Procedures

On Day 0, after confirmation of participant eligibility and randomization, the investigator or designee will dispense study medication to the participant. Before leaving the clinic/hospital, the study staff will ensure that the participant fully understands the dosing instructions. We will dispense a sharps container for each subject discharged before day 14. The clinical trial site investigator may adjust the number of study medication dispensed to the participant based on clinical judgment and visit scheduling to ensure an adequate supply of study medication is available for dosing between clinic visits. An unscheduled distribution of study medication Anakinra/ Placebo may occur on Day 7 if needed to comply with medication changes necessitated by safety lab results.

6.4.2 Blinding

Study medication bottles will be labeled in a blinded manner to ensure that neither participant nor investigator is unblinded. Examples of labels for the initial dispense:

BOTTLE 1, DAY 0

Pharmacy Name, Address, Phone Number
Caution: Federal law prohibits transfer of this drug to any person other than the patient for whom prescribed.

Rx: Number Prescriber:
Patient Name MRN:

Take 1 capsule by mouth daily on Days 1 through 30.

STUDY Prednisone / Placebo 40 mg Capsule
Quantity: 30 Capsules IRB: 1904-650121/AlcHepNet-02
Date Filled:
Do Not Use After: Date

BOTTLE 2, DAY 0

Pharmacy Name, Address, Phone Number
Caution: Federal law prohibits transfer of this drug to any person other than the patient for whom prescribed.

Rx: Number Prescriber:
Patient Name MRN:

Take 1 capsule by mouth daily on Days 1 through 90.

STUDY Zinc Sulfate / Placebo 220 mg Capsule
Quantity: 90 Capsules IRB: 1904-650121/AlcHepNet-02
Date Filled:
Do Not Use After: Date

SYRINGE BAG, DAY 0

Pharmacy Name, Address, Phone Number
Caution: Federal law prohibits transfer of this drug to any person other than the patient for whom prescribed.

Rx: Number Prescriber:
Patient Name MRN:

Inject 1 syringe subcutaneously every morning daily on Days 1 through 14.

STUDY Anakinra / Placebo 100 mg/0.67 mL Prefilled Syringe
Quantity: 90 Capsules IRB: 1904-650121/AlcHepNet-02
Date Filled:
Do Not Use After: Date

6.4.3 Missed Doses

Participants who miss a dose of study medication should be instructed to take it later the same day, as soon as they remember, but no more than 12 hours past normal dosing time for the injectable study medications.-'Missed' doses should not be taken on a subsequent day (i.e., the participant should not take more than the prescribed daily dose). Participants will make note of missed dose on medication compliance log.

6.4.4 Environmental Assessment

Is requested for categorical exclusion for this proposed clinical trial as provided for in 21 CFR.25.31(e) in that the drug shipped under this notice is intended to be used in clinical trials in which the amount of waste expected to enter the environment may reasonably be expected to be non-toxic.

6.5 Randomization

6.5.1 Method of Assigning Participants to Treatment Groups

The trial will be conducted in a double-blind, placebo-controlled manner. Participants will be randomized to the two treatment groups in equal proportions. The randomization plan will be stratified by site and MELD score. Blocks of size 4 will be generated randomly based on a predefined randomization code generated by the AlcHepNet IU data coordinating unit (DCC- IU). Both participants and investigators will be blinded to the randomization plans.

The investigator or designee will be required to register the participant into the REDCap system (AlcHepNet's data management system) and will provide participant data to properly randomize and allocate the participant to treatment. A randomization number will be assigned and study medication dispensing information (e.g., arm assignment) will be provided.

In the unlikely event of a system failure, the randomization will occur outside of REDCap according to the back-up emergency plan detailed in the Manual of Operations. IU DCC will maintain at all times a current, off-line randomization list and will be able to act as the Emergency Support team until the system is restored to its full capacity.

6.5.2 Site Numbers

Each trial site selected to participate in this trial will be assigned a Site Number by the AlcHepNet consortium. The Site Number will be used to categorize participant data and to identify the site and or investigator within trial documents.

6.5.3 Screening Numbers

At the Screening Visit, after the participant has provided written informed consent, the participant will be assigned a unique 5-digit participant ID number.

6.6 Holding and Unblinding Procedures

The site pharmacist will be unblinded to randomization codes and corresponding treatment assignment. Additionally, the randomization codes and corresponding treatment assignment will be maintained by the AlcHepNet DCC-IU. The biostatisticians performing the analyses will also be blinded. The study designated physician (Dr. Gawrieh or his designee), or the individual investigators will not have access to this information. In the event that a medical emergency necessitates unblinding (i.e., in situations where knowledge of the blinded treatment is necessary for further medical management of the participant), the site investigator (or his/her designee) will contact the designated study physician to discuss the rationale for unblinding. If there is a difference of opinion between the designated study physician and the site investigator, then the site investigator will defer to the sub-investigator who retains the final right to seek unblinding. The designated sub-investigator will communicate with the DCC-IU about the decision to unblind the participant and then the DCC-IU will unblind the participant by interacting directly with the sub- investigator.

The Data and Safety Monitoring Board (DSMB) will be able to unblind participants. Cases of premature unblinding (as noted above) will be reviewed by the DSMB.

Access to randomization codes and corresponding treatment assignment will also be made available to the designated study physician who is responsible for reporting SAEs and suspected unexpected serious adverse reactions (SUSARs) to the Regulatory Authorities and should be accessed only in the event that reporting necessitates unblinding. Aside from the IU IDS pharmacist who conducts the drug over labeling, site pharmacy staff will be unblinded to the subject's treatment. Treatment will be assigned through REDCap. Pharmacy staff will be responsible for blinding the subject's treatment before dispensing. Pharmacy will be trained to maintain the blind. No other site personnel will have direct access to blinded participant treatment codes until all trial data have been entered onto the trial database and validated and the database locked.

6.6.1 Treatment and holding/stopping rules for infection and sepsis

Leukocytosis is common in AH and may be further exacerbated by administration of prednisone. Such iatrogenic rise in leukocytosis may alarm participant care teams who are blinded to the treatment arm and may raise concern for development of infection or sepsis.

While many AH participants may develop the systemic inflammatory response syndrome (SIRS) (defined as two or more abnormalities in temperature, increased heart rate, respiration, or white blood cell count), SIRS may not always be indicative of infection in participants with AH and may be reflective of the dysregulated inflammatory state with AH.

Drug Holding Rules (Day 0-Day 30):

If one of the following criteria met, the study drugs should be held for 3 days:

1. Documented Infection
    - Pneumonia defined as new infiltrate by CXR or chest CT scan
    - Positive cultures (blood, ascites) for bacteria or fungus
    - Positive fungal cultures in urine (> 50,000 colonies/ml)
    - CNS infection defined as positive cultures or WBC > 5 in CSF
    - Severe soft tissue or bone infections including clinical diagnosis of cellulitis
2. SIRS (defined as two or more abnormalities in temperature, increased heartrate, respiration, or white blood cell count) with increase in SOFA score ≥2 points.

Participants will be reassessed on 3rd day to determine if study drugs could be resumed or should be stopped.

Drug Stopping rules (Day 0-Day 30):

Participants will be reassessed on third day of the study drugs hold: If infection clears and participant stabilizes, study drugs will be resumed.

1. For participants with documented infection: If infection is responsive to antibiotics and participant is stabilized or improved, study drugs may be resumed at the discretion of the clinical care team. If there is no improvement after 3 days, study drugs will be stopped.
2. For participants meeting only SOFA+SIRS criteria for stopping: if cultures of urine, blood, ascites, stool (including C. diff) are negative and CXR is unchanged, study drugs may be resumed at the discretion of the clinical care team.
3. If the participant has a second hold of study drugs due to infection/SOFA+SIRS criteria, study drugs will be stopped.
4. Participants in either arm who have a Lille score of >0.45 on day 7 will stop bottle 1 (prednisone/ placebo) and will continue with bottle 2 (zinc/zinc placebo.)

Drug stopping Rules (Day 0-Day 30):

- No improvement or stabilization after holding study drugs for 3 days
- 2nd hold of study drugs due to infection/SOFA+SIRS criteria
- Development of severe leukocytosis (≥100,000/mm3).

Treatment holding and stopping rules for Drug Induced Liver Injury (DILI)

Identifying the instances of suspected drug induced liver injury (DILI) in individuals with underlying liver disease with abnormal liver biochemistries is difficult and this issue becomes far more challenging in individuals with severe alcoholic hepatitis because (a) aminotransferases and alkaline phosphatase may not reliably increase due to burnt out hepatocytes in this population and (b) liver test worsening may be due to myriad of other reasons including worsening of underlying alcoholic hepatitis or sepsis.

Therefore, we propose the following:

1) When to hold or discontinue the study medicine in a participant? In individuals with severe alcoholic hepatitis participating in our RCT, we will hold the study medicine if MELD increases by 5 from baseline or Child's score increases by 3 points from baseline plus >3-fold increase in ALT from baseline on two consecutive occasions. These instances may represent either lack of therapeutic efficacy or DILI or some other etiology. If there is a competing etiology and DILI can be excluded, study drug can be resumed within 3-4 days. However, if DILI cannot be excluded, then the study drug will be permanently discontinued. Additional reasons for study drug discontinuation include unexplained elevation in ALT > 5 x baseline or > 500 U/L on two consecutive occasions or unexplained elevation in Alk P > 3 x baseline or >500 U/L on two consecutive occasions. It should be noted that cholestatic DILI may have a devastating impact on participant survival in individuals with advanced liver disease.

2) When to terminate the trial due to DILI concerns? The Data Safety Monitoring Committee (DSMB) should carefully monitor the number of discontinuations due to MELD/Child's Score worsening in the three treatment groups. Since this trial is not powered for rare adverse events, one should not look for statistically significant imbalance among treatment groups, but the DSMB should consider numerical imbalances that its members deem clinically significant. While it may be straight forward in some instances (for example, 2 discontinuations in groups A but 5 discontinuations in group B) but in other instances it may not be as clear cut and one may continue the trial for a growing trend. In any case, the DSMB is advisory in nature and it makes a recommendation to the Sponsor which is the NIAAA for this trial. In addition to seeking the AlcHepNet Steering Committee input, the NIAAA may seek external input to assist in its deliberation if the DSMB recommends that the clinical trial must be terminated due to safety concerns.

Treatment holding/stopping rules for laboratory abnormalities

- If platelet counts drop to < 40,000 while on injectable study drugs, injectable study drugs will be held and resumed when platelet count is ≥40,000.

- Worsening renal function

As all patients are randomized to treatment arms while hospitalized, the site's dispensing pharmacy will check the GFR before dispensing the study drugs for the day. Pharmacy will enter the daily lab values into a REDCap-based decision support form, which will aid the pharmacy by providing guidance on daily dispensation. This REDCap form is in addition to the pharmacy manual of procedures, which also provides guidance on daily dispensation. For weekend coverage, the GFR and platelet values from the Friday labs may be used to generate the dispensation schedule at some sites. If the subject's GFR has dropped to <30 but ≥20 and the subject is in the anakinra group, Redcap will provide instructions for pharmacy to dose reduce to every other day by providing anakinra placebo one day, alternating with active anakinra the following day. If the GFR drops to < 20 mL/min, the instructions from REDCap will hold active anakinra and substitute with anakinra placebo

When the patient is discharged and now outpatient, all injectable drugs will be dose reduced and administered every other day when the GFR drops to <30 but ≥20 mL/min. All injectable drugs

will be stopped if GFR drops to <20 mL/min.

6.6.2 Unscheduled, Emergency Unblinding

6.6.2.1 Rules for unblinding participants for leukocytosis and concern for infection/sepsis (Day 0-Day 30):

In this placebo- controlled, blinded and randomized trial, Principal Investigators, enrolled participants, and any study team member who will/is interpreting data will remain blinded.

1. If the participant develops leukocytosis >) WBC > 30 x 10^9/L or WBC > 30,000/mm^3, the clinical care team may request unblinding if the team believes that knowing the treatment would impact care decisions such as starting antibiotics.

2. If the participant develops hypotension (systolic BP < 90) and/or requires transfer to an ICU, the clinical care team may request unblinding if the team believes that knowing the treatment would impact care decisions such as administration of pressors, fluids or other supportive therapy.
3. If the participant develops any other serious adverse event that is, in the opinion of the investigator, probably related to one of the study drugs, the clinical care team may request unblinding if it would impact care decisions.

Who to unblind?

- Only sub-investigator
- Clinical care team
- DCC contact

Unblinding procedure:

1. Each site will designate a sub-investigator who can be contacted if the participant care team has concerns about rising leukocytosis
2. The contacted sub-investigator will communicate with IU DCC to unblind the participant then inform participant care team of the study drug/s participant is receiving
3. IU DCC will build this into the database system and keep track of unblinded participants
4. The decision to begin antibiotics for possible infection is left to the judgment of the clinical care team.

6.7 Prior and Concomitant Medications or Procedures

Relevant information about all concomitant drugs (including prescribed, over the counter, or herbal preparations) taken prior to 30 days of screening and during the trial must be recorded in the source documents and CRF, as well as any dose or dose regimen changes that occur during the trial. The clinical care team should be advised not to give supplemental zinc to subjects in the trial as that could result in an excessive dosing particularly in subject with renal dysfunction. No interaction between zinc and anakinra is reported. Zinc may interfere with absorption of certain oral antibiotics such as quinolones and tetracyclines. To avoid potential interference with concomitant medications, we will administer the morning blinded capsules (zinc/zinc placebo or prednisone/prednisone placebo) at least 3 hours apart from other medications the participant will take.

6.8 Treatment Compliance

The investigator should assess the participant's compliance with dosing of study medication on an ongoing basis, at least at each visit, and confirm by conducting drug accountability (i.e., count of returned capsules, syringes).

Participants should be instructed to retain all study medication, even if empty, and to return them to the investigator at the subsequent visit. The investigator or designee should perform drug accountability and, if applicable, follow up with the participant to retrieve any study medication that has not been returned.

If the investigator has concerns about a participant's dosing compliance s/he should discuss this with the participant, assess the reasons for noncompliance and the likelihood that the participant will remain noncompliant, and notify the designated study physician accordingly. Continued trial eligibility should be assessed based on the participant's compliance with study medication dosing and clinic visits.

6.9 Study Medication Accountability and Retention

IU Health Investigational Drug Services representative will send study medication to the trial sites under appropriate storage conditions. All shipments of study medication should be unpacked, and the contents reviewed immediately upon receipt. The pharmacist or designee should verify the study medication against the shipment documentation. The pharmacist or designee should contact the designee of IU Health Investigational Drug Services immediately to report any concerns regarding the shipment.

All study medication will be provided for use only in this trial and is not to be used for any other purpose. The investigator or designee will maintain a full record of study medication accountability, including records of individual participant dispensing and final return or disposition.

The monitor from DCC-IU, will review accountability records against study medication dispensed and that remaining in stock, during on-site monitoring visits and when the trial is completed, or if it is prematurely terminated. The CRA will retrieve documentation detailing and confirming the return or destruction of the study medication.

6.10 Trial or Site Termination

The AlcHepNet consortium reserves the right to terminate the trial at any time. It is normal procedure to review the emerging clinical and safety data. As a result of such a review or a recommendation by the DSMB, it may be necessary to stop the trial before all participants have completed the trial.

It is the responsibility of the individual site's PI to report to the Western Institutional Review Board (WIRB) of discontinuation of the trial and the reason for doing so in a timely fashion according to institutional policies.

7. TRIAL PROCEDURES

7.1 Screening

7.1.1 Informed Consent

The investigator or designee will explain the nature, purpose and risks of the trial to the participant or legally authorized representative (LAR/guardian) and will provide him/her with a copy of the written information and informed consent form (ICF). The participant or LAR will be given sufficient time to consider the trial before deciding whether or not to participate. The participant or LAR will be informed that participation is voluntary and that her/his future medical treatment will not be compromised by participation in the trial and that s/he can withdraw from the trial at any time. The participant or LAR must be willing and able to provide written informed consent before entering the trial. The investigator or a medically qualified designee is responsible for administering and documenting the written informed consent of the participant.

7.1.2 Participant Identification Assignment

After the participant has met eligibility criteria and enrolls into the study, the participant will be assigned a unique 5-digit ID number. This 5-digit number will be used to identify the participant throughout the trial and during the data analysis.

7.2 Visit Procedures

Participants should be instructed to fast, when possible, overnight (at least 8 hours) immediately prior to all at- clinic visits, Screening Visit. While fasting, water is permitted.

7.2.1 Screening Period – Fasting for at least 8 hours, when possible

The screening period will consist of ≤ 7 days prior to the randomization visit (Day 0). During the screening period, participants' eligibility criteria must be confirmed. ***Patients with clinical suspicion of sepsis will have an initial run-in period of 48 hours while being screened for eligibility for enrollment. Patients with evidence of sepsis at the end of this run-period will be excluded from participation.*** Participants or LAR must sign an informed consent prior to initiating any of the screening procedures. Participants who do not fulfill all eligibility criteria cannot continue in the protocol (screening failures).

Screening period procedures are as follows:

- The participant or LAR is to review and sign the informed consent document. Informed consent must be obtained from the participant or LAR before performing any trial related procedures, including screening procedures
- Verify inclusion and exclusion criteria for eligibility
- Assess MELD score.
- Physical exam
- Collect medical history including the alcohol consumption specifics
- Have participants completed the TLFB questionnaire, CLDQ, and AUDIT
- Record vital signs, including height and weight
- Record prior (within 30 days of Day 0) and current concomitant medications
- Perform a baseline standard 12-lead electrocardiogram (ECG), if not already done as standard of care within 7 days of screening
- Assess availability of liver biopsy samples (if applicable) which may have been obtained as part of standard of care
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests
- Perform a serum pregnancy test in females of childbearing potential
- Perform a PCR test for COVID-19, if not done as SOC.

**The participant should be reminded that fasting (8 hours) is requested, when possible, prior to his/her next visit.

7.2.2 Day 0 Procedures (Randomization) – Fasting for at least 8 hours, when possible

- Collect medical history including the alcohol consumption specifics
- Perform a physical examination
- Record vital signs, including weight
- Assess and record any pretreatment emergent AEs
- Record prior (within 30 days of Day 0) and current concomitant medications, noting any changes from screening, if applicable. Perform a urine pregnancy test in females of childbearing potential, if greater than one day has elapsed since screening pregnancy test.
- Confirm inclusion and exclusion criteria for eligibility
- Assess MELD score~~s~~
- Randomize the participant only if s/he meets all inclusion criteria and no exclusion criteria
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests, **if greater than one day has passed since screening**.
- Obtain biospecimens for specimen banking and measuring anakinra trough levels as outlined in Table 1.
- Dispense study medication and instruct the participant on medication dosing and administration. Instruct the participant to swallow the capsules whole; s/he must not open, chew, divide, open or crush the capsule. Verbally instruct participant and caregiver (if applicable) on subcutaneous injection procedures and provide IRB-approved instruction handout
- The participant should be reminded that fasting (8 hours) is requested, when possible, prior to his/her next visit.

7.2.3 D3 Procedures (± 2 days) –– Fasting for at least 8 hours, when possible

- Perform a physical examination
- Record vital signs, including weight
- Assess and record any emergent AEs
- Record any changes to concomitant medications
- Assess MELD scores
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests
- Obtain biospecimens for specimen banking and measuring anakinra trough levels as outlined in Table 1.
- The participant should be reminded that fasting (8 hours) is requested, when possible, prior to his/her next visit.
- Assess medication compliance form

7.2.4 D7 Procedures (± 2 days) – Fasting for at least 8 hours, when possible

- Perform a physical examination
- Record vital signs, including weight
- Assess and record any emergent AEs
- Record any changes to concomitant medications
- Assess MELD and Lille scores.
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests
- Obtain biospecimens for specimen banking and measuring anakinra trough levels as outlined in Table 1.
- The participant should be reminded that fasting (8 hours) is requested, when possible, prior to his/her next visit.
- Assess medication compliance.
- Calculate Lille score. If >0.45, participant should stop the bottle Prednisone/placebo.
- Dispense study medication if required by safety lab results (outpatient only.)

7.2.5 Day 14 procedures (± 2 days) –– Fasting for at least 8 hours, when possible

- Collect medical history including the alcohol consumption specifics
- Perform a physical examination, including a urine pregnancy test
- Record vital signs, including weight
- Assess and record any emergent AEs
- Record any changes to concomitant medications
- Assess MELD scores
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests.

- Obtain biospecimens for specimen banking and measuring anakinra trough levels as outlined in Table 1.
- The participant should be reminded that fasting (8 hours) is requested, when possible, prior to his/her next visit.
- Assess medication compliance.

7.2.6 D28, D60 and D 90 Procedures (± 7 days) -– Fasting for at least 8 hours, when possible

- Collect medical history including the alcohol consumption specifics
- Perform a physical examination
- Record vital signs, including weight
- The participant is to complete the Alcohol Timeline Follow back
- Assess and record any emergent AEs
- Record any changes to concomitant medications
- Assess MELD scores.
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests.
- Obtain biospecimens for specimen banking as outlined in Table 1.
- The participant should be reminded that fasting (8 hours) is requested, if possible, prior to his/her next visit.
- Assess medication compliance

7.2.7 Follow up phase D180 (± 7 days) – Fasting for at least 8 hours, when possible

- Collect medical history including the alcohol consumption specifics
- Perform a physical examination
- Record vital signs, including weight
- The participant is to complete the Alcohol Timeline Follow back
- Assess and record any emergent AEs
- Record any changes to concomitant medications
- Assess Liver Scores: MELD
- Obtain blood samples for hematology, hepatic function panel, BMP, and coagulation tests.
- Obtain biospecimens for specimen banking as outlined in Table 1.

7.2.8 Missed Visits

Participants who miss a visit will receive a phone call from the coordinator who will read and collect information from the phone script. If a person is unable to be reached by phone, a medical record review will take place.

7.2.9 Early Termination Procedures

Participants who withdraw consent or discontinue treatment prematurely during the Treatment Phase should complete the assessments required on Day 60, if patient is willing. Participants who discontinue prematurely during the Follow-up Phase should complete the assessments required on Day 180.

7.2.10 Unscheduled Safety Visits

The investigator may schedule an at-clinic Unscheduled/Safety Visit at any time if clinically warranted. Unscheduled and/or repeat assessments may be conducted. As appropriate, the designated study physician should be contacted.

7.2.11 Specimen Banking

Specimens to be banked include genomic DNA, blood mononuclear cells, blood (PBMC, serum and plasma), saliva (only until an adequate number of samples are collected determined by PIs), stool (when possible), urine, and liver biopsy when available.

Type specimen collection is site specific.

8. EFFICACY EVALUATIONS Primary Endpoint Survival at 90 days Secondary Endpoints

- Change in Lille score, change in MELD score, development of AKI, multi-organ failure, SIRS, transfer to ICU, changes in liver function will all be evaluated at 7, 30 and 90 days.

- Organ dysfunction: We will measure changes in Sequential Organ Failure Assessment (SOFA) scores and proportions requiring hemodynamic support for MAP < 65 mm Hg and lactate > 2 mmol/l, renal replacement therapy or mechanical ventilation. We will also modify and re-evaluate the SOFA score without platelet counts given that these are usually low in AH.

- Infections and Sepsis: It is now recognized that the endpoints and case definitions related to sepsis may vary depending on what these will be used for (clinical care, research, surveillance, or quality improvement). Given the central role of sepsis as a driver of outcomes, we will not only measure the types of infection but also identify the proportions of those with sepsis, septic shock or quick-SOFA (qSOFA) criteria based on SEPSIS-3 guidance[2,39]. We will also capture the key parameters needed for various sepsis related endpoint construction aligned by the recent guidance from the European Drug Development Hub (http://eddh-cro.wixsite.com/fdtsfv).

- Renal dysfunction: In alignment with guidance from the acute disease quality initiative[56], we will quantify AKI development and its progression through persistent AKI, acute kidney disease to chronic kidney disease.

- Need for care escalation: Proportion of participants requiring transfer to ICU for care
- Indicators of gut permeability (endotoxin and bacterial 18S DNA) and pro- inflammatory cytokine/chemokines (TNFa, MCP1, IL-6, IL-1ß) will be assessed in serum/plasma samples.
- Survival at day 30 and 180.

Limitations of mortality as an endpoint in AH and plans to overcome these

All-cause mortality is most relevant when mortality is substantial and attributable to a single mechanism in a homogeneous population. Participants with severe AH are highly heterogeneous with respect to disease severity, end organ involvement and the care they receive. Moreover, as in sepsis and heart failure, death involves failure of multiple organs (liver, heart, kidney, lungs) making it hard to ascertain which one is the primary cause of death. This has led to development and regulatory acceptance of composite endpoints including mortality and key parameters driving mortality for acute heart failure trials. We will leverage these guiding principles to construct a novel and innovative key secondary composite endpoint which will include death or worsening of the SOFA score[4,5] by ≥ 2 points and worsening of modified MELD score by ≥ 2 points. These are associated with mortality and capture the organ recruitment that marks progression of AH towards death. This endpoint will also be validated with respect to reliability, construct-, criterion- and content validity to establish this as a primary endpoint in future trials. We will also model changes in SOFA scores and MELD scores against mortality to further optimize this endpoint and cross-validate it in the observation cohort.

9. SAFETY EVALUATIONS

9.1 Adverse Events

Adverse event (AE) data will be collected from Day 0 through the double-blind phase, until the participant fully completes her/his trial participation at the Follow Up visit.

9.2 Physical Examination and Vital Signs

To assess for clinical findings, the investigator or designee will perform a physical examination at the time points specified in the Schedule of Trial Procedures (Table 1). All above physical exams may be extracted from medical records if patient is inpatient. This includes height only at Screening. The physical examination must include the following:

9.2.1 Physical Examination

- General appearance
- Weight
- Eyes
- Ears, nose, and throat
- Neck
- Respiratory system
- Cardiovascular system
- Abdominal region
- Mental status
- Neurological system

9.2.2 Vital signs

The following vital signs will be assessed at indicated visits: body temperature, sitting heart rate, sitting blood pressure (BP), respiratory rate, body weight in kilograms without shoes, and height in centimeters (screening only). When taking heart rate and BP readings, participants should be seated quietly for a minimum of 3 minutes before the reading is taken.

9.3 Clinical Laboratory

Blood samples for serum chemistry, hematology, and coagulation will be collected at every visit as detailed in the Schedule of Trial Procedures. The number and volume of samples to be collected at each visit will be detailed in a separate document. These tests will be conducted at local laboratories at 8 participating sites.

All participants with laboratory tests containing clinically significant abnormal values should be followed regularly until the values return to normal ranges, until a valid reason for the AE (other than study medication related AE) is identified, or until further follow up is deemed medically unnecessary.

9.4 Other Safety Related Clinical Outcomes

The following clinical outcomes will be assessed:

- Death (from hepatic and non-hepatic related causes)
- Complications of portal hypertension including gastroesophageal bleeding, interventions to manage variceal bleeding (e.g., variceal banding/sclerotherapy or TIPS placement) and diuretic resistant ascites
- Complications of Cirrhosis: new-onset ascites, hepatic encephalopathy, hepatorenal syndrome (type I or II), and spontaneous bacterial peritonitis).
- Hospitalization
- Change to a higher level of care (i.e., floor to ICU) is an SAE

Each of these events including hospitalizations will be adjudicated by the site investigator as expected as part of the natural history of severe AH, or unexpected (e.g., suicide, motor vehicle accident, or myocardial infarction).

10. ADVERSE EVENTS

10.1 Definition of Adverse Events & Reporting Period for Adverse Events

Safety will be assessed in terms of AEs. All AEs, whether observed by the investigator, reported by the participant, noted from laboratory findings, or identified by other means, will be recorded from Day 0 until the participant completes trial participation (D-180).

AEs are defined as any untoward medical occurrence associated with the use of the study medication in humans, whether or not considered related to study medication. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with any use of the study medication, without any judgment about causality and irrespective of route of administration, formulation, or dose, including an overdose. For this trial, study medication refers to Anakinra, zinc, Prednisone, or placebo in the double-blind phase.

AEs include but are not limited to: (1) a worsening or change in nature, severity, or frequency of condition(s) present at the start of the trial; (2) participant deterioration due to primary illness; (3) intercurrent illness; and (4) drug interaction.

Participants should be questioned in a general way, without leading the participant or asking about the occurrence of any specific symptom. The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. The diagnosis and not the individual signs/symptoms should be documented as the AE. For example, if the underlying disease process is a stroke, it would not be appropriate to record the AE by describing the symptoms "sudden numbness, dizziness, and difficulty speaking." The AE medical term of "stroke or cerebrovascular accident" should be recorded as it more accurately describes the AE. Following questioning and evaluation, all AEs, whether believed by the investigator to be related or unrelated to the study medication, must be documented in the participant's medical records, in accordance with the investigator's normal clinical practice and on the AE source Document. Each AE is to be evaluated for duration, intensity, frequency, seriousness, outcome, other actions taken, and relationship to the study medication.

10.1.1.1 Pretreatment Event(s)

Untoward events that occur during screening phase (prior to randomization) should be recorded as medical history.

10.1.2 Severity of AEs

AEs must be graded for severity (i.e., intensity). A severity category of mild, moderate, or severe, as defined in Table 2, must be entered on the AE source document. It should be noted that the term "severe" used to grade intensity is not synonymous with the term "serious." For example, severe headache is a severe AE, but may not be a serious AE. The assessment of severity is made regardless of study medication relationship or of the seriousness of the AE.

Table 2: Clinical Description of Adverse Event Severity

Grade	Clinical Description of Severity
1 = Mild	Causing no limitation of usual activities; the participant may experience slight discomfort.
2 = Moderate	Causing some limitation of usual activities; the participant may experience annoying discomfort.
3 = Severe	Causing inability to carry out usual activities; the participant may experience intolerable discomfort or pain.

10.1.3 Relationship of AEs to Study Medication

The investigator will document her/his opinion of the relationship of the AE to treatment with study medication using the criteria outlined in Table 3 An AE for which there is a 'reasonable possibility' that the study medication caused the AE is otherwise referred to as suspected adverse reaction (SAR). 'Reasonable possibility' means there is evidence to suggest a causal relationship between the study medication and the AE.

Table 3. Relationship of AEs to Study Medication

Relationship	Description
Definite	A reaction that follows a reasonable temporal sequence from administration of the drug or in which the study medication level has been established in body fluids or tissue; that follows a known or expected response pattern to the suspected drug; and that is confirmed by improvement on stopping or reducing the dosage of the study medication, and reappearance of the reaction on repeated exposure.
Probable	A reaction that follows a reasonable temporal sequence from administration of the study medication; that follows a known or expected response pattern to the suspected study medication; that is confirmed by stopping or reducing the dosage of the study medication; and that could not be reasonably explained by other factors.
Possible	A reaction that follows a reasonable temporal sequence from administration of the study medication; that follows a known or expected response pattern to the suspected study medication; but that could readily be produced by a number of other factors.
Unlikely	A reaction that does not follow a reasonable temporal sequence from administration of the study medication; that does not follow a known or suspected response pattern to the suspected study medication; and that could reasonably be explained by known characteristics of the participant's clinical state.
Not Related	Any event that does not meet the above criteria.

10.2 Serious Adverse Events

10.2.1 Definition of a Serious Adverse Event

An adverse event or suspected adverse reaction is considered 'serious' if, in the view of the investigator, it results in any of the following outcomes:

(a) Death;

(b) Is life threatening;

(c) Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;

(d) A congenital anomaly/birth defect;

(e) Requires in-participant hospitalization or prolongs an existing hospitalization;

(f) An important medical event that may jeopardize the participant or participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

10.2.2 Serious Adverse Events (SAE) Classifications Common in Severe AH

Acute kidney injury:

- Increase in creatinine of 50% above baseline over a period of 7 days
- Increase in creatinine of 0.3 mg/dl within a period of 48 hrs.
- Onset of renal failure requiring dialysis

Sepsis:

- Life-threatening organ dysfunction caused by a dysregulated host response to infection
- An increase in SOFA score of 2 points of more
- Note: most participants with severe AH have 4 points based on bilirubin only

Infection:

- Pneumonia defined as new infiltrate by CXR or Chest CT scan not explained by "fluid overload"
- Positive blood cultures for bacteria or fungus, not suspected as contaminant
- Positive urine fungal culture > 50,000 colonies/ml
- Positive urine bacterial culture > 100,000 colonies/ml (mixed flora is excluded)
- Soft tissue or bone infections including cellulitis or abscess documented by exam or scan
- CNS infection defined as positive culture of CSF or > 5 WBC/ml
- Ascitic fluid white cell count >500/ml or neutrophils>250/ml. with or without positive bacterial or fungal cultures

Decompensation of liver disease:

- New onset of ascites if not present on admission to study
- New onset of variceal hemorrhage
- New onset of hepatic encephalopathy (HE).

Hepatic failure defined as increase in MELD score ≥ 5 on two consecutive occasions

Cerebral failure defined as worsening HE ≥ Grade 3 if hepatic encephalopathy present on admission

Renal failure defined as serum creatinine ≥3 milligrams per liter or requirement for renal replacement therapy despite adequate volume replacement

Respiratory failure defined as need for BIPAP therapy or ventilation

Circulatory failure defined as shock or requirement of vasopressor support to maintain mean arterial pressure ≥ 60 mm Hg despite adequate volume replacement

Multiple organ failure defined as failure ≥2 organs

Note: Sepsis will often be an SAE that precedes documented infection by 1-5 days. If sepsis is reported as an SAE and infection is never documented, then it should be classified separately, i.e., sepsis with documented infection or sepsis without documented infection.

Sepsis can be diagnosed by minor changes in lab parameters such as decrease in platelet count from 160,000 to 90,000 or increase in creatinine to 1.3 and drop in platelet count to 100-150,000 from normal on admission. However, these changes may also occur due to worsening liver disease without infection as a cause.

10.2.3 Reporting SAEs

In agreeing to the provisions of this protocol, the investigator accepts all legal responsibilities for immediate reporting of SAEs to the study designated physician (Dr. Gawrieh or his designee) as described below.

Table 4: Requirements for Reporting AEs to Various Stakeholders

	WIRB and Local IRB	NIAAA	DSMB	Other centers	Drug Company	FDA
AEs	Continuing Review	Annual Report	Every 6 months	DSMB reports (every 6 months)	DSMB reports (every 6 months)	Annual Report
TEAEs	Continuing Review	Annual Report	Every 6 months	DSMB reports (every 6 months)	DSMB reports (every 6 months)	Annual Report
SAEs	Continuing Review	2 working days	Every 6 months	DSMB reports (every 6 months)	DSMB reports (every 6 months)	Annual Report
SUSAR	5 days	2 working days	2 working days	2 working days	2 working days	7 days if death 15 days if other
Fatality	If unrelated Follow SAE timeline If related follow SUSAR timeline					

AE: Adverse Event; TEAS: Treatment Emergent Adverse Event; SAE: Serious Adverse Event; SUSAR: Serious Unsuspected Adverse Reaction; DSMB: Data and Safety Monitoring Board

The study designated physician for AlcHepNet - 02 is Samer Gawrieh, MD, who as the IND holder will assume the responsibility of monitoring safety concerns internally and serve as the liaison between the AlcHepNet and the DSMB.

All SAEs must be reported to the DCC-IU (i.e., within 2 working days) after the investigator identifies the SAE. SAEs should be entered into the REDCap system within 2 working days of the investigator becoming aware of the event. An initial report by telephone should then be followed, as soon as possible, by completion of the SAE CRF. Any supporting source documentation should be emailed to the assigned contact information as soon as possible. At a minimum the following information should be provided at the time of the initial report: 5-digit Study ID, a description of the event, at least one criterion classifying the event as Serious and the name and title of the reporting individual. Additionally, judgment of causality by the investigator must be provided as soon as possible to ensure timely reporting to regulatory authorities.

Following the initial report, any additional information obtained by the investigator about the SAE must be reported promptly to the DCC-IU.
The site investigator will assess whether the event is causally related to the study medication. The study designated physician (Dr. Gawrieh) will consider the investigator's assessment, however, since Dr. Gawrieh holds the IND and is primarily responsible for the overall safety of the participants, his causality assessment will take precedence over that of the site investigator. The study designated physician will determine if an SAE meets the criteria for an IND safety report (suspected, unexpected serious adverse reaction (SUSAR) in accordance with the FDA regulations, 312.32(c)(1)(i).

We will monitor safety closely, including all SAEs, AKI, and infection. We will provide unblinded data on SAEs including infections and AKI to the DSMB on monthly basis and hold the first DSMB after 3 months from the start of enrollment. We also propose a risk-based monitoring (RBM) approach which will create a more efficient and timely monitoring of accumulating data on the adverse events of interest, namely AKI and infections. The FDA has issued a final guidance in 2013 (Guidance for Industry. Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring. August 2013) with a follow-up draft guidance in March 2019 (A Risk-Based Approach to Monitoring of Clinical Investigations. Questions and Answers. Guidance for Industry) that indicated the acceptability of RBM in studies with FDA oversight. While the details and implementation are left to the trial investigators, the overall concepts are included in these two documents – and in the Transcelerate RBM Position Paper (available at https://www.transceleratebiopharmainc.com/wp-content/uploads/2013/10/TransCelerate-RBM-Position-Paper-FINAL-30MAY2013.pdf). The essential elements of the study-specific AE/SAE monitoring plan would be for the RBM tools to interface with the main REDCap database so that any new AEs or SAEs would be immediately passed to the data driven RBM tools to determine if a trigger threshold has been reached. This interaction between receipt of data by REDCap and the comparison of the data to the triggers (and reporting of it on study dashboards) by the RBM tools will be virtually instantaneous. In addition, levels of thresholds can be set so that data trends of SAEs accumulating within treatment groups can be identified before significance is reached. **A complete monitoring plan, including RBM details, will be developed for reviewby the FDA and the DSMB within three months of study initiation**. We will also include Statistical Process Control (SPC) techniques in the monitoring plan to ensure that appropriate statistical controls are also utilized during the study.

10.2.3.1 Additional Principal Investigator Responsibilities for SAEs

The safety data recorded in the source document represent the official record of all AEs and SAEs reported in the trial. The investigator should comply with requests by the designated study physician to record the SAE on the participant's AE source document and, if necessary, provide a copy of this source document to the DCC-IU. Other supporting documents such as radiology reports, hospital discharge summaries and autopsy reports should also be provided, when appropriate, in anonymized fashion. Additionally, upon request by the designated study physician, the investigator should provide input into the SAE narrative and provide timely information to ensure prompt follow up and closure of the SAE report.

The investigator and supporting personnel responsible for participant care should discuss with the designated study physician any need for supplemental investigations of SAE(s). The results of these additional assessments must be reported to the designated study physician.

10.3 SAEs that are Anticipated to Occur in the Study Population (Expected SAEs)

Certain SAEs are anticipated to occur in this study population at some frequency independent of

drug exposure. Examples of these expected SAEs include variceal bleeding, ascites, spontaneous bacterial peritonitis, jaundice, hepatorenal syndrome, or hepatic encephalopathy. These expected SAEs as well as any related hospitalization will not be expeditiously reported as IND safety reports but will be reported in the manner described above (Table 4.). However, if an aggregate analysis indicates that these events are occurring more frequently in the drug treatment group than in a concurrent or historical control group, the SAEs will be reported as an IND safety report (21 CRF 312.32(c)(1)(c)). Follow up of AEs

All AEs, including clinically significant laboratory values or physical examination findings relative to pretreatment assessments, must be followed during the trial until the AE resolves, is no longer of clinical concern, has stabilized or is otherwise explained, or the participant is lost to follow up.

AEs ongoing at the final visit that are deemed to be 'possibly, probably, or definitely" related or of other clinical significance must be followed for as long as necessary to adequately evaluate the safety of the participant or until the event stabilizes, resolves, or is no longer of clinical concern. If resolved, a resolution date for the AE should be documented on the CRF. The investigator must ensure that follow up includes any supplemental investigations indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, or consultation with other healthcare professionals, as considered clinically appropriate by the investigator.

Note: Some SAEs like AKI, GI bleeding, infections, etc. can be <u>both</u> expected and common

11. STATISTICAL METHODS

11.1 Analysis Sets

The primary analyses of 90-day mortality will be performed in an Intention-to-Treat (ITT) framework using survival analysis. Specifically,

(1) we will use all-cause mortality in the first 90-days as the primary outcome event of interest. We will compare the distributions of time to mortality between the two treatment arms. In the primary analysis, mortality event times greater than 90 days will be censored at the 90 days or at the time of liver transplant, whichever comes first. We will use the Logrank test to compare the survival functions of the two treatment groups. Cox regression analysis can be performed if it is necessary to control for the influences of unbalanced patient characteristics, such as MELD score, between the treatment groups. If evidence suggests a violation of the hazard function proportionality, we will conduct an analysis of the restricted mean survival time (RMST) of the first 90 days.
(2) as a secondary analysis, we will compare transplant-free survival in the first 90 days of treatment initiation as between the two treatment arms, as done in other clinical trials. We will perform logrank test, Cox regression model, and RMST analyses as appropriate.
(3) we plan to conduct one interim analysis and one final analysis for this trial. The interim analysis will be conducted when **129** patients, half of the enrollment number, complete the 90-day follow-up. We use the group sequential method to control the type 1 error rate of the trial.
(4) as another secondary analysis, we will examine the treatment effects in patients with baseline MELD scores ≤25 and >25 by assessing the equality of treatment effects of the anakinra in high and low MELD groups, we will test the interaction between the intervention indicator and baseline MELD score in the Cox regression model.

Complete details of the proposed statistical analyses, including a single interim analysis, can be found in the Revised Statistical Analysis Plan (SAP).

11.2 Determination of Sample Size

The primary analysis will be comparisons of 90-day mortality of Anakinra plus zinc vs Prednisone using survival analysis. Mortality event times greater than 90 days will be censored at 90 days or at time of liver transplant, whichever comes first. The experiment-wise significance level will be 5%. The most recent data from the AlcHepNet consortium suggest a 90-day mortality rate for prednisone is approximately 20%. We estimate that with a total of 258 participants (129 per treatment arm), we will be able to detect a clinically significant hazards ratio of 0.325, which translates to a 13% reduction in 90-day mortality, i.e., 80% survival in prednisone vs 93% in anakinra plus zinc groups with 85% power by using two-sided logrank tests. We use O'Brien and Fleming α-spending function to determine the efficacy stopping boundaries for the sequential tests. Specifically, if the test statistics of the log-rank test is greater than 2.963 or less than -2.963, we will claim a significant difference between the two treatments and stop the trial. Otherwise, we will continue enrolling more patients until the full sample size is reached and then the final analysis will be performed. For the final analysis, if the test statistic value is greater than 1.969 or less than -1.969, we will claim a significant difference between the treatments. Otherwise, we will declare that no statistically significant difference is detected.

Besides the group sequential tests, we have in place an early stopping rule for futility based on conditional power. At the time of interim analysis, we will calculate the conditional power, i.e., the probability of claiming difference at the end of the trial given the data at the interim analysis. We will stop the trial for futility when the conditional power is less than 0.1. In other words, based on data that become available at the interim analysis, assuming that the current trend continues, we should early stop the trial if the probability of declaring anakinra + zinc superior in the final analysis is low

(e.g., less than 10%). We use this early stopping rule to limit patient exposure to a potentially inefficacious treatment.

11.3 Safety Analysis

Descriptive statistics will be used to summarize safety data to allow a comparison between groups. Safety data, including AEs, clinical and laboratory observations and physical examination findings, and other safety related outcomes will be summarized by treatment group.

11.3.1 AEs

AEs will be labeled according to standard medical terminology using the case report forms provided. Summary tables of treatment-emergent AEs will be provided. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity following initiation of study medication. The incidence of TEAEs will be tabulated by system organ class and preferred term for each treatment group, and by severity and relationship to treatment. Tables of TEAEs leading to study medication discontinuation and SAEs will be provided.

11.3.2 Clinical Laboratory Evaluations

Descriptive statistics summarizing central laboratory data (hematology, chemistry, and coagulation) will be presented for all trial visits. Changes from pretreatment to each trial visit will also be summarized by treatment group/arm.

a. The primary analyses of 90-day mortality will be carried out in an ITT framework using the logrank test or Cox regression. Time-to-death will be censored at 90 days or at liver transplant, whichever comes first. In addition to the logrank test, we will perform Cox regression analyses. The models will include baseline MELD score as well as other covariates deemed to be unbalanced or to effect survival. Tests will be performed at a two-sided nominal significance level of 5%. If the proportional hazard assumption is not met, we will compare the restricted mean survival time at 90 days.

   Comparisons of binary survival indicators will be performed as alternative analyses, by using either chi-square tests or logistic regression models. In the logistic regression analysis, MELD score as well as other covariates deemed to be unbalanced or to effect survival will be included as covariates.
b. We will also compare the transplant-free survival functions of the two treatment groups, at 30, 90 and 180 days.
c. We will also secondarily compare the occurrence of events such as sepsis and renal dysfunction by using survival analysis (logrank test and Cox regression).

Before the groups are compared on these instruments, the baseline data will be examined to look for group imbalance among the survivors. Comparisons will be made using linear regression models that include baseline MELD score and other relevant and/or unbalanced covariates.

11.3.3 Additional Safety and Clinical Findings

Additional safety assessments include vital signs, lab results, and physical examinations. Descriptive statistics of the vital sign and lab measures will be provided; comparisons between the two treatment

groups will be made using mixed effects models. Changes of these variables from baseline at each visit will be analyzed similarly.

11.3.4 Other Safety Related Clinical Outcomes

The following clinical outcomes will be assessed:

- Death (from hepatic and non-hepatic related causes)
- Complications of portal hypertension including gastroesophageal bleeding, interventions to manage variceal bleeding (e.g., variceal banding/sclerotherapy or TIPS placement) and diuretic resistant ascites
- Complications of Cirrhosis: new-onset ascites, hepatic encephalopathy, hepatorenal syndrome (type I or II), and spontaneous bacterial peritonitis).
- Hospitalization
- Liver transplantation

11.4 Interim Analyses

Interim analyses for efficacy or futility to determine if the study may be terminated prior to completion will be conducted once after the cohort associated with at least 50% of the participants have been in the study for 90 days. Formal efficacy analyses will be based on the Lan-Demets version of the O'Brien-Fleming boundary to provide an overall two-sided $P = 0.05$ test. Futility will be assessed based on conditional power.[57,58] . For example, termination due to futility may be considered if conditional power is very low (e.g. less than 0.10), under the assumption that the hypothesized treatment difference is correct. In other words, based on data that become available at the interim analysis, assuming that the current trend continues, we should early stop the trial if the probability of declaring anakinra + zinc superior in the final analysis is low (e.g., less than 10%). We use this early stopping rule to limit patient exposure to a potentially inefficacious treatment.

The efficacy interim analyses will be performed on unblinded data. Additional data that will be included in the interim analyses are:

- Tables of laboratory data by group
- Tabulated safety data by group
- Listings of adverse events and serious adverse events by group

11.5 Handling of Missing Data

Missing values will not be imputed and only observed values will be used in data analyses and presentations.

11.6 Data and Safety Monitoring Board (DSMB)

An independent DSMB will be formed to review safety data at periodic intervals from this and all other AlcHepNet studies. Members of the DSMB will not be allowed to participate as investigators in this trial.

The DSMB will review blinded safety data and unblinded safety data (as necessary in their opinion) to ensure the safe and property treatment of participants. The frequency of their standard meetings will be according to the agreed upon DSMB charter. In addition, Chair of the DSMB or the designated study physician may call for an ad-hoc DSMB if deemed necessary by either party. Based on review of these data, the DSMB will send a written report to the designated study physician and to the DCC-IU. It is the responsibility of the designated study physician and the DCC-IU to distribute

these reports to all other stakeholders (NIAAA, FDA, and individual sites)

The DSMB will operate under an appropriate charter (in compliance with relevant regulatory guidance) that will define its organization and operation. The DSMB will prepare written minutes of both its open and closed sessions. The closed minutes will be made available to the investigators only after the database is locked and the blind for the trial has been broken. All investigators and responsible IRBs will be informed of any decisions made which alter the conduct of this trial based on recommendations from the DSMB relating to participant safety. The investigators will inform the participants of such actions and the protocol and ICF will be revised, as appropriate.

Changes to the protocol or to the ICS may be initiated by any investigator, but it is the final responsibility of the designated study physician as the IND holder to approve/finalize any such changes and to communicate to other stakeholder.

12. ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

12.1 Ethical Conduct of the Trial

This trial will be conducted in accordance with 21CFR Part 312, Good Clinical Practice (CPMP/ICH/135/95), and with the ethical principles laid down in the Declaration of Helsinki and applicable regulatory requirements.

12.2 Institutional Review Board (IRB)

Western Institutional Review Board (WIRB) will review and approve this study prior to its initiation. Amendments can be implemented only following the IRB approval unless the amendment is necessary to reduce immediate risk to trial participants. The IRB will be informed of any new safety information that negatively affects the risk assessment of the trial as soon as it becomes available. Investigators will report various categories of the AEs to the IRB according to the schedule described in Table 4.

12.3 Patient Confidentiality and Data Protection

All information obtained during the conduct of the trial with respect to the participant will be regarded as confidential and confidentiality of all participants will be maintained. Clinical monitors (e.g., CRAs), auditors and inspectors will require access to a participant's medical notes for the purpose of source document verification, but the participant's confidentiality will be maintained at all times. An agreement for disclosure of any such information will be obtained in writing and is included in the statement of informed consent. The trial data shall not be disclosed to a third party (with the exception of auditors and/or regulatory authorities) without the written consent of the AlcHepNet consortium. All data shall be secured against unauthorized access.

Apart from the site investigators, no one will have access to participant's identity. Each site will securely maintain the code that links participants' identity to their study numbers to prevent access to unauthorized third parties. Participants will be identified according to their study numbers in the data management system (REDCap) and by the investigators during anycommunications.

The written ICF will explain that, for data verification purposes, authorized representatives of the funding agency, IRB or drug companies which are supplying the study medicine may require direct access to parts of the hospital or practice records relevant to the trial, including participant's medical history.

12.4 Access to Source Documents and Data

12.4.1 Source documents

Source documents may include, but are not limited to, medical records, charts, appointment books, participant questionnaires, original laboratory records, equipment print-outs. All source documents must be made available to the CRA. The following data must be included in the source data:

a) Consent to Participate in Trial
b) Letter to Primary Care Physician, if applicable
c) Participants visit dates
d) Screening and Randomization Numbers
e) Demographic information
f) Medical history
g) Disease history
h) Physical examination
i) Vital signs
j) Laboratory assessments (copy of laboratory reports)
k) AEs and concomitant medications
l) Dates of dispensing study medication
m) ECGs
n) Liver biopsy reports (if applicable)
o) Participant questionnaires
p) Drug accountability
q) Issues with protocol compliance
r) Completion of, or withdrawal from, trial

12.5 Investigator Obligations

The investigator or a medically trained designee will be responsible for obtaining written informed consent and for the care of the participants for the duration of the trial. If the investigator is not present in the clinical research facility during the assessment, he or she will leave instructions for the trial site staff and a telephone number where he or she can be reached.

12.5.1 AE Reporting

The investigator is responsible for recording AEs reported by the participant or discovered by any other means during the trial. In agreeing to the provisions of this protocol, the investigator accepts all legal responsibilities for immediate reporting of SAEs to the designated studyphysician.

12.5.2 Protocol Deviations

The investigator is not permitted to deviate from the protocol in any significant way without proper notification to the DCC-IU and the study designated physician (Dr. Samer Gawrieh). Changes to the protocol or to the informed consent (ICF) may be initiated by any investigator, but it is the final responsibility of the designated study physician, Dr. Samer Gawrieh, as the IND holder to approve/finalize any such changes and to communicate to other stakeholder Although protocol amendments can be initiated by any study personnel, it is the final responsibility of the designated

study physician as the IND holder to approve/finalize any protocol amendments. Any protocol amendments can be implemented at each site only after WIRB approval. The only exception is when the investigator considers a participant's safety to be compromised if immediate action is not taken.

12.5.3 Regulatory Documentation

The following regulatory documentation must be completed or provided, and maintained:

(a) Approved ICF

(b) IRB approvals (of protocol/amendments, participant questionnaires, etc.)

(c) Form FDA 1572 equivalent

(d) Current medical license of the primary site investigator and sub investigators

(e) Financial disclosure forms

(f) GCP CITI Certificate

(g) Delegation of Authority Log

(h) Protocol Training Log

(i) Sponsor Master Trial Files

12.6 Data Quality Assurance and Quality Control

Logic and consistency checks will be performed on all data entered into the source document to ensure accuracy and completeness.

Training sessions, regular monitoring of the trial at the trial sites, instruction manuals, data verification, cross-checking and data audits will be performed to ensure quality of all trial data. Investigators' meetings and/or on-site trial initiations will be performed to prepare investigators and other trial site personnel for appropriate collection of trial data.

12.7 Site monitoring & auditing

12.7.1 Trial Monitoring

Trial records at each site will be monitored at regular intervals by a clinical research associate (CRA). The role of the CRA is to aid the investigator in the maintenance and documentation of complete, accurate, legible, well organized, and easily retrievable data. In addition, the CRA will ensure the investigator's understanding of all applicable regulations concerning the clinical evaluation of the study medication and will ensure an understanding of the protocol, reporting responsibilities and the validity of the data. This will include ensuring that full and appropriate essential documentation is available. Responsibility for properly implementing the protocol and maintaining complete trial records remain with each investigator.

In order to perform this role, the CRA must perform source data verification (SDV) and as such must be given access to the participant's primary source documentation (e.g., paper or electronic medical records) that support data entries in the CRF. The investigator may exercise judgment in allowing the CRA access to particular sections of the participant's medical records if these are deemed irrelevant to the performance, observations or conduct of this trial.

12.7.2 Trial Auditing

The investigator should understand that it may be necessary for the IRB and / or a regulatory agency to conduct one or more site audits during or after the trial and agrees to allow access to all trial related documentation and information and be available for discussion about the trial.

12.8 Archiving and Record Retention

The investigator should retain all correspondence relating to this trial in the Investigator Site File (ISF). Any trial documents stored elsewhere should have their location referenced in the ISF.

All documents relating to the trial including the ISF itself, source documents and participant medical files), completed trial participant log and confidential participant identification list will be retained by the investigator for a minimum period of 15 years, in accordance with regulations. In the event that storage of records becomes a problem at any time during this period, the Sponsor should be consulted for assistance. At the end of the minimum period, the investigator should obtain written authorization from the Sponsor prior to the destruction of any records. The investigator will notify the Sponsor if ownership of documents or responsibility for the trial site is transferred. The Sponsor will inform investigators should it become aware of any changes in storage requirements.

12.9 Publication Policy

No data are to be made public or published without prior knowledge and written approval by the AlcHepNet Consortium Steering Committee.

The AlcHepNet consortium publication policies will be applied.

LIST OF PLANNED LABORATORY ANALYTES

Serum Chemistry

sodium

potassium

calcium

chloride

bicarbonate

albumin

BUN

creatinine

total bilirubin

unconjugated (indirect) bilirubin

conjugated (direct) bilirubin

aspartate aminotransferase (AST; SGOT)

alanine transaminase (ALT; SGPT)

alkaline phosphatase (ALP)

glucose

total protein

MELD Score Calculation

http://www.mayoclinic.org/meld/mayomodel6.html

Lille Score Calculation

https://www.mdcalc.com/lille-model-alcoholic-hepatitis

Hematology

hemoglobin

hematocrit

WBC with differential

platelets

RBC count (incl. MCV, HBE [MCH], MCHC)

prothrombin time (PT and INR)

Urine and/or Serum pregnancy test

Special investigations

Pro-inflammatory cytokine/chemokines measurements (serum TNF-α, MCP1, IL-6, and IL-1β)

Indicators of gut permeability (endotoxin and bacterial 18S DNA) and pro-inflammatory cytokine/chemokines (TNFa, MCP1, IL-6, IL-1ß)

EXPLORATORY EVALUATIONS

The following are the analyses that might be analyzed in the serum/plasma and peripheral blood mononuclear cells (PBMC): Cytokines/chemokines, NK cells, Cytotoxic T cells, Dendritic Cells (DC), Complement activation.

Table 5: Total Blood Volumes¶

	Sample Blood Volume (ml)									
Sample Type	**Screen**	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 28**	**Day 60**	**Day 90**	**Day 180**	**Total Volume (ml)**
Chemistry	5	5	5	5	5	5	5	5	5	40
Hematology	3	3	3	3	3	3	3	3	3	24
Coagulation	3	3	3	3	3	3	3	3	3	24
Serum Pregnancy	3									3
Biospecimen Banking[1]		60		60	60	60	60	60	60	420
Total	14	71	11	71	71	71	71	71	71	522

¶These are estimated volume and may vary from center to center, depending on local laboratory requirements.

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