

Digoxin In Treatment of Alcohol Associated Hepatitis (DIGIT-AlcHep)

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Statement of Compliance

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Protocol Title: Digoxin In Treatment of Alcohol Associated Hepatitis (DIGIT-AlcHep)

Protocol Version: 9.0 dated 12 June 2025

Principal Investigator Name

Principal Investigator Signature

Date:

1. Protocol Summary

1.1 Synopsis

Title:

Digoxin In Treatment of Alcohol Associated Hepatitis (DIGIT-AlcHep).

Study Description:

Prospective, single center, open label, randomized controlled trial.

Objectives:

Primary objective:

To explore whether digoxin treatment affects cytokine levels as biomarkers of inflammation in patients with acute alcohol associated hepatitis

Secondary Objective:

To measure digoxin serum levels and adjust digoxin dose in a timely manner in patients with normal and compromised renal function.

Outcomes:

Primary Outcome:

- 1) Changes in cytokine levels (TNF-A, IL-6 and IL-10) from baseline in patients with acute alcohol associated hepatitis treated with digoxin versus no digoxin at day 3 of the study

Secondary Outcomes:

- 1) Practicality of daily digoxin measurements.
- 2) Feasibility of digoxin dosing in a timely manner.
- 3) Feasibility of digoxin dose adjustments in renal insufficiency.
- 4) Mortality at 7, 14, 28 and 90 days
- 5) Development of arrhythmia
- 6) Ability to recruit 4 patients per month

Tertiary Outcomes:

- 1) Assess trends in:
 - a. Lille score.
 - b. Model for end-stage liver disease (MELD).
 - c. Maddrey's discriminant function (MDF).
 - d. Sequential Organ Failure Assessment (SOFA) score.
 - e. Multi-organ dysfunction score (MODS).
- 2) Development of new or recurrent renal failure.
- 3) Racial and ethnic diversity in subject recruitment and retention.

Study Population:

Sixty patients admitted to the inpatient service at Yale New Haven Health with severe alcohol associated hepatitis, defined by MDF ≥ 32 or MELD ≥ 20 to ≤ 35 .

Phase:

Single-center, open-label randomized control trial.

Description of Sites/Facilities Enrolling Participants:

Yale New Haven Hospital, 20 York Street, New Haven, CT, 06510

St. Raphael's Centre, 1450 Chapel Street, New Haven, CT 06511

Yale New Haven Health, Bridgeport Hospital, 267 Grant Street, Bridgeport, CT 06610

Description of Study Intervention:

The study intervention will be intravenous digoxin (renal-based dosing for maximum of 28 days) versus no digoxin in an open-label 1:1 randomized allocation of patients with severe alcohol associated hepatitis.

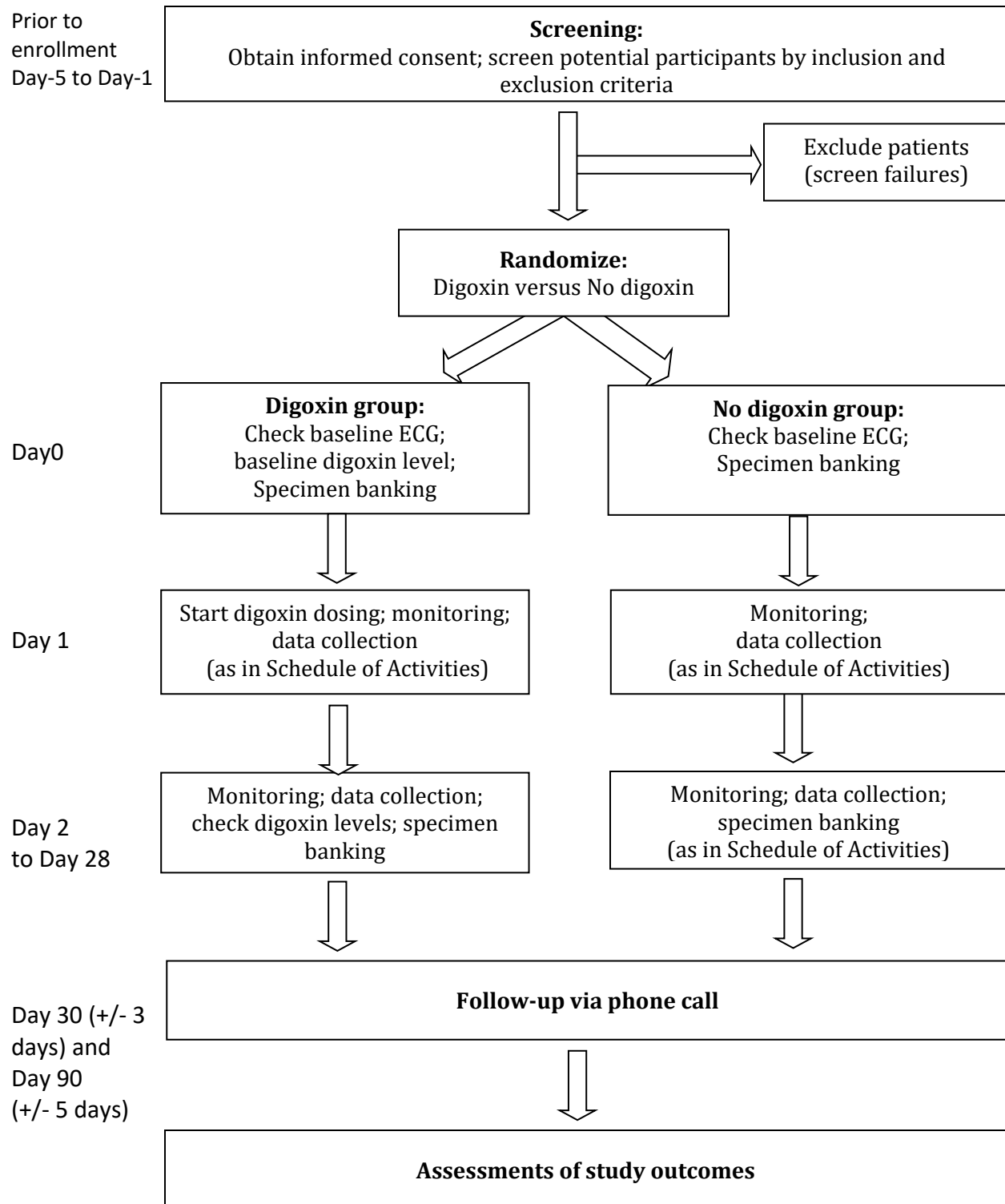
Study Duration:

7 years.

Participant Duration:

3 months.

1.2 Schema



1.3 Schedule of Activities (SoA)

Procedures	Screening	Baseline tests	Treatment period		Post-treatment followup/ phone call	
	Day -5 to Day -1	Day 0	Day 1	Day 2 to Day 28	Day 30 (+/- 3 days)	Day 90 (+/- 5 days)
Informed consent	X					
History and physical	X					
History and physical exam from chart		X	X	X		
Alcohol history and questionnaires ¹	X				X	X
Vital signs, including weight	X	X	X	X		
Height	X					
Concomitant medicines	X	X	X	X		
Chart review for other assessments ²	X	X	X	X		
Blood collection for digoxin level ^{3,4}		X		X		
Specimen banking ⁵		X		X		
Digoxin administration			X	X		
Electrocardiogram	X	X	X	X		
Adverse event review and evaluation		X	X	X	X	X

1. Questionnaires include Alcohol Use Disorders Identification Test (AUDIT), Alcohol Timeline Follow Back (TLFB), and Medical Outcomes Study Questionnaire Short Form 36 Health Survey (SF-36).
2. Includes the following tests, if available: CBC, PT/INR, AST, ALT, alkaline phosphatase, total and direct bilirubin, BMP, magnesium, calcium, phosphorus, thyroid function panel, test for COVID-19, pregnancy test, infection workup including but not limited to urinalysis, urine culture, blood culture, chest Xray, paracentesis fluid cell count, gram stain and culture.
3. Subjects on P-gp inhibitors or spironolactone will have an additional digoxin level performed 12-hours after any dose adjustment. Once digoxin levels are stable, 24-hour blood draws will be performed.
4. Patients treated with diuretics will have daily electrolytes checked to detect magnesium, potassium and calcium.
5. Specimen banking includes blood on Day0, Day3, Day7, Day14, Day 21 and Day28.
6. We will terminate subjects/take them off study at the time of waitlisting for liver transplant with no further data collection. They will have activities performed as per the schedule of events until discontinuation.

2. Introduction

2.1 Study Rationale

Severe alcohol associated hepatitis is a condition of acute on chronic immune liver dysfunction that is associated with high mortality, necessitating a search for drugs that may prove safe and efficacious in treating this disease. Pre-clinical studies suggest that digoxin, which is currently used for treating cardiac conditions, is also effective in improving alcohol-associated liver injury. To date, there have been no clinical studies of digoxin use in patients with alcohol associated hepatitis. The primary objective of this study is to explore the treatment difference in cytokine levels as biomarkers of inflammation between treatment and control group in patients with severe alcohol associated hepatitis.

2.2 Background

In the United States (US), alcohol-related deaths continue to increase. According to 2019 data from the National Survey on Drug Use and Health (NSDUH), 14.1 million adults in the US had alcohol use disorder, with an estimated 95,000 people dying from alcohol-related causes annually [1, 2]. Acute alcohol associated hepatitis, a condition of acute on chronic liver injury caused by alcohol, is associated with a 30-day mortality of 20 to 30% and a 6-month mortality of 30 to 40% in patients with severe disease [3, 4]. Acute alcohol associated hepatitis affects people of all races. A recent analysis preparatory to this study showed the racial distribution of patients with alcohol associated hepatitis admitted to Yale New Haven Hospital (YNHH) as 12% Black, 13% Hispanic, 70% White, and 5% Other/Unidentified.

There are currently no FDA-approved medications to treat the condition. In 2015, a randomized trial of 1103 patients with severe alcohol associated hepatitis showed that prednisolone treatment was associated with a reduced 28-day mortality that did not reach statistical significance; no reduction in mortality was seen at 90 days or at 1 year [5]. Thus, there is an urgent need to find pharmacologic agents that are safe and efficacious in treating acute alcohol associated hepatitis.

The repurposing of presently available drugs would help expedite the search for such an agent. The cardiac glycoside digoxin is currently used in the management of heart failure and supraventricular tachyarrhythmias [6]. Digoxin has also been shown to have hepatoprotective properties. A recent study in a mouse model of alcohol associated hepatitis showed that digoxin improves alcohol-induced liver injury through reduction of hypoxia inducible factor (HIF)-1 α activity and redox pathway signalling [7]. Alcohol upregulates HIF-1 α transcription and translation, and increases the DNA-binding activity of HIF-1 α to its target genes, thereby increasing pathways involved in cell inflammation, proliferation, survival, and a variety of other metabolic processes [8-12]. Hepatocyte-specific deletion of HIF-1 α in mice ameliorates alcohol-induced liver injury [10]. Along these lines, digoxin-mediated downregulation of hepatic HIF-1 α and modulation of intracellular redox status in mice and in human liver tissue leads to improvement in hepatic steatosis, neutrophil infiltration and hepatocellular damage [7]. Extensive literature accrued from using digoxin in the management of cardiac conditions for the

past several decades will guide its use among patients with alcohol associated hepatitis. Digoxin was first isolated from the foxglove plant in the 1930s [13] and is manufactured by several pharmaceutical companies worldwide. Digoxin is dosed based on recipient weight and renal function, and serum digoxin levels can be reliably monitored using blood tests. The drug is known to have a narrow therapeutic window, with levels above this window increasing the risk of digoxin toxicity which may be manifested by a variety of symptoms that are primarily cardiac, neurologic and gastrointestinal [14]. Digoxin toxicity can be minimized by adherence to dosing and monitoring recommendations which have been clearly outlined based on extensive experience with its use in the cardiac population [15, 16].

To assess if digoxin can have immunomodulatory effects in vivo in humans, we initiated a clinical trial of healthy subjects aged between 18yr and 70yrs who were given placebo, low dose (0.15mcg/Kg/day), or regular dose (3mcg/Kg/day) digoxin (ClinicalTrials.gov Identifier: NCT03559868). Blood samples were collected at the initiation of the study (week 0) and then three further occasions weekly (weeks 1, 2, 3) for assessment of serum digoxin level and CBC. ECG was performed weekly at each study visit. There were no adverse reactions or ECG changes noted among subjects in the placebo group or digoxin groups. To test if digoxin in healthy subjects immunoregulates these key pathways involved in alcohol associated liver disease, weeks 0 and 3 peripheral blood monocytes were isolated and plated at a concentration of 2.5×10^6 cells/ml. The next day monocytes were stimulated with LPS (100ng/ml) and supernatant collected at 16hrs, spun to remove debris and snap frozen. LPS stimulated supernatant from week 0 and week 3 blood monocytes was assayed by multiplex for TNF- α , IL-6, and IL-10. To obtain a broader view of any immunomodulatory changes that may be induced by digoxin, the following additional 31 analytes were also tested: IL1RA, GMCSF, IFN- α , IFN- β , IFN- γ , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-7, IL-12, IL-16, IL-17e, CCL19, IL-33, CCL4, PDGFAA, CXCL1, CCL20, B7H1, FGF, CCL2, CXCL10, CCL-11, Flt3-L, VEGF, PDGF, GCSF, Granzyme B. Statistical analysis was performed using an unpaired T-test with Welch's correction. The following results were present seen in pilot data and confirmed in the larger data set, A) Pro-inflammatory cytokines that were significantly decreased: TNF- α , IL-6, IL1RA, GMCSF, IFN- α , CCL4, PDGFAA, CXCL1, CCL20, GCSF, Granzyme B. B) One anti-inflammatory cytokine, IL-10, was significantly increased.

This study of Digoxin In Treatment of Alcohol Associated Hepatitis is informed by substantial preclinical data suggesting potential efficacy of digoxin in treatment of alcohol associated hepatitis, as well as by extensive data on the use and monitoring of digoxin in the clinical setting in patients with heart disease. Our preliminary data regarding the racial distribution of patients with alcohol associated hepatitis sets a framework for participant recruitment that would aim to reflect the diversity of patients admitted to YNHH. Taken together, the available information provides a sound basis for a randomized controlled trial to explore whether digoxin treatment affects cytokine levels as biomarkers of inflammation in patients hospitalized with severe alcohol associated hepatitis.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

While digoxin is currently used for the treatment of atrial fibrillation and heart failure, no studies to date have examined its use in patients with alcohol associated hepatitis. The pharmacokinetics of digoxin are very well known, with digoxin being renally excreted without undergoing hepatic metabolism. Side effects occur occasionally with digoxin use, however digoxin is known to have excellent safety at serum levels below 1 ng/ml. The assay for serum digoxin is routinely available in clinical laboratories to allow for timely dose adjustments. The extensive pharmacokinetic experience with digoxin has also allowed the development of a reliable nomogram to adjust digoxin dosing in the setting of low glomerular filtration rate (GFR). In contrast to the excellent safety profile at serum levels below 1 ng/ml, serum levels above 2 ng/ml are well known to result in a number of side-effects of which an increased risk of bradycardia and tachyarrhythmias are the most serious [14]. It is important to note that serum levels of digoxin can predict the risk of side effects. In a post-hoc analysis of patients with atrial fibrillation randomized to digoxin versus placebo, the risk of cardiac arrhythmia increased in proportion to serum digoxin level [15, 16]. Mortality risk also increased with higher digoxin levels. Patients with serum digoxin ≤ 1.1 ng/ml had no increase in mortality compared to those on placebo [15, 16].

Digoxin has a narrow therapeutic index, increased monitoring of serum digoxin concentrations and for potential signs and symptoms of clinical toxicity is necessary when initiating, adjusting, or discontinuing drugs that may interact with digoxin. Digoxin is a substrate of P-glycoprotein, at the level of intestinal absorption, renal tubular secretion and biliary-intestinal secretion. Therefore, drugs that induce/ inhibit P-glycoprotein have the potential to alter digoxin pharmacokinetics. [14]

The majority of digoxin is renally excreted unmetabolized, and as such renal function dictates digoxin dosing based on well-established nomograms. Notably digoxin does not undergo hepatic metabolism and serum levels should therefore be unaffected by hepatitis.

Several studies have highlighted the existence of endogenous digoxin-like immunoreactive substances (DLIS) in patients with volume-expanded conditions such as liver disease, renal disease, essential hypertension and preeclampsia [17-19]. These substances are known to cross-react with anti-digoxin antibodies and may interfere with measurement of serum digoxin, typically resulting in falsely elevated digoxin levels [17]. To determine the presence of DLIS, baseline serum digoxin levels will be obtained in all study participants prior to the start of digoxin treatment.

A study of serum digoxin levels in 20 patients with cirrhosis versus 20 healthy controls after a 5-day course of digoxin treatment suggested that patients with cirrhosis develop higher serum digoxin levels compared to healthy patients when given the same dose. All 20 patients with liver disease had histologically confirmed cirrhosis and clinical decompensation as demonstrated by a history of ascites; all 40 study participants had normal renal function. Of note, 50% of patients with cirrhosis and 10% of controls exhibited detectable DLIS prior to the start of digoxin treatment. After a 5-day course of 0.25 mg digoxin tablet daily, the average digoxin level in patients with cirrhosis versus healthy patients was 1.28 ± 0.95 nmol/L versus 0.75 ± 0.35 nmol/L, equivalent to 1.0 ± 0.74 ng/mL versus 0.59 ± 0.27 ng/mL, respectively [20, 21]. This study, although limited in sample size, suggests that liver disease facilitates an increase in the serum levels of both DLIS and digoxin.

Table 2.3.1

Side effects of digoxin	Frequent >20% of participants	Occasional 2 - 20% of participants	Rare Less than 2% of participants
Serious		Bradycardia Hyperkalemia Laryngeal edema	Asymmetric chorea Mesenteric ischemia Hemorrhagic necrosis of intestines Vaginal cornification Heart block Thrombocytopenia Delirium Ventricular fibrillation Ventricular Premature beats Depression
Less serious		Nausea Vomiting Diarrhea Headache Dizziness Anxiety Fever Hallucinations	Anorexia Headache Confusion Gynecomastia Xanthopsia, photopsia
Minor		Itchy skin rash	Weakness

2.3.2 Known Potential Benefits

The six-month mortality of patients with severe alcohol associated hepatitis (MDF $\geq$ 32) is approximately 40% [22]. Although there are no FDA-approved medications to treat alcohol associated hepatitis, current guidelines recommend a course of prednisolone in those with severe disease, based on studies that show a non-significant improvement in 30-day mortality and no improvement in 90-day or 1-year mortality [5].

Pre-clinical data demonstrates that digoxin improves alcohol-related inflammation in murine liver and in cultures of human hepatocytes [10]. Digoxin is currently available as a prescription medication in the US and in several other countries. It is easily available and relatively

inexpensive. Digoxin use has been demonstrated to be safe at therapeutic levels in patients with cardiac disease, and a large body of data has been accrued from its use in the clinical setting in cardiac patients. There have been no reports of digoxin-associated drug induced liver disease (DILI), despite the wide use of digoxin over many decades. Its repurposing for alcohol associated hepatitis, if successful, will fast-track the process of delivering much-needed cure to patients with the disease.

2.3.3 Assessment of Potential Risks and Benefits

The primary goal of this study is to explore whether digoxin treatment affects cytokine levels as biomarkers of inflammation in patients hospitalized with severe alcohol associated hepatitis. Participant screening will include a detailed history and physical examination, review of relevant medical records, laboratory test results, and baseline electrocardiogram. Every effort will be taken during participant screening to identify patients who may be at high risk for adverse outcomes, such as those with underlying heart failure and arrhythmia, since the risks of digoxin may outweigh the benefits for such individuals.

Several steps will be taken to minimize potential risks of the study medication. Firstly, given the known risk of potentially serious side effects of digoxin at levels above the therapeutic window, serum levels of digoxin will be monitored on a daily basis.

Secondly, for all subjects on P-gp inhibitors or spironolactone, an additional digoxin level will be performed 12-hours after any dose adjustment. Once digoxin levels are stable, 24-hour blood draws will be performed.

Thirdly, since individuals with liver disease may have DLIS which could interfere with measurement and interpretation of serum digoxin, a baseline digoxin level will be checked prior to the initiation of digoxin treatment.

Fourthly, current cardiology guidelines recommend a goal therapeutic digoxin level between 0.5 to 2.0 ng/ml [14]. To increase the safety profile, a goal therapeutic level between 0.5 and 1.1 ng/ml will be maintained in this trial.

Fifthly, in aiming for a lower therapeutic goal between 0.5 and 1.1 ng/ml, digoxin will be dosed at the lower end of the dosing spectrum recommended by manufactures and dosing guidelines [14].

Sixthly, patients who will be treated with diuretics will have daily electrolytes checked to detect magnesium, potassium and calcium. Any patient found with hypokalemia, hypomagnesemia, and hypercalcemia will have digoxin held until these are corrected.

Finally, by implementing a dosing nomogram, potential variability in dosing will be minimized and the risk of toxicity reduced.

3. Objectives and Outcomes

Primary Objective

To explore the treatment difference in cytokine levels from baseline in patients with acute alcohol associated hepatitis treated with digoxin versus no digoxin at day 3 of the study.

Secondary Objectives

To measure digoxin serum levels and adjust digoxin dose in a timely manner in patients with normal and compromised renal function.

Outcomes	Definition
Primary Outcome	
1) The changes from baseline in biomarkers of inflammation cytokine levels (TNF-A, IL-6 and IL-10) in patients with acute alcohol associated hepatitis at day 3 of the study	Changes in cytokine levels will be calculated for each cytokine individually by subtracting baseline values from those collected at day 3.
Secondary Outcomes	
1) Practicality of daily digoxin measurements.	90% of digoxin levels are checked within the pre-specified time window.
2) Feasibility of digoxin dosing in a timely manner.	90% of patients receive every scheduled dose of the drug.
3) Feasibility of digoxin dose adjustments in renal insufficiency.	90% of necessary dose adjustments were made appropriately in response to digoxin levels.
4) Mortality at 7, 14, 28 and 90 days	Mortality rates at different time points regardless of cause.
5) Development of arrhythmia	Any type of arrhythmia which represents a change from baseline.
6) Ability to recruit 4 patients a month	Number of patients randomized to study
Tertiary Outcomes	
1) Assess trends in: <ul style="list-style-type: none"> a. Lille score. b. Model for end-stage liver disease (MELD). c. Maddrey's discriminant function (MDF). d. Sequential Organ Failure Assessment (SOFA) score. e. Multi-organ dysfunction score 	Lille score will be calculated from labs on day 4 and day 7. Calculated based on creatinine, bilirubin, international normalized ratio and sodium. Calculated from international normalized ratio and bilirubin. Calculated based on a person's liver function, kidney function, nervous system, coagulation, circulation, and respiratory status. Calculated based on a person's liver function,

(MODS).	kidney function, nervous system, coagulation, circulation, and respiratory status.
2) Development of new or recurrent renal failure.	Creatinine rise ≥ 0.5 mg/dL or $\geq 20\%$ from baseline or requiring renal replacement therapy.
3) Racial and ethnic diversity in subject recruitment and retention.	Black and Hispanic patients randomized to study and completing 90-day follow-up.

4. Study Design

4.1 Overall Design

This study of digoxin in patients with alcohol associated hepatitis will be performed using a single center, prospective, open-label randomized control trial design in which patients with severe alcohol associated hepatitis will be randomized to one of two groups:

1. Digoxin
2. No digoxin

Patients with severe alcohol associated hepatitis admitted to Yale New Haven Health will be identified by a MDF ≥ 32 or MELD score ≥ 20 to ≤ 35 . All participants will also receive current standard of care for treatment of severe alcohol associated hepatitis as determined by the primary physician on service in accordance with recent guidelines endorsed by AASLD.

4.2 Scientific Rationale for Study Design

The main rationale of this study is to explore the treatment effect of digoxin on cytokine levels in patients hospitalized with severe alcohol associated hepatitis.

The open-label design of this trial was adopted to facilitate real-time response to the data, given the lack of clinical data on the use of digoxin in this patient population.

4.3 Justification for Dose

Digoxin will be administered via the intravenous route due to a number of factors. Patients with alcohol associated liver disease are known to have alterations in their gastrointestinal tract that may alter the absorption and metabolism of orally administered drugs [23]. Moreover, some patients have gut flora that metabolizes oral digoxin to dihydrodigoxin which is not readily absorbed; while other patients may be taking concomitant medications such as macrolide antibiotics that are known to alter the absorption of digoxin. Furthermore, IV administration of digoxin results in a more rapid digitalization compared to oral administration.

Digoxin is dosed based on weight and renal function. Therapeutic levels of digoxin can be achieved rapidly by administering a loading dose during the first 24 hours of therapy. The

narrow therapeutic window of digoxin and the potentially serious adverse effects of toxic levels necessitate careful dosing and monitoring of digoxin. Current guidelines recommend a goal therapeutic digoxin level between 0.5 to 2.0 ng/ml. However, given the correlation between digoxin levels ≥ 1.2 ng/ml and increased risk of mortality, a goal therapeutic level between 0.5 and 1.1 ng/ml will be targeted in this trial. In aiming for this lower therapeutic goal, doses at the lower end of the recommended dosing spectrum will be utilized (Refer to nomogram; FDA insert)[14].

Digoxin levels will be monitored daily during throughout the treatment period, aiming for a goal serum digoxin between 0.5 to 1.1 ng/ml. In cases where digoxin level rises above the therapeutic window of 0.5 to 1.1 ng/ml, such as in the case of acutely worsening kidney function, appropriate adjustment will be made (as in dose adjustment table below). For levels between 1.2 and 2.0 ng/ml, digoxin administration will be held, levels checked daily and the patient monitored for development of serious adverse side-effects potentially attributable to digoxin toxicity. Patients who do not develop any serious adverse symptoms will be resumed on digoxin per the dosing nomogram when serum digoxin level falls below 0.8 ng/ml (mid therapeutic range). If a patient develops serious adverse side-effects attributable to digoxin toxicity while their digoxin level is between 1.2 and 2.0 ng/ml, digoxin will be permanently discontinued, and levels monitored; in such a situation, digoxin will not be resumed. Permanent discontinuation of digoxin will also occur if a patient's digoxin level rises above 2.0 ng/ml, irrespective of symptom development.

Digoxin dose adjustment

Digoxin range	Serious adverse side-effects due to digoxin toxicity	Action
1.2 to 2.0 ng/ml	No	<ul style="list-style-type: none"> - Hold digoxin - Perform ECG - Check daily digoxin level - Resume digoxin when serum digoxin level falls below 0.8 ng/ml
	Yes	<ul style="list-style-type: none"> - Stop digoxin - Perform ECG - Check daily digoxin level until undetectable - Do not resume digoxin
> 2.0 ng/ml	No	<ul style="list-style-type: none"> - Stop digoxin - Perform ECG
	Yes	<ul style="list-style-type: none"> - Check daily digoxin level until undetectable. - Do not resume digoxin

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the 90-day follow up as shown in the Schedule of Activities (Section 1.3).

5. Study Population

5.1 Inclusion Criteria

Patients included in the study will meet the following criteria:

1. Diagnosis of alcohol associated hepatitis based on clinical criteria or histologic evidence
 - a. Clinical criteria:
 - Onset of jaundice (bilirubin >3 mg/dL) within the prior 8 weeks
 - Regular alcohol use > 6 months, with intake of > 40 g/day (>280 g/week) for women; and > 60 g/day (>420 g/week) for men
 - AST > 50 IU/l
 - AST: ALT > 1.5 and both values < 400 IU/l
 - b. Histological evidence of alcohol associated hepatitis*
2. MDF $\geq$ 32 or MELD $\geq$ 20 to $\leq$ 35 on Day 0 of the trial
3. Age between 21 and 70 years, inclusive

* In patients with possible alcohol associated hepatitis with confounding factors such as possible ischemic hepatitis, possible DILI, uncertain history of alcohol use, or 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 as per discretion of the primary attending physician to confirm alcohol associated hepatitis and exclude competing etiologies. The decision to perform liver biopsy will be made by the primary team and will occur regardless of the study. As per current SOC, a liver biopsy may be obtained to confirm suspected alcohol-associated hepatitis and to rule out other potential etiologies of liver disease.

5.2 Exclusion Criteria

Patients will be excluded from the trial if they meet any one of the following criteria:

- 1 - Currently pregnant or breastfeeding
- 2 - Inability of patient, legally authorized representative or next-of-kin to provide informed consent
- 3 - Allergy or intolerance to digoxin
- 4 - Clinically active C. diff infection
- 5 - Positive test for COVID-19 within 14 days prior to the screening visit
- 6 - Acute hepatitis E, Cytomegalovirus, Epstein Barr Virus, Herpes Simplex Virus
- 7 - 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.
- 8 - Diagnosis of Drug Induced Liver Injury (DILI), or other etiologies seen on liver imaging.
- 9 - History of HIV infection (positive HIV RNA or on treatment for HIV infection)
- 10 - Current diagnosis of cancer
- 11 – Renal failure defined by GFR <30 mL/min
- 12 – Refractory ascites, defined as having more than 4 paracenteses in the preceding 8 weeks despite diuretic therapy

- 13 – Prior exposure to experimental therapies or other clinical trial in last 3 months
- 14 – Current acute or chronic pancreatitis
- 15 – Active gastrointestinal bleeding unless resolved for >48 hours
- 16 – Experiencing withdrawal seizures or considered at high risk for alcohol withdrawal seizures or delirium tremens
- 17 – Heart rate less than 60 bpm at screening visit or at baseline
- 18 – Current diagnosis of atrial fibrillation
- 19 – Cardiomyopathy
- 20 – Heart failure
- 21 - Severe aortic valve disease
- 22 - Presence of Accessory arterio-ventricular pathway (eg Wolf-Parkinson-White syndrome)
- 23 - Complete heart block or second degree arterio-ventricular block without pacemaker or implantable cardiac device
- 24 - Any of the following within the previous 6 months: myocardial infarction, percutaneous intervention, pacemaker/implantable cardiac device implantation, cardiac surgery or stroke
- 25 - Current use of the following medications:
 - Antiarrhythmics (amiodarone, dofetilide, sotalol, dronedarone)
 - Parathyroid hormone analog (teriparatide)
 - Thyroid supplement (thyroid, levothyroxine sodium)
 - Sympathomimetics or inotropic drugs (epinephrine, norepinephrine, dopamine, dobutamine, milrinone)
 - Neuromuscular blocking agents (succinylcholine)
 - Calcium supplement
 - Ivabradine
 - Disulfiram

5.3 Lifestyle Considerations

There are no lifestyle considerations (such diet, weight etc.) for inclusion/exclusion in this trial.

5.4 Screen Failures

Participants se charts are reviewed, but ultimately found not to meet inclusion criteria for participation in the trial during the screening process, will be considered screen failures and excluded. These failures will be evaluated on a case-by-case basis to determine if they can be re-screened at a later period during the same inpatient admission.

Screening period procedure steps are as follows:

- 1) Based on a waiver of consent for chart review, perform chart review to check if patient meets all inclusion criteria (Section 5.1).
 - If patient does not meet *all* the inclusion criteria, patient will be considered a screen failure and excluded at this point.
 - If patient meets *all* the inclusion criteria, continue screening procedure as below.
- 2) The participant, legally authorized representative or next-of-kin will review and sign the informed consent document. Informed consent must be obtained from the participant, legally authorized representative or next-of-kin before

- performing any trial-related procedures, including screening procedures.
- 3) Record the following information if available from the chart
 - Medical history.
 - Current and previous (past 30 days) medications.
 - Results of the following tests, if available: CBC, PT/INR, Liver enzymes, BMP, Magnesium, calcium, phosphorus, test for COVID-19 per hospital's policy, pregnancy test, infection workup including urinalysis, urine culture, blood culture, chest Xray, paracentesis cell count and culture, liver biopsy results.
 - 4) Based on data collected so far, check if patient meets any of the exclusion criteria (Section 5.2).
 - If patient meets *any one* of the exclusion criteria, patient will be considered a screen failure and excluded at this point.
 - If patients does not meet any of the exclusion criteria, continue screening procedure as below.
 - 5) Return to patient and perform history and physical.
 - 6) Complete standardized alcohol history and questionnaires.
 - 7) Perform a baseline standard 12-lead electrocardiogram (ECG).
 - 8) Reassess all the data to confirm that patient does not have *any one* of the exclusion criteria (Section 5.2).
 - If patient meets *any one* of the exclusion criteria, patient will be considered a screen failure and excluded at this point.
 - If patient does not meet any of the exclusion criteria, continue screening procedure as below.
 - 9) Order baseline digoxin level to be drawn on Day 0.
 - 10) Provide blood tubes to RN for specimen banking; this blood will be drawn at the time of baseline digoxin draw. Blood specimen to be saved on ice.

5.5 Blood Sampling

Blood sample	Amount(ml)	Timeframe
Blood collection for digoxin level	2 ml (1/2 teaspoon)	Day 0; Day 2 - Day 28 Daily
Additional blood collection for Digoxin level for subjects on P-gp inhibitors or spironolactone	2 ml (1/2 teaspoon)	Day 0; Day 2 -28 Additional digoxin level performed 12 hours after dose adjustment
Blood collection for magnesium, potassium and calcium levels in subjects on diuretics	2 ml (1/2 teaspoon)	Day 2 -28 Daily
Specimen Banking	20 ml (4 teaspoons)	Day 0, Day 3, Day 7, Day 14, Day 21, Day 28

5.6 Strategies for Recruitment and Retention

Sources of participants:

Identification of participants with severe alcohol associated hepatitis requires collaboration of physicians who manage these participants within the hospital. Since patients with acute alcohol associated hepatitis are generally admitted for further evaluation and management, all patients

involved in the study will be identified in a hospital setting. The trial will involve patients admitted to Yale New Haven Hospital.

Each morning, the admitting diagnosis of patients on the Klatskin inpatient liver service and the liver consult teams will be reviewed to identify potential participants. In accordance with Institutional Review Board Policies, once a potential participant is identified, the primary team caring for the patient must be contacted by the investigators and agree to ask the patient about their interest in participating in a clinical trial to assess digoxin effects on inflammation in patients with alcohol associated hepatitis. Once the patient expresses willingness to be contacted, a member of the study team will meet with the patient to discuss details of the study and obtain informed consent prior to participant screening.

Strategies to increase recruitment and retention of minority participants, including use of interpreter services and a racially diverse study team have been incorporated into the trial design. Participants will receive a monetary compensation of \$25 on Day 1 of the trial, and another \$25 will be paid on Day 90 at completion of the study.

Management of barriers for recruitment:

An email communication will be sent to the Section of Digestive Diseases, General Internal medicine and Hospitalist teams, and ICU staff to 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 the trial.

The Common Rule proposes that selection of study subjects is equitable. Analysis of historical data conducted in preparation to this study showed that among patients with alcohol associated hepatitis admitted to YNHH, 12% identified as Black, while 13% identified as Hispanic. Excluding patients who do not speak English can bias the research and narrow its applicability. Hence, racial minorities admitted with alcohol associated hepatitis will be encouraged to participate, and a Spanish-speaking member of the study team will assist in the recruitment and retention of Hispanic participants.

Contingency plans:

Monthly review of expected versus actual enrollment will be performed to track recruitment. Screen fail rates will also be assessed.

Retention plan:

It is anticipated that some participants will drop out after hospital discharge. This will be minimized by participant education and frequent communication with the participant and their principal care team during their hospital stay.

6. Study Intervention

6.1 Study Intervention Administration

6.1.1 Study Intervention Description

The proposed study is a prospective, open-label randomized control trial to assess the effect of digoxin on inflammation in patients hospitalized with severe alcohol associated hepatitis. Patients with severe alcohol associated hepatitis will be identified by MDF ≥ 32 or MELD score ≥ 20 to ≤ 35 .

In the digoxin arm, the intervention to be administered will be intravenous digoxin dosed by weight and by renal function using an adaption of the established FDA nomogram [14]. Digoxin levels will be monitored daily throughout the participant's hospital stay, to a maximum of 28 days. Digoxin will be discontinued at the time discharge if before 28 days. In the no digoxin arm, no study drug or placebo will be administered.

Concomitant treatment in both arms will be standard care for severe alcohol associated hepatitis as determined appropriate by their inpatient hepatology physician, in accordance with recent guidelines endorsed by AASLD and AGA. This standard of care includes administration of prednisolone unless contraindications to steroid treatment are present. Patients who are not receiving steroids for treatment of severe alcohol associated hepatitis will be excluded from the trial (Section 5.2). The standard of care for patients with alcohol associated hepatitis also includes attention to early diagnosis and treatment of infections, management of portal hypertension and its complications including fluid overload (edema and ascites), gastrointestinal bleeding, hepatic encephalopathy and acute kidney injury. All patient management decisions, including whether to start prednisolone, will be made by the primary team.

6.1.2 Dosing and Administration

FDA approved digoxin injection formulation will be used for the study. All participants (aged 21 to 70 years) randomized to the digoxin arm will receive an intravenous digoxin loading dose administered in 3 doses over 24 hours starting on Day 1. The digoxin loading dose will be determined based on weight and renal function. After completion of digoxin loading in participants randomized to digoxin, and confirmation of digoxin levels in the therapeutic range, these participants will be started on maintenance dose based on weight and renal function. Details of loading and maintenance doses are provided below.

Loading dose

- The total loading dose of digoxin will be determined using the **Loading nomogram**. The FDA-recommended total IV digoxin loading dose range is 8 to 12 mcg/kg. The lowest recommended dose of 8 mcg/kg was used in constructing the digoxin Loading nomogram that will be used in this trial.
- Based on FDA recommendations, the total loading dose of digoxin will be administered in three doses over 24 hours. Administration of digoxin loading dose will start on Day 1 as follows:
 - At Time A: give half of the total loading dose.
 - At Time B (which falls within Time A+6hr to A+8hr): give one-quarter of the total loading dose.
 - At Time C (which falls within Time B+6hr to B+8hr): give the remaining one-

quarter of the total loading dose.

- At Time D (which falls within Time C+6hr to C+8hr): check post-loading digoxin trough.
- If the post-loading digoxin trough is <1.2 ng/ml, proceed as per maintenance dosing as outlined in the next section.
- If the post-loading digoxin trough is ≥1.2 ng/ml, appropriate actions will be taken as indicated in Digoxin dose adjustment table. Digoxin trough will be repeated 6 hours after the last trough was done.
 - If the repeat trough is ≥1.2ng/ml, further administration of digoxin will be discontinued.
 - If the repeat trough is <1.2ng/ml, maintenance dosing will be started as outlined in the next section.

Loading nomogram for total loading dose (to be administered in portions at Time A, B and C as outlined in *Loading dose* section)

Creatinine clearance*	Lean Body Weight (kg)						
	40	50	60	70	80	90	100
30	188 mcg	235 mcg	282 mcg	329 mcg	376 mcg	424 mcg	471 mcg
40	207 mcg	259 mcg	311 mcg	362 mcg	414 mcg	466 mcg	518 mcg
50	226 mcg	282 mcg	339 mcg	395 mcg	452 mcg	508 mcg	565 mcg
60	245 mcg	306 mcg	367 mcg	428 mcg	489 mcg	551 mcg	612 mcg
70	264 mcg	329 mcg	395 mcg	461 mcg	527 mcg	593 mcg	659 mcg
80	282 mcg	353 mcg	424 mcg	494 mcg	565 mcg	635 mcg	706 mcg
90	301 mcg	376 mcg	452 mcg	527 mcg	602 mcg	678 mcg	753 mcg
100	320 mcg	400 mcg	480 mcg	560 mcg	640 mcg	720 mcg	800 mcg

***Creatinine clearance determined as follows:**

Men = $(140 - \text{age}) / \text{serum creatinine}$

Women = $[(140 - \text{age}) / \text{serum creatinine}] \times 0.85$

Maintenance dose

The maintenance dose will be started approximately 24 hours after initiation of digoxin loading. The post-loading digoxin trough will be reviewed prior to starting maintenance dosing.

- If the post-loading digoxin trough is <1.2 ng/ml, maintenance dosing based on lean body weight and renal function will be started using the **Maintenance nomogram** (Table 6.1.2b below).
- If the post-loading digoxin trough is ≥1.2 ng/ml, appropriate actions will be taken as indicated in Digoxin dose adjustment table. Digoxin trough will be repeated 6 hours after the last trough was dose.
 - o If the repeat trough is ≥1.2ng/ml, further administration of digoxin will be discontinued, and the patient withdrawn from the study
 - o If the repeat trough is <1.2ng/ml, maintenance dosing will be started based on the **Maintenance nomogram**.
- After maintenance dosing is started, digoxin trough will be checked daily on blood drawn 8-16 hours after the last maintenance dose. The digoxin trough drawn after the last digoxin maintenance dose will be reviewed daily prior to any subsequent digoxin dose.
 - o If the trough drawn after the last digoxin maintenance dose is ≥1.2ng/ml, appropriate actions will be taken as indicated in Digoxin dose adjustment table.
 - o If the trough drawn after the last digoxin maintenance dose is <1.2ng/ml, maintenance dosing will be continued based on the **Maintenance nomogram**.
- Subjects on P-gp inhibitors or spironolactone, will have an additional digoxin level performed 12-hours after any dose adjustment. Once digoxin levels are stable, 24-hour blood draws will be performed.

Maintenance nomogram

Creatinine clearance*	Lean Body weight (kg)						
	40	50	60	70	80	90	100
30	56 mcg	71 mcg	85 mcg	99 mcg	113 mcg	127 mcg	141 mcg
40	62 mcg	78 mcg	93 mcg	109 mcg	124 mcg	140 mcg	155 mcg
50	68 mcg	85 mcg	102 mcg	119 mcg	136 mcg	152 mcg	169 mcg
60	73 mcg	92 mcg	110 mcg	128 mcg	147 mcg	165 mcg	184 mcg
70	79 mcg	99 mcg	119 mcg	138 mcg	158 mcg	178 mcg	198 mcg
80	85 mcg	106 mcg	127 mcg	148 mcg	169 mcg	191 mcg	212 mcg
90	90 mcg	113 mcg	136 mcg	158 mcg	181 mcg	203 mcg	226 mcg

100	96 mcg	120 mcg	144 mcg	168 mcg	192 mcg	216 mcg	240 mcg
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Creatinine clearance determined as follows:

Men = $(140 - \text{age}) / \text{serum creatinine}$

Women = $[(140 - \text{age}) / \text{serum creatinine}] \times 0.85$

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The Yale Investigational drug pharmacy will purchase digoxin from commercial vendors. All shipments of study medication will be unpacked, and the contents reviewed immediately upon receipt. The pharmacist or designee should verify the study medication against the shipment documentation. The digoxin purchased for use only in this trial will not be used for any other purpose.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Intravenous digoxin is a clear solution for IV infusion. It will be dispensed by the Investigational Drug Pharmacy to participants randomized to digoxin in an open-label manner. The only medication that will be dispensed in this study is digoxin. It will be labeled as an investigational product.

6.2.3 Product Storage and Stability

The storage of digoxin for the clinical trial will be performed according to Good Medical Practice (GMP) standards by the Investigational Drug Pharmacy. Digoxin will be stored at 25°C; excursions permitted to 15°C to 30°C (59°F to 86°F) and protected from light.

6.2.4 Preparation

Intravenous digoxin will be dispensed by YNHH Investigational Drug Pharmacy. There will be no additional preparation of drug involved.

6.3 Measures to Minimize Bias: Randomization and Blinding

The trial is an open-label randomized control trial to explore the treatment effect on cytokine levels as measures of inflammation in patients with acute alcohol associated hepatitis. Digoxin has been used for several decades in patients with heart failure and atrial fibrillation, however there are no previous reports examining its utility and side-effect profile in patients with acute alcohol associated hepatitis. While blinding is considered desirable, we chose to perform an open-label study in this critically ill patient population. Findings from this study will inform the design of a larger, randomized, blinded trial.

Participants who meet all the inclusion criteria and none of the exclusion criteria will be randomized in a 1:1 ratio to the digoxin arm or the control arm using permuted blocks. The randomization will be balanced by using randomly permuted blocks. The randomization scheme will be provided by the study statistician.

The open-label design of the study may affect patient response to the questionnaire. Assessment of several objective measures of disease severity, such as mortality, MELD score, renal function, among others, will reduce the potential for bias in assessing outcomes.

6.4 Study Intervention Compliance

Since the study medication will be administered in the hospital, it is expected that intervention compliance will be high. Digoxin loading, and maintenance doses will be administered as outlined in the protocol. Participants can withdraw from the study at any time if they no longer desire to participate.

6.5 Concomitant Therapy

The main concomitant treatment in this trial is prednisolone, which will be typically administered to all patients with acute alcohol associated hepatitis who do not have contraindication to steroid treatment, as per current standard of care. There are no known interactions between digoxin and prednisolone. The standard of care for severe alcohol associated hepatitis also 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.

The current use of the following drugs is prohibited in the trial: inotropic drugs such as (dopamine, dobutamine, noradrenaline, milrinone), amiodarone, anti-arrhythmics (dofetilide, sotalol, dronedarone), parathyroid hormone analog (teriparatide), thyroid supplement (thyroid), sympathomimetics (epinephrine, norepinephrine, dopamine), neuromuscular blocking agents (succinylcholine), calcium supplement and ivabradine. The current use of disulfiram is also prohibited in the trial.

6.5.1 Rescue Medicine

Digifab is the standard antidote of digoxin which is reserved for cases of life-threatening digoxin toxicity. Digoxin levels will be drawn 6 to 8 hours after the third (ie last) loading dose of digoxin, as well as 8 to 16 hours after each maintenance dose. In this trial, the goal therapeutic digoxin trough is 0.5 to 1.1 ng/ml, which is below the level associated with digoxin toxicity.

However, given that there is sparse data on digoxin use in patients with acute alcohol associated hepatitis, the digoxin antidote Digifab will be available in case any participant exhibits signs or symptoms of severe digoxin toxicity necessitating the administration of a rescue medication. Administration of Digifab will be based on standard of care for management

of digoxin toxicity under the care of the patient's clinical team.

The recommended dosing of Digifab is based on the formula shown below. The nomogram in Table 6.5.1 will provide a quick reference of the number of vials to be administered, which is estimated based on the formula:

$$\text{Digifab dose (in \# of vials)} = ([\text{Serum digoxin concentration in ng/mL}] \times [\text{weight in kg}]) / 100$$

Adult Dose Estimate of Digifab (digoxin immune fab) (in # of vials) from Steady-State Serum Digoxin Concentration*

Patient Weight (kg)	Serum Digoxin Concentration (ng/mL)						
	1	2	4	8	12	16	20
40	0.5 V	1 V	2 V	3 V	5 V	7 V	8 V
60	0.5 V	1 V	3 V	5 V	7 V	10 V	12 V
70	1 V	2 V	3 V	6 V	9 V	11 V	14 V
80	1 V	2 V	3 V	7 V	10 V	13 V	16 V
100	1 V	2 V	4 V	8 V	12 V	16 V	20 V

V= vials

Calculations Based on Steady-State Serum Digoxin Concentrations. Table 6.5.1 provides the estimated number of vials of DigiFab for adult patients for whom a steady state serum digoxin concentration is known.

7. Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

Discontinuation from digoxin does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator will inform the primary team in charge of patient's hospital care in order to determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE). Rules for temporary versus permanent discontinuation of the study intervention are outlined below.

Any patient in whom digoxin is discontinued, whether temporary or permanently, will continue to be followed as per the study protocol.

Temporary discontinuation of digoxin

Infection:

- Similar to the holding rules for the currently on-going NIAAA clinical trial [24], digoxin will be held for 3 days if testing done as SOC yields results that meet any of the following criteria is met:
 - o Pneumonia defined as new infiltrate on imaging (chest Xray or chest CT scan)
 - o Positive cultures (blood, ascites) for bacteria or fungus.
 - o Positive urine culture for bacteria or fungus (> 50,000 colonies/ml).
 - o CNS infection defined as positive cultures or white blood cell > 5 in cerebrospinal fluid.
 - o Severe soft tissue or bone infections including clinical diagnosis of cellulitis.
 - o Clostridium difficile infection.
- Participants will be reassessed on the third day of digoxin hold; if infection is improving, as indicated by decreasing fever curve, improving white blood cell count, etc, digoxin will be resumed.

Digoxin trough level above cutoff

- Participants with digoxin level 1.2 to 2.0 ng/ml will have digoxin placed on hold and actions taken as outlined in Digoxin dose adjustment table.

Overt GI bleeding

- Digoxin will be placed on hold if participant develops overt GI bleeding. The patient will be reassessed daily, and digoxin will be resumed after bleeding has been controlled for >48 hours.

Kidney injury with GFR <30

- Acute kidney injury or worsening of chronic kidney injury in patients with cirrhosis is worrisome owing to high risk of progression. Digoxin will be placed on hold for GFR<30, and serum digoxin levels monitored. Digoxin will be resumed when GFR recovers to >30 on two consecutive daily measurements.

Electrolyte abnormalities in subjects taking concomitant diuretics

- Any subject receiving concomitant treatment with diuretics, with hypokalemia (<3.5 mmol/L), hypomagnesemia (<1.70 mg/dL), and hypercalcemia (>10.20 mg/dL) will have digoxin held until these are corrected.

Permanent discontinuation of digoxin

If patients experience any CTCAE Grade 3 or higher adverse event possibly/probably related to the drug OR CTCAE grade 4 or higher adverse event regardless of attribution to the study drug, they will be discontinued from the trial.

Infection:

- Digoxin will be discontinued permanently if patient is found to be Covid-19 positive.
- Participants with documented infection who are unresponsive to antibiotics, as evidenced by clinical deterioration or persistently positive infectious workup, will have digoxin permanently discontinued after the first 3 days of digoxin hold.

- If the participant requires more than one hold of digoxin due to a subsequent infection after the first digoxin hold, the study drug will be discontinued permanently.

Digoxin trough level above cutoff:

- Participants with post-loading digoxin trough level that is ≥ 1.2 ng/ml on two or more occasions will have digoxin permanently discontinued.
- Participants who develop signs and symptoms attributable to digoxin toxicity will have digoxin permanently discontinued regardless of the trough level as outlined in Digoxin dose adjustment table.
- Participants with digoxin level > 2.0 ng/ml will have digoxin permanently discontinued.

Clinical deterioration:

- During the trial, if Maddrey's Discriminant Factor increases by ≥ 10 points from baseline and also exceeds 32, or MELD score increases by ≥ 10 points from baseline and also exceeds 20, digoxin will be stopped permanently.

Missing data collection during the study:

- Missing an ECG test at baseline will lead to the termination of subject participation.
- Missing an ECG test on two consecutive days will lead to termination of subject participation.

7.2 Participant Discontinuation/Withdrawal from the Study

An investigator may withdraw a participant from the study for the following reasons:

- The participant withdraws consent or requests to be withdrawn from the trial. Participants are free to withdraw from participation in the study at any time upon request.
- The participant refuses to comply with the requirements for trial participation as indicated in the Schedule of Activities.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

The reason for participant discontinuation or withdrawal from the study and the date that this occurred will be recorded on the Case Report Form (CRF).

Participants who sign the informed consent form and are randomized but ultimately do not participate in the study will be replaced. Participants who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

If a participant stays in the study but discontinues study medication (based on participant's preference or due to the investigator's decision), study measurements will be performed according to the Schedule of Trial Procedures. 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 be followed.

7.2.1 Transplant Participant Discontinuation

We will terminate subjects/take them off study at the time of waitlisting for liver transplant with no further data collection. They will have activities performed as per the schedule of events until discontinuation.

7.3 Lost to Follow-Up

Participants will be considered lost to follow up only after documented attempts to reach the participant on three occasions are unsuccessful. A documented effort must be made to determine the reason(s) that a participant cannot be reached or discontinues from the study.

8. Study Assessments and Procedures

8.1 Assessments

Primary outcome:

- The changes in biomarkers of inflammation cytokine levels (TNF-A, IL-6 and IL-10) in patients with acute alcohol associated hepatitis treated with digoxin versus no digoxin at day 3 of the study.

Secondary outcomes:

- Practicality of daily digoxin measurements.
- Feasibility of digoxin dosing in a timely manner.
- Feasibility of digoxin dose adjustments in renal insufficiency.
- Mortality at 7, 14, 28, 90 days. All cause mortality of patients enrolled in the trial.
- Development of ECG abnormalities: Daily ECGs will be performed.
- Ability to recruit 4 patients per month

Tertiary outcomes:

- **Organ dysfunction:** Changes in liver-related function will be determined through assessment of Lille score, MELD, MDF, liver enzymes (including AST, ALT, ALP and bilirubin). Aside from liver dysfunction, dysfunction in other organs will be assessed using SOFA score, MODS, the proportion of participants requiring hemodynamic support for MAP <60 mm Hg, as well as the proportion that develop respiratory dysfunction requiring intubation.
- **Renal dysfunction:** Development of new or recurrent renal failure (creatinine rise ≥ 0.3 mg/dL over 48 hours or $\geq 20\%$ from baseline or requiring renal replacement therapy) will

be quantified (reference).

- **Racial and ethnic diversity in subject recruitment and retention:** Feasibility in recruiting and retaining racially and ethnically diverse participants. The goal is to enroll and retain at least 10% Black and 10% Hispanic participants to study completion.

8.2 Safety and Other Assessments

- **Physical Examination and vital signs:** the investigator or designee will perform a physical examination at the time points specified in the Schedule of Trial Procedures. Vital signs will include the following: temperature, sitting or supine heart rate, sitting or supine blood pressure, respiratory rate, lean body weight in kilograms, and height in centimeters (height obtained at time of screening only). Physical examination will include the following: general appearance, weight, eyes, ears/nose/throat, neck, respiratory system, cardiovascular system, abdomen, mental status, neurological status (asterixis, tremor).
- **Electrocardiogram assessments:** Baseline ECG will be performed during the screening period and will be read by two physicians in the study team prior to initiation of study intervention. ECGs will be performed daily during the treatment period.
- **Special assays:** Serum digoxin levels will be checked daily. All participants with digoxin level ≥ 1.2 ng/ml will undergo evaluation and management as outlined in Digoxin dose adjustment table. Subjects on P-gp inhibitors or spironolactone will have an additional digoxin level performed 12-hours after any dose adjustment. Once digoxin levels are stable, 24-hour blood draws will be performed. Patients on beta-adrenergic and calcium channel blockers will have at least twice a day review of their heart rate and blood pressure by the study team. Patients who will be treated with diuretics will have daily electrolytes checked to detect magnesium, potassium and calcium. Any patient found with hypokalemia, hypomagnesemia, and hypercalcemia will have digoxin held until these are corrected.
- **Infection and Sepsis:** The proportion of participants who develop infection and the types of infection will be determined. In addition, the proportion of patients who develop SIRS, sepsis, septic shock, and their quick-SOFA scores will be assessed.
- **Need for escalation of care:** The proportion of participants requiring escalation of care to ICU will be determined.
- **Duration of hospitalization and readmission rates:** The length of stay and rates of readmission during the 90-day study period will be assessed.
- **Administration of questionnaires:** Patient reported quality of life (SF-36) will be assessed at screening, 30 and 90 days. Alcohol Use Disorders Identification Test (AUDIT), Alcohol Timeline Follow Back (TLFB), and Medical Outcomes Study

Questionnaire Short Form 36 Health Survey (SF-36) will be assessed.

- **Collection of laboratory data from chart review:** Diagnostic tests performed as part of an individual's regular medical care will be used for screening and collected as part of trial data, with adherence to HIPAA rules. Such tests include: CBC, PT/INR, AST, ALT, ALP, total and direct bilirubin, BMP, magnesium, calcium, phosphorus, thyroid function panel, test for COVID-19, pregnancy test, infection workup including but not limited to urinalysis, urine culture, blood culture, paracentesis fluid analysis and culture, chest x-ray, and abdominal imaging tests.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events

Adverse event means any untoward sign (e.g., an abnormal clinically significant laboratory finding), symptom, or disease associated with the use of study medication, whether or not considered intervention-related. 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 (Day 90). The Principal Investigator will determine if an abnormal laboratory finding is considered clinically significant.

Adverse events 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.

Results of laboratory testing, imaging and other tests done during hospitalization will be reviewed. The Principal Investigator will determine if an abnormal result is considered clinically significant. Only clinically significant abnormal findings as assessed by Principal Investigator will be appropriately documented and captured in the electronic data capture.

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.

8.3.2 Definition of Serious Adverse Event

An AE or suspected adverse reaction is considered “serious” if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization, prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include symptomatic bradycardia or arrhythmia requiring transfer to the intensive care unit.

Serious adverse events commonly encountered in severe alcohol associated hepatitis

A number of serious adverse events, including those listed below, are encountered in patients with severe alcohol associated hepatitis. Results of laboratory testing, imaging and other tests done during hospitalization will be reviewed. As stated previously, the Principal Investigator will determine if an abnormal result is considered clinically significant. Only clinically significant abnormal findings as assessed by Principal Investigator will be appropriately documented and captured in the electronic data capture.

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 or more

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 neutrophils > 250/ml, with or without positive bacterial or fungal cultures

Decompensation of liver disease:

- New onset of ascites if not present prior to start of study
- New onset of variceal hemorrhage
- New onset of hepatic encephalopathy (HE)

Organ failure

- Hepatic failure defined as increase in MELD score ≥ 5 on two consecutive occasions.
- Cerebral failure defined as worsening HE $\geq$ Grade 3 if HE was present prior to start of study.
- Renal failure defined as GFR < 30 or requirement for renal replacement therapy despite

adequate volume replacement.

- Respiratory failure defined as need for BIPAP therapy or invasive 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.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

- **Mild:** Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate:** Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".
- **Life-Threatening:** (1) diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted and (2) diseases or conditions with potentially fatal outcomes, where the endpoint of clinical trial analysis is survival.
- **Fatal:** Death

The Common Terminology Criteria for Adverse Events (CTCAE V5) will be used to grade adverse events.

8.3.3.2 Relationship of Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician and temporal relationship determined based his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 Expectedness

The study investigators will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 Time Period and Frequency of Event Assessment and Follow-up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product, and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The trial coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until 90 days after the trial onset.

8.3.5 Adverse Event Reporting

All AEs, whether observed by the investigator, reported by the participant, noted from abnormal laboratory findings that are clinically significant, or identified by other means, will be recorded from Day 0 until the participant completes trial participation (Day 90). Adverse event means any untoward sign (e.g., an abnormal clinically significant laboratory finding), symptom, or disease associated with the use of study medication, whether or not considered intervention-related.

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.

8.3.6 Serious Adverse Event Reporting

The study clinician will immediately report to the project manager any serious adverse event, whether or not considered study intervention related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

The Investigator will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Investigator’s initial receipt of the information. In addition, the Investigator must notify FDA in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.3.7 Events of Special Interest

Events of special interest that will be noted include: missing a timepoint for digoxin level collection; supratherapeutic digoxin level; digoxin dose adjustment indicated but not performed; digoxin dose adjustment performed but not indicated.

8.3.8 Reporting of Pregnancy

Not applicable to this study. As the study drug will be administered during inpatient hospitalization, it is anticipated that the risk of pregnancy occurring during the study period will be low. However, patients who are sexually active are advised to be on contraception for the duration of enrollment.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (Ups) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI’s name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, Ups will be reported using the following timeline:

- Ups that are serious adverse events (SAEs) will be reported to the IRB within 7 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 14 days of the investigator becoming aware of the problem.

- All Ups should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), and the Office for Human Research Protections (OHRP) within 7 days of the IRB's receipt of the report of the problem from the investigator.

9. Statistical Considerations

9.1 Statistical Hypotheses

The primary objective of this randomized control study of digoxin versus no digoxin in patients with severe alcohol associated hepatitis is to explore whether digoxin treatment affects cytokine levels as biomarkers of inflammation in patients hospitalized with severe alcohol associated hepatitis. A priori, the following objectives are specified:

- The primary objective will be exploring the treatment's effect on inflammatory cytokines and estimating the treatment differences and the associated 95% confidence intervals. The secondary feasibility objective will be considered successful if: 90% of patients had digoxin levels checked within the pre-specified times; 90% of patients received every scheduled dose of the drug; 90% of necessary dose adjustments were made appropriately in response to digoxin levels.

9.2 Sample Size Determination

Given the pilot nature of this study, no power analyses for sample size were performed. Instead, the sample size was based on practical considerations including participant flow, budget limitations, and the number of participants required to accurately assess goals. The general flat rule of using 30 participants per study arm was in line with these considerations. The findings from this study will pave the way for a larger scale efficacy trial. For reference, group sample sizes of 30 and 30 achieve 63% to 97% power to reject the null hypothesis of no difference when the standard effect size varies from 0.6 to 1 and the significance level is 0.05 using a two-sided to sample equal variance t-test.

9.3 Populations for Analyses

Sixty patients admitted to the inpatient service at Yale New Haven Health with severe alcohol associated hepatitis, defined by MDF ≥ 32 or MELD ≥ 20 to ≤ 35 , will be studied. Participants will be assigned in an open-label 1:1 randomized allocation of digoxin versus no digoxin. The population for analysis will be 30 patients who received digoxin and 30 patients who did not receive digoxin.

9.4 Statistical Analyses

9.4.1 General Approach

A priori, the following objectives are specified for the study:

- The primary objective will be the exploring of treatment differences on the changes of inflammatory cytokine levels at day 3 of the treatment. The secondary feasibility objective will be considered successful if: 90% of patients had digoxin levels checked within the pre-specified times; 90% of patients received every scheduled dose of the drug; 90% of necessary dose adjustments were made appropriately in response to digoxin levels.

All analyses will be carried out on the intention-to-treat population (i.e. all the randomized patients) with sensitivity analyses conducted in the per protocol population. A pre-analysis review will be conducted prior to the analyses, with the aim of establishing rules for a per protocol analysis and to reconsider the analysis plan (for instance adjustment for potential confounders). A per protocol analysis involves looking at the major protocol violations such as withdrawals and lost to follow-up. The per protocol population will consist of all randomized patients who have complied with the treatment allocation, were not lost to follow-up, and who do not have major protocol deviations pertaining to eligibility.

The number of patients who enroll, discontinue, or complete the study and Digoxin administration will be summarized. Reasons for premature study discontinuation and treatment discontinuation will be listed and summarized. Major protocol deviations, including violations of inclusion/exclusion criteria will also be listed and summarized.

For binary outcomes, count and percentage will be reported for each study arm. For continuous outcomes, the number of patients, mean, median, interquartile range (Q3-Q1), standard deviation, minimum, and maximum values will be reported for each study arm. This study is not powered to perform hypothesis tests, however, point estimates and confidence intervals for individual group means and proportions and between group differences in means and proportions will be reported to inform the design of future studies. Statistical analyses will be conducted using SAS 9.4 (SAS Institute, Cary NC) and the latest version of R (currently 4.0.4)[25].

9.4.2 Analysis of the Primary Outcome

The primary outcome is the changes in cytokine levels (TNF-A, IL-6 and IL-10) from baseline in patients treated with digoxin versus no digoxin at day 3 of the study. To estimate the treatment difference at day 3, we will use all available longitudinal data.

The change of each cytokine level over time will be calculated by subtracting baseline values from those at each time point. The change of cytokine level from all time points will be analysed using mixed effects model for repeated measures (MMRM), with baseline cytokine level, treatment group, time and interaction between treatment group and time as fixed effects assuming missing at random (MAR). With the randomized design and adequate sample size, confounding of the treatment groups by covariates is not anticipated. However, if confounding were to occur, the identified confounders will be added the MMRM as covariates. Correlation among residual errors will be directly modelled by using unstructured covariance and this variance-covariance matrix will be estimated across treatment groups. Treatment difference and associated 95% confidence interval at day 3 will be generated using linear contrast. Due to the exploratory purpose of these analyses, multiple comparisons problems will not be corrected.

Missing pattern on outcomes will be explored and sensitivity analyses will be conducted to assess the robustness of the study's conclusions to different assumptions of missing data.

9.4.3 Analysis of the Secondary and Tertiary Outcomes

Secondary Outcomes

The secondary endpoints will be binary variables at the participant level, with population-level summaries determined as the percentage of participants who have met the binary endpoint. Each participant will be evaluated by a yes/no answer, which will be used for the counts and percentages in the population summaries.

Practicality of daily digoxin measurements.

Feasibility of digoxin dosing in a timely manner.

Feasibility of digoxin dose adjustments in renal insufficiency.

To assess the above secondary feasibility objectives, the count and percentage of patients that (1) had digoxin levels checked within the pre-specified times, (2) received every scheduled dose of the drug and (3) had necessary dose adjustments made appropriately in response to digoxin levels will be summarized in the digoxin group.

Mortality at 7, 14, 28, 90 days: The mortality rates at different time points in the digoxin group and in the control group will be calculated. Kaplan-Meier survival curves will be used to summarize the survival probabilities during and over 90 days in the two arms. Percentiles of survival time (i.e., median survival time) will also be reported, as appropriate.

Development of ECG abnormalities: The number and proportion of patients in the digoxin and control groups with ECG changes compared to baseline will be reported with 95% confidence interval and compared between treatment group using Fisher's exact test or Chi-squared test as appropriate.

Ability to recruit 4 patients a month: The number of subjects recruited per month will be summarized to assess this recruitment feasibility objective.

Tertiary Outcomes

Organ dysfunction: Changes in liver-related function will be determined through assessment of Lille score, MELD, MDF, liver enzymes (including AST, ALT, ALP and bilirubin) over the course of the study. Aside from liver dysfunction, dysfunction in other organs will be assessed using SOFA score, MODS, the proportion of participants requiring hemodynamic support for MAP <60 mm Hg, as well as the proportion that develop respiratory dysfunction requiring intubation. Average change from baseline in continuous measures over the study period will be reported for each study arm. For longitudinally collected and normally distributed variables, mixed-effects model for repeated measures (MMRM) will be built to evaluate the group difference with similar set up as for the primary outcomes. For longitudinally collected binary or count variables, generalized linear mixed-effects model will be built to evaluate the group difference.

Renal dysfunction: The number and proportion of patients developing new or recurrent renal failure (creatinine rise ≥ 0.3 mg/dL over 48 hours or $\geq 20\%$ from baseline or requiring renal replacement therapy) in the digoxin and control groups will be reported and compared between groups using Fisher's Exact test or Chi-squared test as appropriate.

Racial and ethnic diversity in recruitment and retention: Race and ethnicity of enrolled subjects and subjects who completed the study will be summarized using count and proportion to assess the study's objective of enrolling and retaining at least 10% Black and at least 10% Hispanic participants to study completion (90-days follow-up).

9.4.4 Safety Analyses

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

9.4.5 Baseline Descriptive Statistics

Descriptive statistics of baseline characteristics, including demographics and laboratory measurements, will be presented by treatment group. No inferential statistics will be presented.

9.4.6 Planned Interim Analyses

Not applicable to current study.

9.4.7 Sub-Group Analyses

The primary outcome and secondary outcomes will be analyzed within subgroups defined by age, sex, race/ethnicity. Point estimates and confidence intervals for individual group means and proportions and between group differences in means and proportions will be reported for each level of the subgroup variable.

9.4.8 Tabulation of Individual Participant Data

Individual participant data will be tabulated and available for meta-analysis.

9.4.9 Exploratory Analyses

Biochemical analysis will be conducted from biobank specimens, including other cytokines/chemokines levels, gene expression and protein analysis.

20ml of blood will be collected at the time of daily blood collection on days 0, 3, 7, 14, 21 and 28. Blood will be used to extract germline DNA as well as to extract serum/plasma/peripheral blood mononuclear cells (PBMC). The biobank specimens may be shared with other investigators in a de-identified manner.

10.Supporting Documentation and Operational Considerations

10.1 Regulatory, Ethical, and Study Oversight Consideration

10.1.1 Informed Consent Process

10.1.1.1 Consent and Other Informational Documents Provided to participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol (Informed Consent Form). For patients who are Spanish speakers, a certified translation of the ICF will be presented and signed.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Participants who are temporarily or permanently impaired that may occur in alcoholic hepatitis patients, consent will be sought from the legally authorized representative, if not available, then the next-of-kin would be contacted for informed consent. If the subject regains capacity to consent, they will be reconsented with the latest version of the consent.

A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be

informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and/or Food and Drug Administration (FDA).

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the password protected Yale Secure Box. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analysed and stored at the password protected Yale Secure Box. After the study is completed, the de-identified, archived data will be transmitted to and stored at the password protected Yale Secure Box, for use by other researchers including those outside of the study.

Biospecimen banking will occur at days 0, 3, 7, 14, 21, and 28. Serum samples will be obtained at the time of collection of digoxin and stored de-identified at in a -80 degree Celsius biospecimen repository.

When the study is completed, access to study data will be provided through the password protected Yale Secure Box.

10.1.5 Key Roles and Study Governance

Principal Investigator	
Name, degree, title	Bubu Banini, MD PhD Assistant Professor of Medicine
Institution Name	Yale School of Medicine
Phone Number	203-785-4138
Email	Banini.Research@yale.edu
Co-Investigator	
Name, degree, title	Wajahat Mehal, MD PhD Professor of Medicine
Institution Name	Yale School of Medicine
Phone Number	203-737-6063
Co-Investigator	
Name, degree, title	Simona Jakab, MD Associate Professor of Medicine
Institution Name	Yale School of Medicine
Phone number	203-785-4138

10.1.6 Safety Oversight

The Data and Safety Monitoring Board (DSMB) will evaluate study data on an ongoing basis to assure study participant safety and data integrity. The DSMB periodically monitors study data and makes recommendations based on the data that is reviewed. The DSMB will act in an advisory capacity to the Sponsor – Investigator, Bubu Banini, MD, PhD and the Institutional Review Board (IRB) of record to monitor safety of the study.

The DSMB will receive safety data bi-annually. This will include a review of the number of patient evaluations, visits, enrollment, dropouts, protocol deviations, adverse events, medication dosing, and measures of primary and secondary outcome measures. The board will use these reports to evaluate the progress of the study and the quality of data capture. Confidentiality will be maintained during all phases of the studies, with possible exceptions when the DSMB deems it necessary (e.g., serious adverse events, etc.). The DSMB will conduct a thorough review of all this available data to make a recommendation about proceeding with the protocol as planned, making modifications to the study, suspending the study and/ or terminating the study.

The Sponsor – Investigator may inform the DSMB if, at any time, prompt review of safety data is warranted. The DSMB Chair may call an emergency meeting at any time should questions of

study participant safety arise. Any occurrence of a serious and unexpected adverse event will trigger a pause in enrollment to allow for a safety review by the DSMB. For additional information on DSMB processes, please refer to the DSMB Charter.

10.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, that the facilities and staffing are adequate for continued study conduct, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Council for Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s) including federal, state and local regulations and institutional policies and procedures.

Clinical site monitoring will be performed by the Yale Center for Clinical Investigation (YCCI). Monitoring visits will include a site initiation visit, regularly scheduled on-site interim monitoring visits and/ or remote interim monitoring visits while subjects are on study, and a site close-out visit at the site. Following each monitoring visit, a visit report will be generated containing information on site activities and a summary of pertinent points and action items. The report will be provided with a follow-up letter. Site-specific data status reports will be distributed to the site regularly to outline planned, missing or incomplete case report forms and any outstanding data queries.

During monitoring visits, the following may be reviewed:

- Protection of the rights, safety and welfare of subjects through review of informed consent process and documentation, adverse events (AEs) and serious adverse events (SAEs) and safety procedures
- Subject eligibility
- Source verification
- Protocol compliance
- Deviations and Non-compliance
- Investigator Site File
- ICH GCP compliance
- Investigational Drug Storage and Accountability (including quantity and disposal procedures)
- Laboratory Facilities
- Study progress and/or follow-up on issues with Site Principal Investigator (PI) and relevant members of the study team

The Study PI and YCCI will define the required study monitoring activities in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10.1.8 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct as well as data collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be clarified/ resolved.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 Study Records Retention

Study documents and biological samples should be retained until at least 3 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the

responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from researchers 2 years after the completion of the primary endpoint by contacting banini.research@yale.edu.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Yale Center for Clinical Investigation has established policies and procedures for all study group members to

disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Abbreviations

AASLD	American Association for Study of the Liver
AE	Adverse Event
AGA	American Gastroenterology Association
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
BMP	Basic metabolic panel
CBC	Complete blood count
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
CRF	Case Report Form
DILI	Drug-induced liver injury
DLIS	Digoxin-like immunoreactive substances
eCRF	Electronic Case Report Forms
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HE	Hepatic encephalopathy
HIF	Hypoxia Inducible Factor
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
INR	International Normalized Ratio
IRB	Institutional Review Board
MELD	Model for End Stage Liver Disease
MDF	Maddrey's Discriminant Function
MODS	Multi-organ dysfunction score
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NSDUH	National Survey on Drug Use and Health
OHRP	Office for Human Research Protections
PT	Prothrombin time
PI	Principal Investigator
QC	Quality Control
SAE	Serious Adverse Event
SF-36	Medical Outcomes Study Questionnaire Short Form 36 Health Survey
SOA	Schedule of Activities
SOFA	Sequential Organ Failure Assessment

SOP	Standard Operating Procedure
TLFB	Alcohol Timeline Follow Back
UP	Unanticipated Problem
US	United States
YNHH	Yale New Haven Health
SRC	St. Raphael's Centre

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