Title: A Multicenter Randomized, Double-Blind, Placebo-controlled, Dosing, Safety, and Efficary Study of IMM 124-E (Hyperimmune Bovine Colostrum) for Patients with Severe Alcoholic Hepatitis.

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CLINICAL STUDY PROTOCOL

A Multicenter Randomized, Double-Blind, Placebo-Controlled, Dosing, Safety and Efficacy Study of IMM 124-E (Hyperimmune Bovine Colostrum) for Patients with Severe Alcoholic Hepatitis

Protocol Number: TREAT IMM 124-E
IND Number: 15675

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Developmental Phase: 2a

Date of Protocol: August 15, 2016

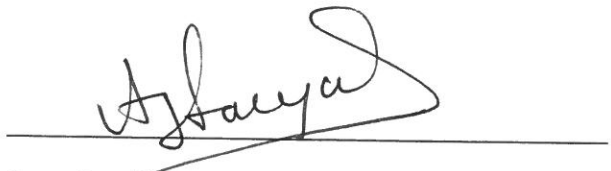
Disclosure

This study is conducted by the TREAT Consortium which is funded by the National Institute of Alcohol Abuse and Alcoholism (NIAAA) to pursue translational investigations in alcoholic hepatitis. The TREAT Consortium is made up of investigators from Indiana University School of Medicine (Indianapolis, IN), Mayo Clinic (Rochester, MN) and Virginia Commonwealth University (Richmond, VA). Study drug and matching placebo are kindly provided by Immuron, Ltd, Australia.

This study will be performed in compliance with Good Clinical Practice, including the archiving of essential documents.

SPONSOR'S Approval of the Protocol

Reviewed and Approved by:



8/23/2016

Arun Sanyal, MD — Date

Primary Investigator (Virginia Commonwealth University)

Table of Contents

ABBREVIATIONS

Abbreviation	Definition
AH	Alcoholic Hepatitis
AE	Adverse Events
ALT	Alanine Aminotransferase (also known as glutamate-pyruvate trasaminase-SGPT)
ANA	Anti-nuclear Antibody
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase (also known as glutamate-oxaloacetate transaminase-SGOT)
AUDIT	Alcohol Use Disorder Identification Test
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CHMP	Committee for Medicinal Products for Human Use
cm	Centimeter
CMV	Cytomegalovirus
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CRSU	Clinical Research Services Unit
CPS	Cytokine Release Syndrome
CPK	Creatine Phosphokinase
CRF	Case Report Form
DF	Discriminant Function
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
EOT	End of Treatment
FACS	Fluorescence Activated Cell Sorter
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMT	Geometric Mean Titer
H	Hours
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HB Ab	Hepatitis B Antibody
HCT	Hematocrit

HCV Ab	Hepatitis C Virus Antibody
HDPE	High-density polyethylene
HGB	Hemoglobin
hCG	Human Chorionic Gonadotropin
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-12	Interleukin-12
INR	International Normalized Ratio
IP	Investigational Product
IMP	Investigational Medicinal Product
IR	Immediate Release
IRB	Institutional Review Board
K	Potassium
kg	Kilogram
LAR	Legally Authorized Representative
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
MELD Score	Model for End Stage Liver Disease Score
MLN	Mesenteric Lymph Nodes
µg	Microgram
mg	Milligram
Min	Minute
mL	Milliliter
N/A	Not Applicable
Na	Sodium
NOAEL	No Observable Adverse Event Level
NYHA	New York Heart Association
°c	Degrees Centigrade
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PI	Principal Investigator
PT	Prothrombin Time
QA	Quality Assurance
R&D	Research and Development
RBC	Red blood cells

SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF	Tumor Necrosis Factor
ULN	Upper Limit of Normal
WBC	White Blood Cells
WHO	World Health Organization
w/w	Weight for weight

PROTOCOL SYNOPSIS

TITLE: A Multicenter Randomized, Double-Blind, Placebo-controlled Dosing, Safety and Efficacy Study of IMM 124-E (Hyperimmune Bovine Colostrum) for Patients with Severe Alcoholic Hepatitis

INVESTIGATIONAL PRODUCT: IMM 124-E Hyperimmune Bovine Colostrum

INDICATION: Treatment of Severe Alcoholic Hepatitis (SAH)

PHASE OF DEVELOPMENT: 2a - Proof of Mechanism

INVESTIGATIONAL SITES/LOCATIONS:

Lead Principal Investigator: Dr Arun Sanyal/Virginia Commonwealth University, Virginia

Investigators: VCU: Dr. Puneet Puri, Dr. Velimir A Luketic, Dr. Mohammad S Siddiqui
Indiana Univ: Dr. Naga Chalasani, Dr. Suthat Liangpunsakul, Dr. David Crabb, Dr. Saurabh Agrawal, Dr. Margaret Sozio, Dr. Marco Lacerda
Mayo Clinic: Dr. Vijay Shah, Dr. Gregory J Gores, Dr. Patrick S. Kamath

OBJECTIVES:

To determine:

1. The safety of orally administered Imm 124-E in patients with severe alcoholic hepatitis being treated with steroids

2. If orally administered Imm 124-E reduces endotoxemia and markers of activation of the innate immune system in patients with severe alcoholic hepatitis being treated with steroids
3. The efficacy of orally administered Imm 124-E in patients with severe alcoholic hepatitis being treated with steroids

STUDY DESIGN: This is a multi-center, randomized, placebo-controlled, double-blind, multiple dose study comprising three groups, 22 patients in each group.

TREATMENT: Subjects with severe AH (MELD ≥20 but ≤28) about to receive prednisolone/prednisone (40 mg/day x 28 days)[1] will be randomized 1:1:1 to receive either Imm 124-E at 2400 mg/day or 4800 mg/day orally or matching placebo for 28 days. Standard of care nutrition support and alcohol cessation recommendations will be provided to all subjects. Alcohol withdrawal will be managed per standard of care.

Eligible patients will be randomly allocated to Imm 124-E or placebo as follows:

- Group A: Imm 124-E 2400 mg in two divided doses daily (plus placebo, see below for further details)
- Group B: Imm 124-E 4800 mg in two divided doses daily

- Group C: Placebo twice daily

NUMBER OF SUBJECTS (PLANNED): 66 patients with 22 patients in each of the above defined study groups

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Diagnosis: Severe Alcoholic Hepatitis (SAH)[1] is defined as:

Well accepted clinical criteria of jaundice and abnormal liver chemistry in appropriate setting with other corroborative findings such as hepatomegaly and leukocytosis

History of heavy alcohol use: Average daily alcohol intake of > 40 g in women and > 60 g in men for a minimum period of 6 months

MELD score > 20

Key inclusion criteria:
Eligible patients must fulfil the following inclusion criteria:

- Men and women age 21 and above
- Severe Alcoholic hepatitis with MELD score ≥ 20 and ≤ 28
- About to initiate prednisolone treatment,< 7 days of steroid treatment, or treatment naïve.
- Actively consuming alcohol within 6 weeks of entry into the study
- Willing and able to comply with study requirements
- Subjects or their legally authorized representatives (LAR) who have provided voluntary written informed consent.

Key exclusion criteria:

- Cow milk allergy, severe lactose intolerance and/or known or suspected allergy to any of the ingredients in IMM 124-E
- Other or concomitant cause of liver disease (Viral hepatitis, autoimmune liver disease, metabolic liver disease, vascular liver disease).
- Subjects who are known to be HIV positive.
- Active infection or sepsis (pneumonia by X-ray, positive blood or urine culture) Untreated spontaneous bacterial peritonitis based on > 250 polymorphonuclear (PMN) cells or positive culture. All subjects with enough ascites to be deemed safe for paracentesis, will undergo this procedure (as standard of care) to exclude spontaneous bacterial peritonitis.
- Acute kidney injury with serum creatinine > 1.5 mg/dl at time of randomization
- Evidence of acute pancreatitis (by imaging and lipase > 5 x ULN)
- Subjects who are pregnant or lactating
- Significant systemic or major illness that, in the opinion of the Investigator, would preclude the patient from participating in and completing the study.

- Subjects who are non-cooperative or unwilling to sign consent form.
- Active Gastrointestinal bleeding (e. g. hematemesis, melena)

(See body of protocol for full list of exclusions)

TEST PRODUCT(S), DOSE AND MODE OF ADMINISTRATION: The placebo and IMM-124E will both be presented as 1200mg of powder for reconstitution in Plastic Aluminium Foil Poly Laminate Pouches (sachets). The appearance of the active and placebo will be indistinguishable. Each subject will take two sachets each morning and then again in the evening. Consequently, each arm of the study will take the same number of doses per day. Subjects in:

- Group A (IMM-124E 2400mg/day) will receive one sachet of active and one sachet of placebo in the morning and again in the evening.
- Group B (IMM-124-E 4800 mg/day) will receive two sachets of active in the morning and again in the evening.
- Group C (Placebo - High protein milk powder) will receive two sachets of placebo in the morning and again in the evening.

DURATION OF TREATMENT: 28 Days

DISCONTINUATION FROM TREATMENT OF INDIVIDUAL SUBJECT:

Reasons for interruption/discontinuation include the following:

- Grade 3 or 4 toxicity considered related to study medication (laboratory abnormalities must be confirmed within 3 days)
- The investigator may withdraw a subject from the study at any time if he/she considers that remaining in the study compromises the subject's health or the subject is not sufficiently cooperative.
- The study DSMB may ask that a subject be withdrawn from the study if it considers that remaining in the study compromises the subject's health and after discussing the issue with the Principal Investigator.

DISCONTIUATION OF ENTIRE STUDY

- The entire study may be discontinued due to unexpected adverse events and with guidance from DSMB and NIAAA.

ENDPOINTS:

Mechanistic Endpoints:

- The sample size is based on a decrease in plasma endotoxin levels (adjusting for baseline) as measured by analysis of covariance across the study groups. This was

chosen based on the mechanism of action of Imm 124-E. The hypothesis is that both active-drug groups are superior to placebo. In prior studies, we have found the standard deviation of endotoxin levels to be 8-10%

- markers of gut permeability
- intestinal inflammation (calprotectin)
- TNF-α and other immune-inflammatory markers
- microbiome-metagenome

Efficacy related endpoints:

- number of subjects meeting Lille failure criteria[3] at day 7
- mortality at 30 days, 90 days and 180 days,
- time to drop in conjugated bilirubin by 50%
- change in MELD and sequential organ failure assessment scores[4] and Discriminant Function
- serum bile acids
- liver function tests

Safety related endpoints:

- incidence and severity of gastrointestinal events, including nausea, vomiting and diarrhea
- Proportion of subjects who develop renal failure, encephalopathy or pulmonary compromise
- Frequency of sepsis
- Frequency of any other adverse events

SAFETY ENDPOINTS:
No unexpected treatment related SAEs are expected throughout the participation period and the follow up period. It is anticipated that majority of the patients will be initially hospitalized; thus, the initial hospitalization or prolongation of hospitalization will not be considered as serious adverse event in this study. Please see "Adverse Events" section of the protocol for further information concerning AE's and SAE's.

STATISTICAL ANALYSIS:
This study is designed to evaluate dose response and appropriateness of endpoints for further work. All measured variables and derived parameters will be listed individually and, if appropriate, tabulated by descriptive statistics. For descriptive statistics, summary tables will be provided giving sample size, absolute and relative frequency by study group and sample size, arithmetic mean, standard deviation, coefficient of variation (if appropriate), median,

minimum and maximum, percentiles, p-values, and 95% CI (Confidence Interval) by study group for means of continuous variables.

Chi-square test or Fisher's Exact test (as appropriate) will be applied for testing the statistical significance of the difference in percent of subjects reporting adverse events between the study groups.

Paired T-Test or Signed Rank test (as appropriate) will be applied for testing the statistical significance of the changes from baseline in laboratory results within each study group.

ANOVA model will be applied for testing the statistical significance of the difference in the changes in laboratory results between the study groups.

ANOVA model will be applied for testing the statistical significance of the difference in immunological markers and the secondary endpoint parameters between the study groups.

Dunnett's method will be used for comparing each of the active groups to the Placebo group. Changes in immunological markers and efficacy parameters may be assessed across each individual subject.

Investigator Statement of Agreement

I agree to:

Implement and conduct this study diligently and in strict compliance with the protocol, the Principles of Good Clinical Practice, and all applicable laws and regulations.

Maintain all information supplied by Immuron Ltd. in confidence and, when this information is submitted to an Institutional Review Board (IRB) or any other group, it will be submitted with a designation that the material is confidential.

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

I have read this protocol in its entirety and I agree to all aspects.

_____ _____

Investigator Signature Date

_____

Printed Name of Investigator

ETHICAL CONDUCT OF THE STUDY AND REGULATORY REQUIREMENTS

Institutional Review Board (IRB)

The study protocol and any amendments will be reviewed by an Institutional Review Board (IRB). The IRB will review the informed consent form, their updates (if any), and any written materials given to the subjects. A list of all IRBs and contact information will be included in the study report.

Ethical Conduct of the Study

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

Subject Information and Consent

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

The Investigator will explain that the subjects are completely free to decline participation the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify.

The subject will receive a copy of the research subject information and consent form.

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

Each subject will be informed that a monitor or an authorized regulatory inspector, in accordance with applicable regulatory requirements, may review the portions of their source records and source data related to the study. Data protection and confidentiality will be handled in compliance with local laws.

INTRODUCTION

Background

Alcoholic Hepatitis

Alcoholic hepatitis[1] is a specific clinical syndrome in patients with history of heavy (on an average > 40 g/day for women and > 60 g/day for men) alcohol consumption for a minimum of 6 months. It is characterized by jaundice with conjugated hyperbilirubinemia, right upper quadrant pain, variable fever, hepatomegaly and frequent leucocytosis. The clinical spectrum of alcoholic hepatitis is quite variable and extends from hepatomegaly with only minimal jaundice to severe jaundice, altered mental status and leukocytosis. The hepatic histology in alcoholic hepatitis includes steatosis, cytologic ballooning, scattered inflammation, extensive Mallory-Denk bodies

and pericellular fibrosis. Alcoholic hepatitis is also sometime characterized by perivenular fibrosis and fibro-obliterative lesions of the centrilobular veins. The severity of cholestasis is variable and can range from none to severe. Clinically, a liver biopsy is usually not necessary to make the diagnosis which rests on the development of jaundice and hepatomegaly without an alternative cause in a subject with a prolonged history of alcohol consumption. In such cases, the serum AST is higher than the ALT and is often more than 2-fold higher than the ALT. In many cases, alcoholic hepatitis is superimposed on underlying alcohol related cirrhosis.

Severe alcoholic hepatitis (AH) is diagnosed by jaundice, hepatomegaly and an AST> ALT in a person with a history of consumption of > 40-60 g of alcohol daily for a prolonged period of time and at least up to 6 weeks prior to clinical presentation. It is also defined by the Maddrey discriminant function (DF) of 32 or higher. This score is based on the serum bilirubin and prothrombin time. Since most hospitals report the INR rather than the prothrombin time, a Model for End Stage Liver Disease (MELD) score of 20 or higher has also been used to define severe AH which corresponds to DF ≥ 32[2]. AH is a major public health problem[5, 6] and accounts for many alcohol related admissions for liver disease. The mechanisms underlying the development of severe AH are not well understood and there is a need to develop more effective treatment. These gaps in the field currently limit the ability to predict, prognosticate, prevent and treat AH.

The severity of alcoholic hepatitis will be assessed by the MELD score (≥20 and ≤ 28) for this study.

Current Treatment

Current standard medical treatment for severe AH is corticosteroid therapy[7]. A fair proportion of patients do not respond to treatment. Unfortunately, no other treatment than steroids has been rigorously evaluated for severe AH in clinical trials. While pentoxyfylline has also been used for severe AH, there are only limited data to support its use[7, 8]. Steroids work best in those with encephalopathy and are contraindicated in those with infection or GI bleeding. The limited therapeutic options for severe AH along with the > 40% mortality associated with it, make severe AH a major public health problem. This gap led the NIAAA to fund a consortium to identify novel targets for therapy and perform early stage proof of mechanism studies for AH. Despite medical treatment, severe AH results in high morbidity and mortality[1, 9, 10].

Role of Lipopolysaccharide (LPS) and Intestinal Microbiome in AH

LPS has been implicated as a central driver in the pathogenesis of AH[11-15]. Increased intestinal permeability with increased systemic exposure to endotoxin activates Kupffer cells. Following a subsequent challenge these cells release inflammatory cytokines which induce cholestasis and hepatic dysfunction. Activation of the innate immune system in both the intestine and systemically has also been implicated in the pathogenesis of severe AH. Recently, specific changes in the intestinal microbiome have also been reported in alcoholic liver disease[16, 17]. However, it is unclear how the altered microbiome and its metabolites might lead to AH, particularly severe AH. Currently, the principal role of the intestinal microbiome in the genesis of AH is as a source of endotoxin and activator of the innate immune system.

Imm 124-E Hyperimmune Bovine Colostrum Powder

Imm 124-E

Imm 124-E contains hyperimmune bovine colostrum and is lactose- and fat-reduced, high in protein and contains no artificial additives. The product contains antibodies targeted against outer antigens of Enterotoxogenic *E. coli* (ETEC). Imm 124-E is harvested from the first milking of dairy cows that have been immunized with a killed ETEC to produce high levels of specific antibodies against selected surface antigens from the most common strains of ETEC, the main causative agent of Traveler's diarrhea. Immuron hyperimmune colostrum powder contains approximately 30% antibodies (immunoglobulins) in the dry powder. The main classes of immunoglobulins found in bovine colostrum are IgG (mainly IgG1) and IgA with small amounts of IgM and IgE. The immunoglobulins in Imm 124-E have high binding activity against the lipopolysaccharide (LPS) of Gram-negative bacteria. Binding of LPS is assayed by Immuron using a standardized ELISA and immuno-blotting detection systems, the Immuron's hyperimmune colostrum powder product.

Imm 124-E is currently marketed as a complimentary medicine through pharmacies in Australia under the name Travelan®. It has been sold since September 2004 for the prevention of traveler's diarrhea at a dose of 200mg three times a day for use by adults and children over 6 years of age. Approximately 140,000 packets (30 tablets each packet) have been sold and no treatment related adverse events have been reported.

In 2001 Immuron Ltd provided full risk assessment information to the Australian Therapeutic Goods Administration (TGA) and Imm 124-E was approved by the TGA as suitable for use as an active ingredient in listable therapeutic goods and a compositional guideline was published in September 2003. Immuron's Imm 124-E Bovine Colostrum Powder is produced to international dairy GMP standards suitable for export of product for human consumption and export from Australia. These standards are recognized in the USA.

Pre-clinical Safety

AMES testing of hyperimmune bovine colostrum powder demonstrated neither cytotoxicity nor mutagenesis. The safety of hyperimmune colostrum powder was evaluated in a non-GLP study in mice. Hyperimmune colostrum powder (10% w/w, test diet) or milk whey powder (10% w/w, control diet) was added to ground commercial feed for laboratory mice. The test and control batches were pelleted and fed for the duration of the trial (10 days). All mice survived and the weight of mice in all four groups increased. Clinical observations did not reveal any adverse effects. The first milking hyperimmune colostrum powder manufactured by the most current collection and processing techniques used to produce its hyperimmune colostrum powder. Potential toxic effects of the hyperimmune colostrum powder were studied in mice at the Department of Microbiology, University of Melbourne, Australia. Five female and five male mice per test group were given a bottle containing either hyperimmune colostrum powder (50mg/ml) or skim milk (SM); controls were given water. Mice were weighed daily, as a group, bottles were changed daily and replaced with fresh hyperimmune colostrum powder, SM or water. Daily consumption of hyperimmune colostrum powder, SM and water was measured as the volume remaining in each bottle after one day. Mice were kept on this diet for a total of eight days. Each

test group of mice was given 100 ml of hyperimmune colostrum powder, SM or water in their bottles daily; food pellets were given *ad libitum*.

All mice showed a steady increase in weight over the eight-day period of testing indicating that the hyperimmune colostrum powder did not have a toxic effect on the mice. Mice health and behavior were the same between the test and control groups. The average intake of hyperimmune colostrum powder for the eight-day test period was 20 mg per gram of mouse per day for females and 19.5 mg per gram of mouse per day for males. The average intake of SM for the eight-day test period was 15 mg per gram of mouse per day for females and 10 mg per gram of mouse per day for males. The slight bias for the colostrum powder may be due to taste or satiety; however this cannot be confirmed from this mice trial. The equivalent intake for an average person of 70 kg body weight would be 1400 g per day for hyperimmune colostrum powder and 1050 g per day for SM.

Pharmacology

Effect of Imm 124-E in Leptin Deficient Ob/Ob Mice

Leptin deficient Ob/Ob mice were fed for 6 weeks with 0.1 mg of hyperimmune colostrum powder prepared from non-immunized cows or with 0.1mg of ETEC colostrum raised against an ETEC extract, or with 0.001, 0.1 and 1mg of IgG-enhanced fraction of ETEC colostrum (Immuron, Australia). Hepatic injury and insulin resistance were measured by fasting glucose levels, glucose tolerance tests (GTT) and liver enzymes. Fat accumulation in the liver and plasma lipids were measured. Serum TNF-alpha was determined by ELISA; and the staining of Tregs in the spleen and liver was performed by flow cytometry.

Oral administration of high dose (1mg) of IgG-enhanced fraction of ETEC colostrum significantly decreased serum ALT ($P<0.05$) and serum and hepatic triglycerides ($P<0.009$ and $P<0.05$, respectively) compared with control animals. Glucose intolerance measured by GTT, was alleviated after 90 and 120 min ($P<0.05$). Oral administration of low and high doses of IgG-enhanced fraction of ETEC and ETEC colostrum lowered glucose levels within 3 weeks of treatment. These results were associated with a decrease in serum TNF-alpha levels following oral treatment with 0.1 and 1mg of Travelan® colostrum. The beneficial effect of IgG-enhanced fraction of ETEC and ETEC colostrum was associated with an increase in the number of CD4+CD25+ cells ($P<0.01$, $P<0.05$, for 0.001, 0.1 and 1mg Imm 124-E hyperimmune colostrum powder, and ETEC, respectively), CD4+CD25+Foxp3 cells ($P<0.001$, $P<0.05$ for 0.001mg of Imm 124-E, and 0.1mg of ETEC, respectively) and CD3+NK1.1 cells ($P<0.05$ for 0.1mg of Imm 124-E, and 0.1mg of ETEC).

Oral administration of IgG-enhanced fraction of ETEC colostrum induced Tregs and ameliorated the chronic inflammatory state in the metabolic syndrome, alleviating insulin resistance and liver injury.

Clinical Data

Imm 124-E was evaluated in double-blinded, randomized, placebo controlled, phase 2 clinical trials in Europe and the USA. These studies are summarized below.

Phase 2 Clinical Trial: Central Public University Hospital, Warsaw, Poland

The double blinded, randomized, placebo controlled, challenge trial with 60 healthy volunteers divided randomly into 4 groups to test a variety of dosage combinations. Each group of 15 was given either 1 or 2 Immuron hyperimmune colostrum powder or placebo tablets 3 times a day (with or without bicarbonate) before meals for 7 days and was then challenged with a pathogenic dose of O78 ETEC (1-2 x 10^9 bacteria). Group 1 had 3 x 2 Immuron hyperimmune colostrum powder tablets per day with bicarb; group 2 had 3 x 2 placebo with bicarb; group 3 had 3 x 1 Immuron hyperimmune colostrum powder tablets per day with water and group 4 had 3 x 2 Immuron hyperimmune colostrum powder tablets per day with water. Participants were seen 3 times a day by clinical staff and submitted a diary that noted the number of bowel movements, a score of the type of movement and any clinical signs such as abdominal cramps and stomach pain. Bacterial analysis was carried out on stools collected before, during and after the challenge. It was found that the active tablet formulations containing high titers of antibodies against ETEC antigens were significantly better ($p<0.01$) than the placebo treatments in protecting the volunteers against the development of diarrhea after the challenge with pathogenic doses of ETEC. There was 86% protection against the development of diarrhea for group 1 volunteers, 80% for group 4 and 64% protection for group 3, compared to 14% for the placebo group (group 2). There was no significant difference between bicarbonate buffered and unbuffered treatments. ETEC O78 was detected in the feces of most volunteers after the challenge, independent of the treatment, showing that the mode of action was blocking the bacteria from binding to the gut epithelium, rather than a bactericidal effect. Study demonstrated that Immuron tablet of hyperimmune colostrum powder, containing antibodies against ETEC bacterial antigens, significantly reduced the risk of susceptible subjects developing diarrhea, following oral challenge with a very high dose of ETEC.

Phase 2 Clinical Trial: Central Public University Hospital, Warsaw, Poland

A double blind, randomized, placebo controlled, challenge trial was conducted with 30 healthy volunteers divided randomly into 2 groups. Each group of 15 was given either 2 Immuron hyperimmune colostrum powder or placebo tablets 3 times a day before meals for 7 days, before challenge with 1-2 x 10^9 O78 ETEC. It was found that the active tablet formulations, containing high titers of antibodies against ETEC antigens, were significantly more effective ($p<0.001$) than the placebo treatments in protecting the volunteers against the development of diarrhea after challenge with pathogenic doses of ETEC. There was 93% protection against the development of diarrhea in the group receiving Immuron hyperimmune colostrum powder tablets, compared to 27% in the group receiving placebo tablets. Bacterial output in stools was not significantly different between placebo and treatment groups, indicating that the mode of action was blocking the bacteria from binding to the gut epithelium, rather than a bactericidal effect.

Phase 2 Clinical Trial: Center of Hyperimmune Colostrum Powder Development, University of Maryland, Baltimore, Maryland, USA

Subjects were treated with ten tablets of active immunoglobulin concentrate containing an equivalent of 1.4 g of colostral immunoglobulins, or equivalent placebo, taken 3 times a day, with bicarbonate solution. In this double blinded, randomized, placebo controlled, challenge trial, 22 healthy volunteers were divided randomly into 2 groups. Each group of 11 was given either 10 immunoglobulin or placebo tablets 3 times a day before meals for 7 days, before challenge with

1 x 10^9 O78 ETEC. The bovine immunoglobulin product reduced the attack rate for diarrhea by 44% following a vigorous experimental challenge with virulent ETEC strain O78, and reduced the severity of the illness, as measured by diarrheal stool volume. There was 64% protection against the development of diarrhea for volunteers who received active tablet, compared to 36% for the placebo group. No adverse events were reported.

Toxin-mediated Intestinal Conditions

In a study of 10 volunteers challenged orally with a concentrate of ETEC, the administration of a bovine antibody concentrate obtained by immunizing cows with the corresponding E. coli strains prevented the development of diarrhea in all 10 participants who received the product; by contrast, 9/10 controls developed diarrhea[18]. In another study, the administration of milk-derived antibodies against the ETEC colonization factor protected 14/15 subjects from diarrhea, compared to 7/10 subjects given placebo[19]. Another disease with a similar pathogenesis is pseudomembranous colitis. A study evaluated the effect of immune whey protein, obtained by immunizing cows with *C. difficile* inactivated toxins and whole-cell killed *C. difficile* to prevent relapse of *C. difficile* disease. Sixteen patients received the product after standard treatment for a confirmed episode of *C. difficile* colitis for two weeks. In all but one case, *C. difficile* toxin was not measurable in the stool, and there were no recurrences after a median follow-up of 333 days[20]. Collectively, these observations suggest that bovine-derived colostrum preparations deliver biologically active concentrations of specific antibodies to the intestinal lumen when taken orally, and might be capable of blocking various forms of bacterial toxins in the gut by that mechanism.

Phase 1 Clinical Trial of Hyperimmune Colostrum Powder in Patients with NASH, Hadassah Medical Center, Jerusalem.

An open-label trial was conducted comprising 10 subjects with biopsy proven NASH and insulin resistance. These subjects were orally treated for 30 days with IgG-enhanced fraction of Enterotoxigenic E.coli (ETEC) colostrum (Immuron, Australia). Subjects were monitored for safety, serum levels of adiponectin, GLP-1, and regulatory T cells (Tregs). The clinical effect was determined by OGTT, liver enzyme tests, and lipid profile. The comparison was done between day 0 and day 30 for each patient.

Oral administration of ETEC colostrum was well tolerated. Alleviation of insulin resistance was determined by the following measures: a decrease in fasting glucose levels (6.9 vs. 6.05 mmol/L, $p<0.06$); elevation in the early peak of insulin secretion following glucose administration (278 vs. 470 pmol/L, $p<0.07$); Improved OGTT (AUC of 2492 vs. 2252, $p<0.08$); improved insulin secretion during the OGTT (AUC of 99177 vs. 117784, $p<0.08$); improved HOMA score (6.71 vs. 4.82, $p<0.06$) and improved HbA1c levels (7.19 vs. 6.20, $p<0.001$). Treated patients showed a decrease in serum levels of triglycerides (1.88 vs. 1.32 µmol/L, p=NS), total cholesterol (5.28 vs. 4.44 µmol/L, $p<0.04$), and LDL cholesterol (3.7 vs. 2.49 µmol/L, $p<0.07$). A decrease in liver enzymes was noted in most treated patients (ALT: 54.5 vs. 43.16, u/L, $p<0.04$; AST: 50.58 vs. 45.5 u/L, $p<0.01$; Alkaline phosphatase: 82.1 vs. 72.4 u/L, $p<0.001$; GGT: 84.3 vs. 58.6 u/L, $p<0.05$). These effects were associated with increased serum levels of GLP-1 and adiponectin noted in 60% and 80% of treated patients, respectively (58.816 vs. 62.828 pM, for GLP1, $p<0.07$; and 6181 vs. 7068, ng/ml, for adiponectin, $p<0.08$), the Adiponectin/IL-6 ratio increased in 60% of patients (1569.51 vs. 1884.74 $p<0.03$) An increased in CD4+CD25+ CD4+CD25+Foxp3+

Tregs (4.6% vs. 6.3% and 1.95% vs. 2.28% respectively, $p<0.002$) and in CD4+CD62+ and CD4+CD25+ HLA-DR (34.4% vs. 38.4% and 1.9% vs. 3 $p<0.002$).

Oral administration of ETEC colostrum was well tolerated and exerts an immunomodulatory effect in patients with type 2 diabetes, hyperlipidemia and NASH. The anti-inflammatory effect and promotion of Tregs were associated with alleviation of insulin resistance, hyperlipidemia, and liver damage in these patients.

Rationale

The objective of the current trial is to provide "proof of concept" that oral administration of Imm124-E is well tolerated and will improve pathophysiological/clinical parameters related to severe AH.

Imm124-E is a simple and rational way to deliver anti-LPS to the intestine to reduce the burden of LPS in severe AH. There is no direct evidence in humans that decreasing the burden of LPS improves AH. In this proposal, we will use the innovative approach of targeting LPS in the gut by oral administration of anti-LPS that is relatively protected from digestion to improve AH. This study will generate novel data to further clarify the role of altered microbiome and its metagenome in AH.

Imm 124-E is a "first in class" product for AH. Conventional wisdom, summarized in FDA guidance [21], dictates the need to identify the tolerability, safety profile and the best dose based on safety, pathophysiology, and efficacy-related endpoints, in a proof of concept trial prior to embarking on a trial powered to evaluate clinical efficacy endpoints.

Severe AH is associated with the greatest risk of mortality and thus is a logical target for new therapeutics. Also, in this sick population, the TREAT consortium felt that the principle of "primum non nocere" would be best served with Imm 124-E which has a supportive safety profile. This initial study will be performed against a background of steroid therapy because it would be unethical to do a pure placebo controlled study in this sick population.

Risk-Benefit Considerations

The risk:benefit of Imm 124-E in severe AH has yet to be elucidated. There is a plausible rationale for possible reduction of liver damage secondary to AH but this has not been established. Weighed against these potential benefits are potential risks inherent in the protocol:

1. Like any drug, subjects receiving the drug could experience unanticipated side effects. Based on current experience, Imm 124-E has been well tolerated. Possible side effects of the gastrointestinal tract include nausea, abdominal pain, flatus or diarrhea. It is possible that other, unexpected unanticipated side effects may occur and there may be long latency periods before AEs are detected.
2. There is a possibility of pain and bruising in areas where blood is drawn.
3. If a subject becomes pregnant during the study, there may be risks that are currently unforeseeable that may cause damage to the fetus. The study requires women and men to undertake two forms of contraception.
4. Each subject will be instructed to inform the research physician about any new medical problem that may develop during participation in the study. After the study is completed,

the subjects may contact the study Investigator(s) with any study-related medical problems that may occur.

5. There is a possibility that the immune modulatory effect noted may not have a sustained beneficial effect, or alternatively may exert a deleterious effect on the tested disease parameters.

STUDY OBJECTIVES AND ENDPOINTS

Primary Objective

The primary objective of the study is to determine the safety of orally administered Imm 124-E and to determine if it reduces endotoxemia and markers of activation of the innate immune system in patients with severe AH being treated with steroids.

Secondary Objective

The secondary objective is to determine the safety of orally administered Imm 124-E in patients with severe AH being treated with steroids.

Exploratory Objectives

The exploratory objectives are to determine the preliminary efficacy of orally administered Imm 124-E in patients with severe AH being treated with steroids.

Primary Endpoint

The primary endpoint is the safety profile of Imm 124-E in this pilot study.

Secondary Endpoints

The secondary endpoints will include:

- Frequency, severity and relatedness of adverse events
    - Proportion of subjects with nausea and emesis
    - Proportion of subjects with diarrhea
    - Proportion of subjects who develop renal failure, encephalopathy or pulmonary compromise
    - Frequency of sepsis
- Changes in endotoxin levels adjusted for baseline levels at day 7 and 28
- Change in laboratory parameters

Exploratory Endpoints

The exploratory endpoints will include:

- sCD14 levels (ELISA test to be measured at VCU)
- Intestinal permeability Intestinal inflammation (stool calprotectin by ELISA -to be measured at VCU)
- Proportion meeting Lille criteria for futility (Lille score > 0.45) at day 7[3]
- Mortality at day 7, day 30, day 90 and day 180

- Change in MELD score and discriminant function
- Change in liver function
- Intestinal microbiome-metagenome (end of treatment (EOT) comparison) [22]
- Activation of systemic inflammation: (EOT across group comparison, baseline-EOT comparison)
    - Markers of oxidative stress (measured at IU)
    - T-cell phenotypes (measured at IU)
    - Cytokines for specific T cell subtype and activation of innate immune system (measured at IU)
- Hepatic transcriptome (real time PCR, gene array Illumina in all cases-EOT across group analyses).RNA will also be available for baseline data and gene array with PCR confirmation of key targets will be performed so that variances at baseline across groups can be accounted for.

INVESTIGATIONAL PLAN

Overall Study Design and Plan – Description

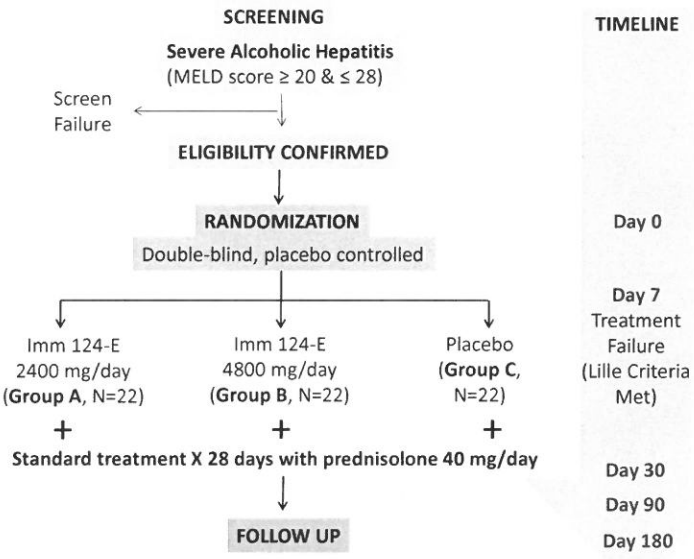


Figure 1: Study Schema

Subjects with severe AH (MELD score ≥ 20 and ≤ 28) about to receive prednisolone (40 mg/day x 28 days)[1] will be randomized to receive either:

- Group A: 2400 mg/day Imm 124-E as 1200mg bid PO
- Group B: 4800 mg/day Imm 124-E as 2400mg bid PO
- Group C: Identical appearing placebo bid PO

Treatment will be for 28 days **(Fig. 1, above)**. Given variable outcomes and response to corticosteroid therapy in patients with severe AH, a Lille model was developed by French investigators to identify subjects early in the course of treatment that are unlikely to benefit from continued treatment and in fact might have worse outcome. The Lille model takes into account patient's age, and at the start of therapy total bilirubin, albumin, creatinine, prothrombin time values and total bilirubin level on day 7 of therapy. It projects a 6-month survival probability of patients with a Lille score above 0.45 to be about 25% contrary to patients with a Lille score below this cutoff (85%)[3]. Subjects who meet the Lille criteria[3] for failure of treatment on Day 7 or side effects requiring discontinuation of steroids will be removed from the study. Standard of care nutrition support and alcohol cessation recommendations will be provided to all subjects. Alcohol withdrawal will be managed per standard of care.

Selection of Study Population

The subjects will be screened to determine eligibility criteria. Criteria are to be determined at Screening unless otherwise indicated. Those that meet all of the inclusion and none of the exclusion criteria will be enrolled.

Inclusion Criteria

- Men and women aged 21 and above
- Severe AH with:
  - MELD score ≥ 20 and ≤ 28
  - About to initiate prednisolone treatment, < 7 days of steroid treatment, or treatment naïve.
- Actively consuming alcohol within 6 weeks of entry into the study
- Willing and able to comply with study requirements
- Voluntary written informed consent

Exclusion Criteria

- Cow milk allergy, severe lactose intolerance and/or known or suspected allergy to any of the ingredients of IMM 124-E
- Concomitant cause of liver disease:
  - Hepatitis B: positive surface antigen
  - Hepatitis C: positive HCV antibody and/or PCR
  - Autoimmune hepatitis: (positive ANA >1:160 titer, ALT > AST, and ALT>300u/L
  - Budd Chiari syndrome: Hepatic vein occlusion by Doppler sonography
  - Subjects who are known to have Wilson's disease
- Subjects who are known to be HIV positive

- Active infection and sepsis (pneumonia by X-ray, positive blood or urine culture)
- Untreated spontaneous bacterial peritonitis based on >250 polymorphonuclear (PMN) cells or positive culture
- Acute kidney injury with serum creatinine >1.5 mg/dL at the time of randomization
- Evidence of acute pancreatitis (by imaging and lipase >5 x ULN)
- Active gastrointestinal bleeding (e.g. hematemesis or melena)
- Subjects who are pregnant or lactating
- Significant systemic or major illness, that, in the opinion of the Investigator would preclude the patient from participating in and completing the study
- Patients requiring the use of vasopressors or inotropic support in 12 hours prior to randomization
- Liver biopsy, if carried out for clinical indications, showing findings not compatible with alcoholic hepatitis
- Any patient that has received any investigational drug or device within 30 days of dosing or who is scheduled to receive another investigational drug or device in the course of the study
- Treatment for alcoholic hepatitis within 1 month of study entry with corticosteroids or corticosteroid use >1 week immediately prior to the time of entry into the study.

Removal of Subjects from Therapy or Assessment

Each subject will be informed of their right to withdraw from the study at any time and for any reason. Subjects may be withdrawn from treatment or assessment:

- If continuing in the study does not appear to be in the best medical interest of the subject, his/her participation may be terminated by either the Principal Investigator or the study director. Removal of an individual by the Principal Investigator will be reported to the Institutional Review Board and the appropriate regulatory agencies according to the guidelines of these groups.
- Subjects considered as treatment failure i.e. those who meet Lille criteria for failure will be removed from the study and will be offered alternative standard therapy by their treating physician.

Reasons for permanent discontinuation include the following:

- The subject decides to withdraw from the study.
- The Investigator may withdraw a subject from the study at any time if he considers that remaining in the study compromises the subject's health or the subject is not sufficiently cooperative.
- The study DSMB on behalf of NIH and the TREAT consortium may ask that a subject be withdrawn from the study if it considers that remaining in the study compromises the subject's health and after discussing the issue with the Principal Investigator.

The reasons for any subject withdrawal will be recorded on the study completion form of the case report form (CRF). The Investigator will inform the Sponsor in writing of the subject's early withdrawal for any reason.

Upon withdrawal from the study, any time after dosing has taken place, the subject will have a further hematology, serum biochemistry, urinalysis, and medical examination. Laboratory tests will be those outlined for the follow-up safety tests in Appendix 1.

If withdrawal is caused by an adverse event that the Investigator considers may be related to the Imm 124-E, it will be reported to the IRB and to the Sponsor.

Any serious unexpected adverse event which is thought to be possibly or probably related to the study drug must be reported to Sponsors by telephone within 24 hours of being informed of the SAE or on the next business day after a holiday or weekend, and then, a Serious Adverse Event form must be completed and faxed to the sponsors within 48 hours. The event must be reported to the IRB within 5 days.

In the event of any abnormalities considered to be clinically significant by the investigating physician, subjects will be followed up with appropriate medical management until the outcome is determined or stabilized, according to the Principal Investigator's clinical judgment. All follow-up information will be recorded in the subject's CRF until full subject recovery is achieved and the PI has stated that the subject is dismissed from the medical care. Subsequent follow-up will be documented in the subject's personal file.

The TREAT steering committee reserves the right to discontinue the study at any time for any reason. If applicable, the Investigator must implement NIAAA request to terminate the study in a time frame that is compatible with the subjects' well-being.

STUDY PRODUCT

Study Medication Supply

Imm 124-E will be provided to each site by Immuron. The medications will be stocked and dispensed from the investigational drugs pharmacy at each site.

Description of Study Product

Name:	Imm 124-E
Characteristics & Physical State:	Off white powder equivalent to 1200mg of Imm 124-E hyperimmune bovine colostrum
Formulated & Supplied by:	Immuron Ltd
Storage Conditions:	Store in cool and dry place. Temperature controlled between 2˚C and 8˚C
Shipments:	Temperature controlled between 2˚C and 8˚C

Package:	Plastic Aluminium Foil Poly Laminate Pouches

Description of Comparator Product

Name:	Placebo
Characteristics & Physical State:	Off white high protein milk powder, ProMilk85®
Supplied by:	Immuron Ltd.
Storage Conditions:	Store in cool and dry place. Temperature controlled between 2˚C and 8˚C
Shipments:	Temperature controlled between 2˚C and 8˚C
Package:	Plastic Aluminium Foil Poly Laminate Pouches

Packaging and Labeling

All clinical supplies will be packed and labeled in compliance with the Good Manufacturing Practices of drugs used in clinical trials. All study medications provided will be appropriately documented.

Imm 124-E will be supplied in Plastic Aluminium Foil Poly Laminate Pouches (sachets). The sachets will be labeled as following:

1. Investigational pharmacy at each recruiting center will label each sachet with the subject's study ID, subject's name, IMM-124E/placebo, batch number, and expiry date before dispensation. It will also state that the medication is only for the intended subject and is not to be shared.

2. Labels of the outer packaging (the package in which the sachet will be placed) will bear the details of the name of Immuron Ltd., the address and telephone number of the main contact for information on the product, route of administration, batch number of Drug Product and Placebo, a trial reference code (i.e., TREAT), the Study ID, Investigator name, directions for use, and the phrase "For Clinical Trial Use Only". Expiration date will be given in month/year format, and label will include storage conditions.

Method of Assigning Subjects to Treatment Groups

At enrollment and after an ICF is signed, subjects will be identified by their subject ID. Subjects with severe alcoholic hepatitis will be screened for this trial from the inpatient services of the participating centers. Informed consent will be obtained after confirming eligibility. Baseline studies will be performed and subjects will be started on prednisolone/prednisone (40 mg/day) and randomized to the study drug 2400 mg, 4800 mg/day, or placebo. Once enrolled at the Screening visit, each subject will be given a unique identification number which will identify both the site and the individual subject. At the time of randomization, the randomization code will be linked to this identifier and this information will be provided to the clinical center by the data coordinating center. This information will be used by the investigational drug pharmacy to dispense the appropriate study drug/placebo to the individual subject.

Subjects will be randomized sequentially, as they become eligible for randomization. If a subject discontinues participation from the study, their unique identifier code will not be reused, and the subject will not be allowed to re-enter this study.

The appearance and packaging of active and placebo powders for reconstitution are identical. Sachets of study drug will be packed in groups of two sachets into sealed cardboard boxes supplied by Immuron Ltd. Each box will have a code which will be matched to the randomization code at the data coordinating center. Following reconciliation of the randomization code with the box code, the investigational drugs pharmacy will dispense enough medication to last 28 days (56 packs, a total of 112 sachets).

A sealed master list of "box codes i.e. the box set number" vs. randomization code will be securely held by Immuron Ltd., the data coordinating center and also in a sealed envelope by each site pharmacy.

To maintain the double-blind design, drug supplies will be dispensed only by the investigational drug pharmacist, according to a written procedure provided by the Sponsor. The pharmacist will not participate in any other study procedure.

Randomization Procedures

This is a double blind study and therefore the study staff and the subjects will remain blinded to the code assignments throughout the study. Prior to administration, each participant will be assigned with an individual number and will be provided with IP packs according to the predetermined computer generated randomization list.

Unblinding Procedures

The Investigator will promptly document and explain to the data coordinating center, the NIAAA and the data safety and monitoring board any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s). Reasons for premature unblinding will be clearly documented by all research personnel involved and will include reason(s) of unblinding. In cases of emergency where written permission cannot be obtained, the site PI will make the decision to unblind based on the perceived harm to the subject if that is not done and then document that unblinding has occurred.

Upon completion of the study, emergency code envelopes will be returned to the Sponsor.

Dispensing, Compliance and Accountability

Eligible subjects will be randomly assigned to each of the study groups. Subject will be instructed to take the contents of two sachets of powder reconstituted with water twice daily. Subject ID pack dispensing will be documented in the Case Report Forms and on the Investigational Drug Administration Records.

To prepare the study drug immediately before administration, add the powder to a cup and add approximately four tablespoons of water to the cup, stir gently for 1 minute until dissolved. Each subject will be required to drink the contents of both sachets in the morning before breakfast. Additional water may be added to the powder to ensure all the powder is taken. Subjects will be required not to eat for 2 hours after taking the drug. If a subject forgets to take the drug in the morning, he/she can take the drug during the day. He/she will be required not to ingest food for

2 hours before and 2 hours after taking the drug. The subject will also be instructed to take the contents of two sachets in the evening, at least 2 hours after eating.

The Investigator/designee is responsible for maintaining accountability for the receipt, dispensing, and return of all study medication.

The Investigational Pharmacy will be responsible for recording the receipt of all drug supplies and for ensuring the supervision of the storage and allocation of these supplies. When a shipment is received, the pharmacist verifies the quantities received and the accompanying documentation (e.g. certificate of analysis, date of manufacturing/expiry, storage instructions etc.) and return the acknowledgment of receipt to the Sponsor.

The Investigator/designee is responsible for keeping records of receipt of study medications, where the study medication is stored after arrival to the clinic and for maintaining detailed records, indicating dates and amounts of drug dispensed to whom ("subject by subject accounting").

Subjects will be requested to return all the used and unused sachets. That shall be recorded by the Investigator/designee in the CRF. The records should:

- distinguish between unopened (not dispensed) and unused (by study subjects) supplies;
- explain broken or lost drug supply; and
- account for all supplies and stock that may have been used.

The subjects will be properly educated about these procedures. If unused drug is returned, the Investigator/designee must follow protocol instructions regarding documentation.

The Monitor will check the drug account, and all unused drug will be sent to the pharmacy or drug depot at the site and destroyed at the end of the study. Products deliberately and/or accidentally destroyed by the Investigator should also be accounted for. Used packs should be kept until the Clinical Monitor has performed drug accountability.

At study termination, unless otherwise specified (in writing), all unused drug supplies, including partially used containers and the study drug accountability log, should be returned to:

Immuron Ltd.

Level 1, 18 Kavanagh Street

Southbank VIC 3006, Australia
Tel: +61 3 9018 4880; Fax: +61 3 9018 4881

When the supplies are returned, the Investigator or pharmacist signs the Immuron's Investigational Drug Return Form, to verify that all unused or partially used supplies have been returned and that no study supplies remain in the Investigator's possession. One copy of all inventory records and the return statement are retained by the Investigator for the study files.

Prior and Concomitant Therapy

Any concomitant medication should be reported to the study team and will be recorded in the subject's files and CRF.

Clinical Laboratory

Clinical chemistry and hematology tests will be flagged when outside of the reference range. The responsible Investigator will assess its significance as "Clinically significant" (CS) or "Not clinically significant" (NCS).

Safety tests (biochemistry, hematology, urinalysis and stool analysis: See Table 2) will be performed along with the safety assessment.

Safety tests will be carried out by accredited clinical laboratory. All tests are to be collected and performed according to written instructions. The study coordinator will notify the laboratory when samples have been collected and it is the responsibility of laboratory to pick up these samples on the same day.

Test results will be forwarded to the study team within 48-72 hours.

Table 1: Protocol Thresholds for Clinical Significance of Abnormal Safety Labs

Table 1: Laboratory Cutoffs That Would Lead to Drug Discontinuation in an Individual Subject
Hematological • Absolute neutrophil count < 500/mm^3 • Platelet count < 20,000/mm^3 **Hepatic safety labs** • AST or ALT > 20x upper limit of normal • AST or ALT doubling with absolute values > 400 IU/L Please note that bilirubin is highly elevated to start with and may rise as part of the disease process in subjects with severe alcoholic hepatitis and therefore cannot be used as a test to detect drug induced liver injury Also, INR is prolonged in subjects with severe alcoholic hepatitis and there cannot be used to evaluate drug related liver injury in this setting

Evaluation of Adverse Events

Adverse events will be assessed in the scheduled visits (Day 7, day 30, day 90 and day 180), and in unscheduled visits. The intensity of rash, pruritus, malaise, arthralgia and myalgia will be evaluated on a scale of 0 to 3 (see table below) and recorded in the CRF.

Assessment Scale for Systemic Reaction Severity

Symptoms	Score
Absence of symptoms	0
Slightly bothersome	1
Bothersome and interferes with normal activity	2
Prevents normal activity	3

Adverse Events

Adverse Events (AE), Serious Adverse Events (SAE), and Suspected Adverse Reactions

An adverse event (AE) is any untoward medical occurrence in a subject participating in a clinical trial. An adverse event can be any unfavorable and unintended sign, symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication. AEs will be collected from the start of treatment until 24 weeks following the initial dose of study medication. Any events occurring prior to treatment will be recorded on the medical history page with the event name, onset date and end date if not continuing. Pre-existing, known clinically significant conditions observed at screening should be recorded as medical history.

This definition also includes accidental injuries, reasons for any change in medication (drug and/or dose) other than planned titration, reasons for admission to a hospital, or reasons for surgical procedures (unless for minor elective surgery for a pre-existing condition). It also includes adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the study medication.

A **treatment emergent adverse event** is any adverse event occurring after start of study medication and within the time of residual drug effect, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of study medication and within the time of residual drug effect.

Causality Assessment

For this study, all AEs developing any time from administration to 7 days after discontinuation will be evaluated for relationship to study drug. For Imm 124E, the closer it is to study administration and if it recurs on rechallenge (depending on the nature of the event), it will be considered to be study drug-related.

Adverse events should be recorded as diagnoses, if available. If not, separate sign(s) and symptom(s) are recorded. One diagnosis/symptom should be entered per record.

Hospitalization will not be considered an event; however, the reason for hospitalization will be documented as an adverse event. Procedures are not events; the reasons for conducting the

procedures are. In general, only the reason for conducting the procedure will be captured as an adverse event. However, if deemed necessary by the Investigator, a procedure can be captured along with the reason for conducting the procedure.

An overdose or medication error is not an adverse event unless it is temporally associated with an unfavourable or unintended sign or symptom.

Each AE is to be classified by the Investigator as serious or non-serious. A serious adverse event (SAE) is any untoward medical occurrence or effect that occurs at any dose:

- Results in death
- Is life-threatening (i.e., an immediate risk of death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is associated with a congenital anomaly/birth defect
- Is an important medical event

An adverse event caused by an overdose or medication error is considered serious if a criterion listed in the definition above is fulfilled.

Important adverse events that may not result in death, may not be life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject's safety or may require medical or surgical intervention to prevent one of the outcomes listed above.

Serious adverse events also include any other event that the Investigator or sponsor judges to be serious or which is defined as serious by the regulatory agency.

A **suspected adverse reaction** means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

The Investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject using concise medical terminology. In addition, each trial subject will be questioned about adverse events. The question asked will be "Since you began taking the study medication, have you had any health problems?"

Procedures for Assessing, Recording, and Reporting Adverse Events and Serious Adverse Events, in Accordance with 21 CFR 312.32(a) FDA Guideline for Safety Reporting

Throughout the duration of the study, the Investigator will closely monitor each subject for evidence of drug intolerance and for the development of clinical or laboratory evidence of adverse events. All adverse events (expected or unexpected) which occur during the course of the study, whether observed by the Investigator or by the subject, and whether or not thought to be drug-related, will be reported and followed until resolution or until they become stable.

All subjects will be under close medical supervision during their stay in the CRSU or hospital. During the entire course of the study, subjects will be encouraged to report any adverse events which may occur.

Any adverse event or other unwanted response which occurs in the course of the study should be monitored and followed up until:

- It has resolved or stabilized according to the judgment of the Principal Investigator.
- There is a return to normal or baseline values.
- It has been shown to be unrelated to the study drug.

All adverse events noted during the course of the study will be included in the final study report.

The description of the adverse event will include description of event, start date, stop date, intensity, if it was serious, relationship to test drug, change in test drug dosage, if the subject died, and if treatment was required.

Events will be coded to one of the following intensity categories below:

Toxicity Grade	Severity	Definition
1	Mild	Awareness of signs or symptoms, but no disruption of usual activity
2	Moderate	Event sufficient to affect usual activity (disturbing)
3	Severe	Event causes inability to work or perform usual activities (unacceptable)
4	Life Threatening	

Events will be coded into one of the following causality categories as defined below:

Category	Definition
Not Related	This category applies to those adverse experiences, which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)
Unlikely	In general, this category can be considered applicable to those adverse experiences which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug.
Possibly	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty.
Probably	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug.
Almost Certainly	

Adverse events with the causality assessed as unrelated or unlikely are categorized as not related to study medication.

Adverse events with the causality assessed as possible or probable are categorized as related to study medication and are called adverse drug reactions.

Any Serious Adverse Event, whether deemed drug-related or not, must be reported to the IRB, and to the Sponsor by telephone and by Fax, as soon as possible after the Investigator has become aware of its occurrence even if not all the information is available at the time of initial contact:

Primary Sponsor (NIAAA) Dr. Gary Murray

Secondary Contact (Immuron) information:

Contact person: Dan Peres, Director of Clinical Research
Email: dan@immuron.com

The Investigator must complete a Serious Adverse Event (SAE) Form, and send it, via fax, to the Sponsor within 24 hours of becoming aware of the event. If the event occurs over the weekend, it will be reported on the first business day after the weekend. Accompanying documentation, such as copies of hospital case reports, autopsy report, and other documents when applicable, should be sent as soon as they are available.

Subjects who have had an SAE must be followed clinically until all parameters (including laboratory) have either returned to normal or are stabilized.

Any serious and unexpected adverse reaction (not consistent in severity, specificity, outcome or frequency, with the relevant safety information), including all deaths, must also be reported to the IRB by the Investigator according to local regulations.

STUDY PROCEDURES AND FLOW CHART

The total expected duration of the study for each subject is up to 180 days and tabulated below:

Screening: Up to 7 days

Treatment: 28 days

Follow-ups: Day 30, day 90 and day 180 post treatment

Table 2: Schedule of Study Procedures

Schedule of Study Procedures	Screening	Treatment Phase		Follow-up Phase		
	Day-7 to 0	D0	D7	D30	D90	D180
Informed Consent	X					
Eligibility Criteria/Demographics	X					
Medical and Surgical History/Updated	X		X	X	X	X
Medication Record /Concomitant meds	X		X	X	X	X
Physical Exam/Vital signs	X		X	X	X	X
Anthropometric Measurements/Nutrition	X					X
Urine Pregnancy Test	X		X	X	X	
Adverse Event Assessment			X	X	X	X
Liver biopsy (if performed)	X					
Study Drug Compliance Assessment			X	X		

Assessment of Meld, DF, and Child Pugh Scores	X		X	X	X	X
Assessment of Lille Score			X			
Alcohol Addiction Advice per SOC	X		X	X	X	X
Dispense Study Drug		X				
Alcohol AUDIT and NIAAA Six Question Set	X					X
Time Line Follow Back	X					X
Tobacco/Marijuana Use Questionnaire	X					
CBC, Hepatic panel, INR, Basic metabolic panel	X		X	X	X	X
Serum-WBC-endotoxin, FACS analysis of cell types, cytokines, oxidative stress markers, soluble CD14		X	X	X		
Plasma-Metabolomics		X	X	X		
Stool 16 multi-tag pyrosequencing (microbrome), fecal calprotectin		X	X	X		
Urine-Metagenome		X				

EXPLORATORY EVALUATIONS

The following are the analyses that might be analyzed in the serum/plasma and peripheral blood mononuclear cells (PBMC). Collected at Day 0 and Day 180.

Table 3: Exploratory Evaluations

Cytokines/chemokines	Serum TNF-α, IL-1, IL-6, IL-8, and others.
NK cells	Flow cytometry of PBMC for CD3–CD56+ cells. Cytotoxic activity will be measured by assessing intracellular perforin and granzyme expression.
Cytotoxic T cells	Flow cytometry of PBMC for CD3+CD56+ cells. Cytotoxic activity will be measured by assessing intracellular perforin and granzyme expression.
Complement activation	Plasma C3a levels and plasma circulating immune complexes

Interpretation of Labs

All of these are continuous variables. We will compare end of treatment levels across the treatment arms correcting for baseline values. We will also compare the drop from baseline to end of treatment in these arms. We hope to demonstrate that both active arms will have lower levels of pro-inflammatory cytokines compared to those treated with steroids alone. However, no formal hypothesis testing is planned because we have no prior data to project the magnitude of change and thus cannot develop a meaningful sample size calculation. These are in many ways hypothesis generating analyses to guide future studies.

Intestinal microbiome: **These studies are exploratory and will be contingent on funding from NIAAA**. 16 S pyrosequencing is planned to describe the diversity of the microbial populations in

the stool at baseline and at end of study. There are no data on the impact of steroids or Imm-124 on the microbiome. Therefore no formal hypothesis testing is planned.

Unscheduled Visits

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the Investigator. The date and reason for the unscheduled visit will be recorded. At the unscheduled visit, vital signs are required. All other assessments are optional and to be conducted at the discretion of the investigator.

Early discontinuation visit will occur if a subject prematurely discontinued study participation and will be similar to the Termination Visit.

STATISTICAL METHODS PLANNED AND SAMPLE SIZE

Determination of Sample Size

Given the potential for changes in clinical status we used a S.D. of 25% for our sample size calculations and estimated the size required to obtain 80-90% power for changes in LPS *in placebo vs. either arm* with active drug in four difference scenarios shown in **Table 4**. Due to multiple comparisons, the p value is set at 0.025.

Table 4	Scenario 1	Scenario 2	Scenario 3	Scenario 4
α (p value)	0.05	0.025	0.025	0.025
power (%)	90	80	90	90
placebo mean/SD drop in LPS (%)	25/10	25/25	25/25	25/25
Imm-124 E mean/SD drop in LPS at either dose (%)	50/10	50/25	70/25	50/25
N required (each group)	5	20	7	26

22 subjects will be recruited to each of the three arms.

Other Mechanism of action related endpoints

- Intestinal permeability (stool α1 antitrypsin): Previous studies have indicated that alcoholic liver disease is associated with increased intestinal permeability. We will measure intestinal permeability, as measured by the stool α1 antitrypsin level across the two study arms correcting for baseline values using analysis of covariance. We will not plan any formal hypothesis testing because there are no prior data to guide sample size estimates etc.
- Intestinal inflammation (stool lactoferrin and calprotectin, ELISA): These are considered to be markers of intestinal inflammation. They will also be analyzed using a similar approach as noted above. We will compare the levels across both arms correcting for baseline values for these continuous variables, which we anticipate will be normally distributed.
- Systemic inflammation: We will measure the following:

A: C reactive protein
B: 4-OH nonenal and malondialdehyde (oxidative stress markers)
C: cytokines: Innate immunity- TNF-α, IL6, Inflammasome- IL1β, TH1- interferon-gamma, TH2- IL-10, IL-4, Treg-IL17, IL23 (these will be run on a multiplex machine but are subject to ongoing funding from NIAAA for these analyses).

Null Hypothesis
The null hypothesis is that there will be no significant differences between the steroid + placebo arm, versus either steroid + active drug arm, with respect to the plasma endotoxin level.

Analysis Population
For the mechanistic endpoints, we will perform an intent-to-treat analysis. An intent to treat analysis will also be performed for the following endpoints:

- Proportion meeting Lille criteria for futility (Lille score > 0.45)
- Mortality at day 30, 90 and 180
- Change in MELD score and discriminant function adjusting at day 7 and 30 for baseline values
- Change in liver functions adjusting for baseline values on day 7 and 30.

For mechanistic and safety related endpoints, we will include an efficacy evaluable set which will only include those who have received drug for the planned duration within the study.

Subject Populations

All subjects who receive at least one dose of Imm 124-E or placebo will be included in the safety analysis while those who receive at least 7 days of treatment will be considered for the primary pathophysiology-based endpoint

Subject Disposition

Missing values will be explained in individual data tables. No procedures for replacing missing values by estimates will be implemented.

If there is scientific evidence or explanation for one or more outliers, this will be presented together with a statistical test for outlier identification. In case of outliers, the corresponding statistical analysis will be provided with and without these values.

Any deviation from the original statistical plan will be described and justified in the study report.

Primary endpoint: The incidence and severity profile of adverse events will be reported for those receiving Imm-124 versus placebo. It is expected that there will be no signals for increased adverse events with Imm-124. The adverse event rates will be enumerated for each group. Proportions of subjects with specific adverse events will be compared using Fisher Exact test. However, no formal hypothesis testing is planned as there is no basis to expect any changes across groups.

Principal secondary endpoint: The principal secondary endpoint will be the decrease in plasma LPS (endotoxin) levels (adjusting for baseline) as measured by analysis of covariance across the study groups. This was chosen based on the mechanism of action of Imm 124-E. The hypothesis is that both active-drug groups are superior to placebo. In prior studies, we have found the standard deviation of LPS levels to be 8-10% [22, 23].

Subgroup Analysis: We will perform subgroup analysis based on gender, race, baseline MELD and SOFA scores, and presence or absence of encephalopathy. Given the overall sample size, we do not anticipate having enough power to do meaningful hypothesis testing. We will also do

a multivariable regression analysis of outcomes with these as covariates to determine if they have an independent impact on outcome, and to identify any interaction terms between these covariates and treatment arm. These are primarily designed for hypothesis generation.

Safety Analysis

Safety will be assessed by descriptive statistics of AEs and laboratory tests. The incidence of reported AEs and the values of laboratory tests from all subjects will be presented with and without regard to causality based on the Investigator's judgment.

Adverse events will be coded according to MedDRA and presented in tables by system organ class, MedDRA preferred term and study group. Adverse events will be summarized by treatment group to provide visual comparison among the treatment groups with respect to incidence of adverse events (the number of subjects reporting at least one episode of a specific adverse event), incidence of adverse events by severity within body system, incidence of adverse events by attribution within body system, and incidence of adverse events causing withdrawal and incidence of serious adverse events. Regarding severity and attribution summaries, the most extreme outcome (highest severity and closest to study drug related) will be used for those subjects who experience the same adverse event on more than one occasion.

Written narratives will be provided for all serious, unexpected or other significant adverse events that are judged to be of special interest because of their clinical importance.

Chi-square test or Fisher's Exact test (as appropriate) will be applied for testing the statistical significance of the difference in percent of subjects reporting adverse events between the study groups.

Paired T-Test or Signed Rank test (as appropriate) will be applied for testing the statistical significance of the changes from baseline in laboratory results within each study group.

ANOVA model will be applied for testing the statistical significance of the difference in the changes in laboratory results between the study groups. Dunnett's method will be used for comparing each of the active groups to the Placebo group. Adverse Events

All tests will be two-tailed, and a p value of ≤5% will be considered statistically significant.

The data will be analyzed using the SAS ® version 9.1 (SAS Institute, Cary North Carolina).

The measurement of serum bovine and anti-LPS antibody levels at baseline, Day 7, and Day 28-30 of dosing with test colostrum/placebo, and the fold increases over baseline will be assessed. Typically, an antigenic response is defined as 4-fold above baseline. A specialist laboratory can utilize commercially available ELISAs to measure for the presence of serum anti-bovine and anti-LPS IgGs, such as the bovine IgG ELISA kit from Genway (no reactivity with IgG from mouse, human, rat and rabbit) and human LPS ELISA kit from Cusabio, respectively. Samples drawn at baseline, Day 7, and Day 28-30 will then be analyzed, all together, at the end of the study.

Clinical Laboratory

Clinical laboratory results will be summarized with descriptive statistics at baseline, each study time point, and with shifts from baseline.

Data Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) to be established by the NIH and TREAT consortium to assess at intervals the progress of a clinical trial, the safety data, and the critical safety endpoints, and to recommend to the NIH whether to continue, modify, or stop a trial.

Anticipated Outcomes: Means for continuous variables at end of study will be analyzed adjusting for baseline values using ANCOVA. Proportions will be compared using chi square or Fisher's Exact test. We expect to show a greater decrease in mean circulating LPS levels in those receiving Steroids+ Imm 124-E compared to steroids + placebo. We further expect to demonstrate that those receiving Imm 124-E will have a reduction in MELD, DF, intestinal permeability, markers of intestinal inflammation, markers of dendritic cell activation, pro-inflammatory T-cell and cytokine profile, cholestasis (serum bile acids) and bilirubin.

Additional pathophysiologic insights: Microbiome-metagenomic-immunologic-transcriptomic-histologic data from will lend themselves to analyses using a "systems biology approach" to determine how interactions between these contribute to severe AH and how Imm 124-E can modify this. These will provide novel insights on the role of the metagenome in AH and provide additional targets for therapy in the future.

QUALITY CONTROL AND QUALITY ASSURANCE

Source Data and Records

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, and data sheets which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IRB review(s) and regulatory inspection(s), with direct access to all the required source records.

All study records will be retained for a period of time as defined by the regulatory authority for the country in which the investigation is conducted. Generally, this means at least 2 years following the date on which the drug is approved by the regulatory authority for marketing for the purposes that were the subject of the investigation. In other situations (e.g., where the investigation is not in support of or as part of an application for a research or marketing permit), a period of 2 years following the date on which the entire clinical program is completed, terminated or discontinued or the investigational application under which the investigation is being conducted is terminated or withdrawn by the regulatory authorities.

In the event the Investigator retires, relocates or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to any other person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor. The Investigator must contact the Sponsor prior to disposal of any records related to this study.

Reporting of Results

The Case Report Form (CRF) is an integral part of the study and subsequent reports. The CRF must be used to capture all study data recorded in the subject's medical record. The CRF must be kept current to reflect subject status during the course of the study. Only a subject identification code will be used to identify the participant.

The monitor is responsible for performing on-site monitoring at regular intervals throughout the study to verify adherence to the protocol; verify adherence to local regulations on the conduct of clinical research; and ensure completeness, accuracy, and consistency of the data entered in the CRF.

The sponsor will monitor completed Case Report Forms (CRFs). A case report form will be provided for each screened patient.

All protocol-required information collected during the study must be entered by the Investigator, or designated representative in OnCore® electronic data collection and management of bio-specimen, data and patient registry.) OnCore® is 21CFR Part 11 compliant.

If the Investigator authorizes other persons to make entries in the CRF, the names, positions, and signatures of these persons must be recorded on the study signature log.

The Investigator, or designated representative, should complete the eCRF as soon as possible or at least within 7 days, after information is collected but preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. By design, an explanation must be provided for all missing data, altered data, and/or out of range data.

The completed case report form must be reviewed and signed by the Investigator named in the study protocol or by a designated sub investigator.

Final monitored and audited eCRFs will be provided to the sites at the end of the study in the format of a PDF file.

Confidentiality of Subject Data

The Investigator will ensure that the confidentiality of the subjects' data will be preserved. In the CRF or any other documents submitted to the sponsor, the subjects will not be identified by their names, but by an identification system, which consists of their subject ID in the study. The Investigator will maintain documents not meant for submission to the sponsor, e.g., the confidential subject identification code and the signed informed consent forms, in strict confidence.

REPORTING AND PUBLICATION

Confidentiality of Study Data

Any information relating to the study product or the study, including any data and results from the study, will be the exclusive property of the NIAAA. The Investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to Immuron under a confidentiality agreement. All results will be shared with Immuron before they are made public and Immuron will have the opportunity to make comments, queries and recommendations for additional analyses to the Investigators. The relationship between Immuron

and each site will be controlled by a contract with each participating institution. The FDA may also review, inspect, and audit the study records and source documents to support the study data.

Publication Policy

Immuron will have 30 days to review any proposed publication of the data for accuracy and proprietary information.

Laboratory Safety Parameters

SCREENING LABORATORY SAFETY TEST PARAMETERS

SERUM BIOCHEMISTRY

Total Protein

Albumin

Total bilirubin

ALT

AST

Alkaline phosphatase

Glucose

Sodium

Potassium

Calcium

Phosphate

BUN

Creatinine

HEMATOLOGY

Red Blood Cell Count

Hemoglobin (Hgb)

Hematocrit (Hct)

Mean Cell Hemoglobin (MCH)

Mean Cell Hemoglobin Concentration (MCHC)

Mean Corpuscular Volume (MCV)

White Blood Cell (WBC) Count and Differential

Platelet Count

PT/INR

URINALYSIS

Protein

Glucose

Specific Gravity

Ketones

Urobilinogen

Bilirubin

pH

Blood (Hemoglobin)

Leukocytes

SEROLOGY
HBSAg

HCAb

FOLLOW-UP AND POST-STUDY SAFETY TEST PARAMETERS

SERUM BIOCHEMISTRY
Total Protein

Albumin

Total bilirubin

ALT

AST

Alkaline Phosphatase

Glucose

Sodium

Potassium

Calcium

Phosphate

BUN

Creatinine

HEMATOLOGY

Red Blood Cell Count

Hemoglobin (Hgb)

Hematocrit (Hct)

Mean Cell Hemoglobin (MCH)

Mean Cell Hemoglobin Concentration (MCHC)

Mean Corpuscular Volume (MCV)

White Blood Cell (WBC) Count and Differential

Platelet Count

PT/INR

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Appendix 1 – Summary of Changes to Protocol – 04/21/2014

1. Summary of Changes to current protocol.
2. Overall: Font corrections, typographical errors.

The following revisions were made to the current protocol.
(Note: Differences have been indicated in **bold font or strike through for removal)**

Section	Original Text	Revised Text
All pages	Date: 10/1/13	**04/21/2014**
Page 11 (currently Page 6)	SF 36 Short Form 36	~~SF 36 Short Form 36~~
Page 34 (currently page 32)	Alcohol Addiction Counseling, Alcohol Audit, SF 36, Epworth and Berlin Sleep Questionnaires	Alcohol Addiction **Advice per SOC,** Alcohol **AUDIT Removed SF 36, Removed Epworth and Berlin Sleep Questionnaires**
Page 42 (currently Page 38)	Drugs of Abuse: Entire list, HIV-Ab	~~Drugs of Abuse list, HIV-Ab~~
Page 44 (currently Page 39)	Follow Up Labs: Urinalysis: Entire list	**Urinalysis and list of tests removed**

Appendix 2 – Summary of Changes to Protocol – June 26, 2014

1. Summary of Changes to current protocol.
2. Overall: Font corrections, typographical errors.

The following revisions were made to the current protocol.
(Note: Differences have been indicated in **bold font**).

Section	Original Text	Revised Text
All pages	Footer: Bold line, CONFIDENTIAL, 04/21/2014, Page	**TREAT IMM 124-E, June 26,** 2014, Confidential**, page number only**
Page 1	Title page	**Protocol Number** (deleted study product) **IMM 124 E, IND: 15675, Study PI's added, and Disclosure Statement reworded.**
Page 2	Inserted Sponsor signature page	**SPONSOR'S Approval of the Protocol**
Page 3	Re-numbered Table of Contents	**Table of Contents, entire table**
Page 7	Title "Synopsis"	**PROTOCOL** SYNOPSIS
Page 12	Document Approval	**Investigator Statement of Agreement**
Page 12	This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirements. Sponsor Representative, Principal Investigator.	**I agree to:** **Implement and conduct this study diligently and in strict** compliance with the protocol, the Principles of Good Clinical Practice,

		and all applicable **laws and** regulations. **Maintain all information supplied by Immuron Ltd. in confidence and, when this information is submitted to an Institutional Review Board (IRB) or any other group, it will be submitted with a designation that the material is confidential.** **I understand that no data are to be made public or published without prior knowledge and written approval by the TREAT Steering Committee.** **I have read this protocol in its entirety and I agree to all aspects. Investigator Signature, Printed Name of Investigator**
Page 32	Table 1: Samples/information to be collected at baseline, Days 7, 30, 90, and 180	Table 1: **Schedule of Study Procedures – Table revised for ease of reading.**
Page 33		Added **Table 2: Exploratory Evaluations for PBMC's.**
Page 38	Appendix 1	Changed to **Summary of Changes to the Protocol on page 43,** "Laboratory Safety Parameters" kept on page 38.

Appendix 3 – Summary of Changes to Protocol – July 31, 2014

1. Summary of Changes to current protocol.

   The following revisions were made to the current protocol.
   (Note: Differences have been indicated in **bold font**).

Section	Original Text	Revised Text
All Pages	June 26, 2014	**July 31,** 2014
Page 1	Date of Protocol: June 26, 2014	Date of Protocol: **July 31,** 2014
Page 3		Added **Appendix 2, Appendix 3**

Page 5		Added **HDPE, High Density Polyethylene**
Page 9	Imm 124-E caplets containing freeze dried bovine colostrum "hyper immunized" with ETEC (Enterotoxigenic *E.coli*) at dose of 600 mg/caplet and identical placebo caplets will be supplied in amber colored bottles, to be administered orally twice daily: ▪ Group A: Imm 124-E 2400 mg per oral in divided doses twice a day, ▪ Group B: Imm 124-E 4800 mg per oral in divided doses twice a day. Group C: microcrystalline cellulose (placebo) per oral twice a day.	Imm 124-E **powder for reconstitution** at dose of 600 mg **per bottle and matching** placebo will be **supplied in high-density polyethylene (HDPE) bottles,** to be administered **to subjects in one of 3 treatment groups:** ▪ Group A: Imm 124-E 2400 mg per oral **daily in two divided doses** ▪ Group B: Imm 124-E 4800 mg per oral **daily in two divided doses** Group C: **High protein milk powder** (placebo) per oral twice a day.
Page 24	Off white caplets containing hyperimmune bovine colostrum as 600mg of Imm 124-E	Off white **powder equivalent to** 600mg of Imm 124-E hyperimmune bovine colostrum
Page 25	Storage: Temperature should not exceed 25˚C Shipments: Temperature should not exceed 25˚C Package: Amber glass bottles with screw cap Characteristics & Physical State: Off white caplets containing methylcellulose Imm 124-E will be supplied in amber glass bottles	Storage: Temperature **controlled between 15˚C and 25˚C** Shipments: Temperature **controlled between 15˚C and 25˚C** Package: **HDPE** bottles with screw cap Characteristics & Physical State: Off white **high protein milk powder, ProMilk85®** Added under Supplied By: **Storage Conditions: Store in cool and dry place. Temperature controlled between 15˚C and 25˚C, Shipments: Temperature controlled between 15˚C and 25˚C, Package: HDPE bottles with screw cap**

	Investigational pharmacy at each recruiting center will label each bottle with the subject's study ID, subject's name, drug name/placebo	Imm 124-E will be supplied in **HDPE** bottles. Investigational pharmacy at each recruiting center will label each bottle with the subject's study ID, subject's name, **IMM-124E**/placebo
Page 26	The appearance and packaging of active caplets and placebo caplets are identical. Bottles of caplets to last 7 days will be packed into cardboard boxes sealed by Immuron Ltd. Each box will have a code which will be matched to the randomization code at the data coordinating center. Following reconciliation of the randomization code with the box code, the investigational drugs pharmacy will dispense the medication. Eligible subjects will be randomly assigned to each of the study groups. Subject will be instructed to take the caplets twice daily. Subjects will take the drug in the morning before breakfast	The appearance and packaging of active and placebo **powders for reconstitution** are identical. Bottles of **study drug will be packed in groups of two bottles into sealed** cardboard boxes supplied by Immuron Ltd. Each box will have a code which will be matched to the randomization code at the data coordinating center. Following reconciliation of the randomization code with the box code, the investigational drugs pharmacy will dispense **enough medication enough medication to last 28 days (56 packs, a total of 112 bottles).** Eligible subjects will be randomly assigned to each of the study groups. Subject will be instructed to take the **contents of two bottles of powder reconstituted with water** twice daily. **To prepare the study drug immediately before administration, add approximately two tablespoons of water to each of the two bottles, recap and swirl gently for 1 minute until dissolved. Each** subject will **be required to drink the contents of both bottles** in the morning before breakfast. **Additional water may be added to the bottles to ensure all the powder is taken.**

Page 27	and will be required not to eat for 2 hours after taking the drug.	**Subjects** will be required not to eat for 2 hours after taking the drug. Added at end of this paragraph: **The subject will also be instructed to take the contents of two bottles in the evening, at least 2 hours after eating.**
Page 28	Day 7	Day 7 - **Highlighted**
Page 31	Secondary Sponsor (Immuron) contact information: Contact person: Dr. Gerhard Rank Email: Gerhard.rank@immuron.com	Secondary **Contact** (Immuron) information: Contact person: **Sally Kinrade, Director of Clinical Research** Email: Sally@immuron.com

Appendix 4 – Summary of Changes to Protocol – August 29, 2014

1. Summary of Changes to current protocol.
2. Overall: Typographical errors.

The following revisions were made to the current protocol.
(Note: Differences have been indicated in **bold font,** or strike through for removal).

Page 8	Key Exclusion: Under point four, "Active infection or sepsis…"	Added: **All subjects with enough ascites to be deemed safe for paracentesis, will undergo this procedure (as standard of care) to exclude spontaneous bacterial peritonitis.**
Page 9	Test Product: Imm 124-E powder for reconstitution at dose of 600 mg per bottle and matching placebo will be supplied in high-density polyethylene (HDPE) bottles, to be administered to subjects in one of 3 treatment groups: □ Group A: Imm 124-E 2400 mg per oral daily in two divided doses □ Group B: Imm 124-E 4800 mg per oral daily in two divided doses □ Group C: High protein milk powder (placebo) per oral twice a day.	Test Product: **The placebo and** IMM-124E **will both be presented as 1200mg of** powder **for reconstitution in a** high-density polyethylene (HDPE) bottle. **The appearance of the active and placebo will be indistinguishable. Each subject will take two bottles each morning and then again in the evening. Consequently, each arm of the study will take the same number of doses per day. Subjects in:** • Group A (IMM-124E 2400mg/day) **will receive one bottle of active and one bottle of placebo in the morning and again in the evening.** • Group B (IMM-124-E 4800 mg/day) **will receive two bottles of active in the morning and again in the evening.**

		• Group C (**Placebo -** High protein milk powder) **will receive two bottles of placebo in the morning and again in the evening.**
Page 9	DISCONTINUATION FROM TREATMENT:	DISCONTINUATION FROM TREATMENT **OF INDIVIDUAL SUBJECT:**
Page 9	A pattern of adverse events in more than one subject indicating a potential safety concern	~~A pattern of adverse events in more than one subject indicating a potential safety concern~~
Page 9	Under – DISCONTINUATION FROM TREATMENT	**DISCONTIUATION OF ENTIRE STUDY: The entire study may be discontinued due to unexpected adverse events and with guidance from DSMB and NIAAA.**
Page 10	Safety Endpoints:	Added: **Please see "Adverse Events" section of the protocol for further information concerning AE's and SAE's.** (To end of paragraph)
Page 21	Exploratory Endpoints: Activation of systemic inflammation: (EOT across group comparison, baseline-EOT) comparison)	Under this section, removed: ~~Neutrophil and Dendritic cell activation (measured at IU)~~
Page 22	Under – "INVESTIGATIONAL PLAN" **(Fig. 1)**	(Fig. 1, **above)**
Page 24	Under – "Description of Study Product": Off white powder equivalent to 600mg of Imm 124-E hyperimmune bovine colostrum	Off white powder equivalent to **1200mg** of Imm 124-E hyperimmune bovine colostrum
Page 27	Subjects will be requested to return all the used and unused blisters.	Subjects will be requested to return all the used and unused **bottles.**
Page 28	Under – "Clinical Laboratory": Safety tests (biochemistry, hematology, urinalysis and stool analysis, see Appendix 1) will be performed along with the safety assessment.	Safety tests (biochemistry, hematology, urinalysis and stool analysis**: See Table 2)** will be performed along with the safety assessment.
Page 28	Under – "Clinical Laboratory"	**Table 1: Protocol Thresholds for Clinical Significance of Abnormal Safety Labs** **Table 1: Laboratory Cutoffs That Would Lead to Drug Discontinuation in an Individual Subject** **Hematological** • **Absolute neutrophil count < 500/mm3** • **Platelet count < 20,000/mm3** **Hepatic safety labs** • **AST or ALT > 20x upper limit of normal**

		• **AST or ALT doubling with absolute values > 400 IU/L** **Please note that bilirubin is highly elevated to start with and may rise as part of the disease process in subjects with severe alcoholic hepatitis and therefore cannot be used as a test to detect drug induced liver injury** **Also, INR is prolonged in subjects with severe alcoholic hepatitis and there cannot be used to evaluate drug related liver injury in this setting**
Page 29	Adverse Events (AE), and Serious Adverse Events (SAE)	Adverse Events (AE), ~~and~~ Serious Adverse Events (SAE)**, and Suspected Adverse Reactions**
Page 29	A treatment emergent adverse event is…	A **treatment emergent adverse event** is… (Words are bolded)
Page 29		**Causality Assessment** **For this study, all AEs developing any time from administration to 7 days after discontinuation will be evaluated for relationship to study drug. For Imm 124E, the closer it is to study administration and if it recurs on rechallenge (depending on the nature of the event), it will be considered to be study drug-related.**
Page 30	Under – "Adverse Events"	**A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.**
Page 30	Procedures for Assessing, Recording, and Reporting Adverse Events and Serious Adverse Events	Procedures for Assessing, Recording, and Reporting Adverse Events and Serious Adverse Events, **in Accordance with 21**

		CFR 312.32(a) FDA Guideline for Safety Reporting
Page 32	Table 1	Table **2 – Schedule of Study Procedures** (added to Table 2)
Page 33	Under – "Exploratory Evaluations" - Collected at screening and Day 180	Collected at **Day 0** and Day 180
Page 33	Table 2	Table **3**
Page 33	Table 3: Dendritic Cells (DC) Flow cytometry for the number and phenotype (HLA-DR level). DCs in PBMCs lack CD3, CD14hi, CD19, and CD56, but strongly express HLA-DR. Spontaneous ex-vivo cytokine production will be analyzed.	~~Dendritic Cells (DC) Flow cytometry for the number and phenotype (HLA-DR level). DCs in PBMCs lack CD3, CD14hi, CD19, and CD56, but strongly express HLA-DR. Spontaneous ex-vivo cytokine production will be analyzed.~~
Page 33		**Interpretation of Labs** **All of these are continuous variables. We will compare end of treatment levels across the treatment arms correcting for baseline values. We will also compare the drop from baseline to end of treatment in these arms. We hope to demonstrate that both active arms will have lower levels of pro-inflammatory cytokines compared to those treated with steroids alone. However, no formal hypothesis testing is planned because we have no prior data to project the magnitude of change and thus cannot develop a meaningful sample size calculation. These are in many ways hypothesis generating analyses to guide future studies.** **Intestinal microbiome: <u>These studies are exploratory and will be contingent on funding from NIAAA</u>. 16 S pyrosequencing is planned to describe the diversity of the microbial populations in the stool at baseline and at end of study. There are no data on the impact of steroids or Imm-124 on the microbiome. Therefore no formal hypothesis testing is planned.**
Page 34	Table 5	Table **4 (Change made to sentence and Table)**

Page 34	Under "Determination of Sample"	**Other Mechanism of action related endpoints** **•Intestinal permeability (stool α1 antitrypsin): Previous studies have indicated that alcoholic liver disease is associated with increased intestinal permeability. We will measure intestinal permeability, as measured by the stool α1 antitrypsin level across the two study arms correcting for baseline values using analysis of covariance. We will not plan any formal hypothesis testing because there are no prior data to guide sample size estimates etc.** **•Intestinal inflammation (stool lactoferrin and calprotectin, ELISA): These are considered to be markers of intestinal inflammation. They will also be analyzed using a similar approach as noted above. We will compare the levels across both arms correcting for baseline values for these continuous variables, which we anticipate will be normally distributed.** **•Systemic inflammation: We will measure the following:** **A: C reactive protein** **B: 4-OH nonenal and malondialdehyde (oxidative stress markers)** **C: cytokines: Innate immunity- TNF-α, IL6, Inflammasome- IL1β, TH1- interferon-gamma, TH2- IL-10, IL-4, Treg-IL17, IL23 (these will be run on a multiplex machine but are subject to ongoing funding from NIAAA for these analyses).** **Null Hypothesis** **The null hypothesis is that there will be no significant differences between the steroid + placebo arm, versus either steroid + active drug arm, with respect to the plasma endotoxin level.** **Analysis Population**

		For the primary analysis, we will perform an intent-to-treat analysis. An intent to treat analysis will also be performed for the following endpoints: **•Proportion meeting Lille criteria for futility (Lille score > 0.45)** **•Mortality at day 30, 90 and 180** **•Change in MELD score and discriminant function adjusting at day 7 and 30 for baseline values** **•Change in liver functions adjusting for baseline values on day 7 and 30.** **For mechanistic and safety related endpoints, we will include an efficacy evaluable set which will only include those who have received drug for the planned duration within the study.**
Page 35	Under – Subject Disposition	**Subgroup Analysis: We will perform subgroup analysis based on gender, race, baseline MELD and SOFA scores, and presence or absence of encephalopathy. Given the overall sample size, we do not anticipate having enough power to do meaningful hypothesis testing. We will also do a multivariable regression analysis of outcomes with these as covariates to determine if they have an independent impact on outcome, and to identify any interaction terms between these covariates and treatment arm. These are primarily designed for hypothesis generation.**
Page 36		**The measurement of serum bovine and anti-LPS antibody levels at baseline, Day 7, and Day 28-30 of dosing with test colostrum/placebo, and the fold increases over baseline will be assessed. Typically, an antigenic response is defined as 4-fold above baseline. A specialist laboratory can utilize commercially available ELISAs to measure for the presence of serum anti-bovine and anti-LPS**

		IgGs, such as the bovine IgG ELISA kit from Genway (no reactivity with IgG from mouse, human, rat and rabbit) and human LPS ELISA kit from Cusabio, respectively. Samples drawn at baseline, Day 7, and Day 28-30 will then be analyzed, all together, at the end of the study.
Page 39	Last sentence under – "Confidentiality of Study Data"	**The FDA may also review, inspect, and audit the study records and source documents to support the study data.**

Appendix 5 – Summary of Changes to Protocol – February 9, 2015

1. Summary of Changes to current protocol.

The following revisions were made to the current protocol.
(Note: Differences have been indicated in **bold font or strike through for removal**)

Section	Original Text	Revised Text
All pages	August 29, 2014	**February 9, 2015**
Page 1	Protocol Date: August 29, 2014	Protocol Date: **February 9, 2015**
Page 3		Added **Appendix 5, 56**
Page 8	About to initiate prednisolone treatment	About to initiate prednisolone treatment, **< 7 days of steroid treatment, or treatment naïve.**
Page 22	About to initiate prednisolone treatment	About to initiate prednisolone treatment, **< 7 days of steroid treatment, or treatment naïve.**
Page 23	Wilson disease: low ceruloplasmin (10mg/dL)	**Subjects who are known to have** Wilson**'s** disease ~~low ceruloplasmin (10mg/dL)~~
Page 32	Table 2 : Nutrition Assessment	Anthropomorphic Measurements**/Nutrition** ~~Assessment~~
Page 33	Lille Assessment – Screening Day	~~X~~ removed
Page 40	GGT, LDH, CPK	~~GGT, LDH, CPK~~
Page 41	GGT, LDH, CPK	~~GGT, LDH, CPK~~

Appendix 6 – Summary of Changes to Protocol – August 15, 2016

1. Summary of Changes to current protocol.
2. Overall: Font resizing for appendices.

The following revisions were made to the current protocol.
(Note: Differences have been indicated in **bold font or strike through for removal**)

Section	Original Text	Revised Text
All Pages	February 9, 2015	**August 15, 2016**
Page 1	Protocol Date: February 9, 2015	Protocol Date: **August 15, 2016**
Page 3		Appendix 3, Page **46** Appendix 5, Page **55**
Page 3		Added Appendix 6, Page **55**
Page 7	If orally administered Imm-124 reduces endotoxemia and markers of activation of the innate immune system in patients with severe alcoholic hepatitis being treated with steroids	**The safety of orally administered Imm 124-E in patients with severe alcoholic hepatitis being treated with steroids**
Page 7	The safety of orally administered Imm 124-E in patients with severe alcoholic hepatitis being treated with steroids	**If orally administered Imm 124-E reduces endotoxemia and markers of activation of the innate immune system in patients**
Page 9	Under "Test Product" - a high-density polyethylene (HDPE) bottle; Each subject will take two bottles; Group A…receive one bottle of active and one bottle; Group B…receive two bottles; Group C…receive two bottles	**Plastic Aluminium Foil Poly Laminate Pouches (sachets);** Each subject will take two **sachets;** Group A…receive one **sachet** of active and one **sachet;** Group B…receive two **sachets;** Group c…receive two **sachets**
Page 10	Primary Endpoint: primary endpoint will be the	~~Primary Endpoint: primary endpoint will be the~~. Added: **sample size is based on a**
Page 11	**Dunnett's method** will be used for comparing each of the active groups to the Placebo group.	~~**Dunnett's method** will be used for comparing each of the active groups to the Placebo group.~~
Page 20	Under "Primary Objective" – if	**the safety of… and to determine if it**
Page 20	The primary endpoint will be plasma LPS (endotoxtin) measured by ELISA	The primary endpoint **is the safety profile of Imm 124-E in this pilot study.**
Page 20	Under "Secondary Endpoints"	Added: **Changes in endotoxin levels adjusted for baseline levels at day 7 and 28**
Page 24-25	Under "Study Product" – Storage: Temperature controlled between 15˚C and 25˚C; Shipments: Temperature controlled between 15˚C and 25˚C; Package: HDPE bottles with screw cap	Storage: Temperature controlled between **2**˚C and **8**˚C; Shipments: Temperature controlled between **2**˚C and **8**˚C; Package: **Plastic Aluminium Foil Poly Laminate Pouches**
Page 25	Under "Comparator Product" - Storage: Temperature controlled between 15˚C and 25˚C; Shipments: Temperature controlled between 15˚C and 25˚C; Package: HDPE bottles with screw cap	Storage: Temperature controlled between **2**˚C and **8**˚C; Shipments: Temperature controlled between **2**˚C and **8**˚C; Package: **Plastic Aluminium Foil Poly Laminate Pouches**
Page 25	Under "Packaging" - Imm 124-E will be supplied in HDPE bottles. The bottles	Imm 124-E will be supplied in **Plastic Aluminium Foil Poly Laminate Pouches (sachets).** The **sachets**

Page 25- cont.	Investigational pharmacy at each recruiting center will label each bottle	Investigational pharmacy at each recruiting center will label each **sachet**
Page 25	Labels of the outer packaging (the package in which the bottle	Labels of the outer packaging (the package in which the **sachet**
Page 26	Under "Method" - Bottles of study drug will be packed in groups of two bottles; a total of 112 bottles	**Sachets** of study drug will be packed in groups of two **sachets;** a total of 112 **sachets**
Page 26	Under "Dispensing" - Subject will be instructed to take the contents of two bottles; add approximately two tablespoons of water to each of the two bottles, recap and swirl gently for 1 minute until dissolved. Each subject will be required to drink the contents of both bottles in the morning before breakfast. Additional water may be added to the bottles	Subject will be instructed to take the contents of two **sachets;** add **the powder to a cup and add** approximately **four** tablespoons of water **to the cup, stir** gently for 1 minute until dissolved. Each subject will be required to drink the contents of both **sachets** in the morning before breakfast. Additional water may be added to the **powder**
Page 27	The subject will also be instructed to take the contents of two bottles; Subjects will be requested to return all the used and unused bottles.	The subject will also be instructed to take the contents of two **sachets**; Subjects will be requested to return all the used and unused **sachets**.
Page 32	Under "Secondary Contact" - Sally Kinrade; Email: Sally@immuron.com	**Dan Peres**; Email: **dan@immuron.com**
Page 35	Under "Analysis Population" – primary analysis	**mechanistic endpoints**
Page 35	Under "Subject Disposition" – Primary Endpoint	Added: Primary endpoint: **The incidence and severity profile of adverse events will be reported for those receiving Imm-124 versus placebo. It is expected that there will be no signals for increased adverse events with Imm-124. The adverse event rates will be enumerated for each group. Proportions of subjects with specific adverse events will be compared using Fisher Exact test. However, no formal hypothesis testing is planned as there is no basis to expect any changes across groups.**
Page 35	Under "Subject Disposition" – Primary endpoint: The primary endpoint will be the decrease in plasma LPS (endotoxin) levels (adjusting for baseline) as measured by analysis of covariance across the study groups. This was chosen based on the mechanism of action of Imm 124-E. The	**Principal secondary** endpoint: The **principal secondary** endpoint will be the decrease in plasma LPS (endotoxin) levels (adjusting for baseline) as measured by analysis of covariance across the study

	hypothesis is that both active-drug groups are superior to placebo. In prior studies, we have found the standard deviation of LPS levels to be 8-10% [22, 23].	groups. This was chosen based on the mechanism of action of Imm 124-E. The hypothesis is that both active-drug groups are superior to placebo. In prior studies, we have found the standard deviation of LPS levels to be 8-10% [22, 23].