**Study Title:** A phase II, randomized, double-blind, placebo-controlled study of IMM-124E for patients with non-alcoholic steatohepatitis

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**Protocol Version/Date:** Version 1.3 Date: 08 May 2016

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## STUDY ACKNOWLEDGEMENT

A phase II, randomized, double-blind, placebo-controlled study of IMM-124E for patients with non-alcoholic steatohepatitis.

**IMM-124E-2001, Version 1.3 (incorporating amendment 3), 08 May 2016**

This protocol has been approved by the *Sponsor*. The following signature documents this approval.

| | |
|---|---|
| Dan Peres,MD Head of Medical |  |
| Name (Printed) | Signature |
| | 9 May 2016 |
| | Date (dd mmm yyyy) |

## INVESTIGATOR STATEMENT

I have read the protocol entitled "A phase II, randomized, double-blind, placebo-controlled study of IMM-124E for patients with non-alcoholic steatohepatitis" dated 24 June 2015.

I will conduct this study in accordance with this protocol and will make all reasonable efforts to complete the study within the time designated. I agree to disclose the financial interests of all investigators or sub-investigators who are directly involved in the treatment or evaluation of research subjects.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Immuron. I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Immuron. I will discuss this material with them to ensure that they are fully informed about the investigational product(s) and the study.

| | |
|---|---|
| Principal Investigator's Name (Printed) | Signature |
| | Date (dd mmm yyyy) |

## 1. STUDY SYNOPSIS

| | |
|---|---|
| **Protocol No.:** | IMM-124E-2001 |
| **Study Title:** | A phase II, randomized, double-blind, placebo-controlled dosing study of IMM-124E (bovine colostrum) for patients with non-alcoholic steatohepatitis (NASH). |
| **Investigational Product:** | IMM-124E: hyperimmune bovine colostrum containing polyclonal antibodies to bacterial lipopolysaccharide (LPS) and matching placebo. |
| **Indication:** | NASH |
| **Development Phase:** | 2 |
| **Primary objectives:** | To evaluate the safety and preliminary efficacy of two dose levels of IMM-124E in reducing liver fat evaluated by MRI compared with placebo. |
| **Secondary objectives:** | Determine the pharmacokinetic profile of IMM-124E.<br><br>Assess the impact of treatment with IMM-124E on markers of glucose metabolism and serum lipid profile.<br><br>Assess the impact of treatment on liver function over 24 weeks.<br><br>Establish the recommended dose. |
| **Exploratory objectives** | Investigate the impact of treatment on potentially relevant immune and metabolic biomarkers associated with NASH. |
| **Primary outcomes measures:** | Safety: Incidence and severity of adverse events and changes in vital signs and clinical laboratory tests.<br><br>Efficacy:<br>• Mean change from Baseline in the percentage fat content of the liver measured by Magnetic Resonance Imaging (MRI) at Week 24. |
| **Secondary outcome measures:** | Pharmacokinetics:<br>• Serum concentration of IMM-124E bovine antibodies over time (if measurable) including, but not limited to, maximum observed concentration (Cmax), minimum observed concentration (Cmin) and area under the concentration-time curve (AUC), and, if feasible, elimination half-life (t1/2)<br><br>Metabolic markers:<br>• Body Mass Index (BMI), waist circumference and waist:hip ratio, Hemoglobin(HB)A1C and the homeostatic model assessment of insulin resistance (HOMA-IR)<br>• Serum lipid profile: total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions<br>Liver function: |

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| | • Mean serum ALT, aspartate aminotransaminase (AST), bilirubin, albumin and gamma-glutamyl transpeptidase (GGT) at Weeks 4, 8, 12, 16, 20 and 24<br>• Proportion of subjects whose ALT at Week 24 is within the normal reference range defined a ≤19IU/L for women and ≤30IU/L for men. |
| **Exploratory outcome measures** | • Mean serum concentrations of: lipopolysaccharide (LPS), C-reactive protein (CRP), cytokeratin (CK)-18 fragments, glucogon-like peptide (GLP)-1 and adiponectin and subsets of inflammatory cytokines: interleukin (IL)-6, IL-1α, IL1β, IL-2, IL-3, IL-4 IL-10, IL-13, IL-12, IL-17α, IL-23 interferon gamma (IFNγ), Transforming Growth Factor Beta (TGF-β) and tumor necrosis factor alpha (TNFα)<br>• Serum metabolomics<br>• Relative levels of regulatory T cells in peripheral blood mononuclear cells (PBMC) samples, including CD4, CD8, CD25, FoxP3, NKT, CD62 and CD69 T cells;<br>• Characterization of the gut microbiome<br>• Explore a dose effect |
| **Study design:** | This is a randomized, double blind, placebo controlled, 3-arm parallel group, multi-dose, multi-center study. |
| **Study duration / participant:** | Up to 45 days for Screening and 24 weeks of treatment.<br><br>Subjects terminating study drug will be followed-up post-treatment for 28 days. |
| **Number of participants:** | At least 120, randomized 1:1:1<br><br>Treatment allocation will be stratified by the following:<br>• Baseline MRI Liver Fat, according to the following scale: ≤10%, 10%<X≤20%, 20%<X<30% and ≥30%<br>• Diabetic status defined as being normal (HBA1C ≤6.0) or diabetic (having a diagnosis of Type II diabetes and/or HBA1C >6.0 and ≤ 9.0 whether or not receiving allowable medication). |
| **Study drug:** | IMM-124E 600mg tablet |
| **Control group:** | Placebo to match |
| **Dose Regimens** | Arm A: IMM-124E 600mg three times daily;<br>Arm B: IMM-124E 1200mg three times daily;<br>Arm C: Matching placebo three times daily.<br><br>All treatment dose regimens are administered per orally |
| **Inclusion criteria:** | 1. Age ≥ 18 years<br>2. Provision of written informed consent<br>3. Diagnosis of NASH, histologically proven within 12 months of Screening with all of the following criteria met:<br>• NASH activity score (NAS) of 4 or more |

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| | • Cytologic ballooning score of at least 1<br>• 10% or more macrovesicular steatosis<br>• Hematoxylin & Eosin (H&E) stained slides and/or paraffin block available for independent assessment<br>4. HBA1C of ≤9.0<br>5. Agree to the use of effective contraceptive measures if either male or female of child bearing potential |
| **Exclusion criteria:** | 1. Presence of vascular liver disease or cirrhosis<br>2. Presence of liver disease with other cause (autoimmune, metabolic, medication induced)<br>3. BMI ≤25kg/$m^2$<br>4. Alcohol use ≥30g/day<br>5. Type 1 diabetes<br>6. History of major bariatric surgery (not including balloon / sleeve gastrectomy)<br>7. Weight loss or gain of 5kg or more in the past 6 months or ≥10% change in bodyweight in the past 12 months<br>8. Contraindication for MRI<br>9. Inadequate venous access<br>10. Lactating/breastfeeding/pregnant at Screening or Baseline<br>11. HIV antibody positive, hepatitis B surface antigen positive (HBsAg) or Hepatitis C virus (HCV)-RNA positive<br>12. Receiving an elemental diet or parenteral nutrition<br>13. Concurrent conditions:<br>• Inflammatory bowel disease<br>• Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of Screening<br>• Ongoing infectious disease<br>• Ongoing multi-systemic immune-mediated disease<br>• Concurrent or past malignant disease<br>• Any other concurrent condition which, in the opinion of the investigator, could impact adversely on the subject participating or on the interpretation of the study data |

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| | 14. Concurrent medications including:<br>• Anti-NASH therapy(s) taken for more than 10 continuous days in the last 3 months. These include S-adenosyl methionine (SAM-e), betaine, milk thistle, probiotic supplements (other than yoghurt), vitamin E and gemfibrozil.<br>▪ NOTE: If determinant biopsy is performed while on stable treatment – subject is eligible<br>▪ Wash out for any of the anti-NASH therapies is as follows: under 10 days no washout required, more than 10 days and up to 3 months treatment requires 6 weeks washout. Any treatment of over 3 months would require to re-biopsy to ensure histological eligibility<br>• Thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or glucagon-like peptide-1 analogs in the last 6 months.<br>▪ NOTE: If determinant biopsy is performed while on stable treatment of at least 6 months, subject is eligible<br>▪ NOTE: Allowable anti-diabetic treatment includes metformin and/or sulfonylureas administered at constant dose for at least 2 months prior to study entry<br>▪ NOTE: Subjects treated with Insulin are eligible if clinically stable on insulin treatment (i.e. no recurrent acute hypo-/hyperglycemic episodes diagnosed clinically and by Glucose serum levels of <50 mg/dL and >200 mg/dL respectively) for at least 2 months prior to study entry<br>○ immune modulatory agents including:<br>▪ Within 3 months of study entry:<br>• systemic steroids for more than 7 days<br>• daily treatment with multiple non-steroidal anti-inflammatory drugs (such as aspirin (>100mg/day), ibuprofen, naproxen, meloxicam, celecoxib) for more than 1 month<br>▪ In the last 12 months:<br>• azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, anti-TNFα therapies (infliximab, adalimumab, etanercept) or anti-integrin therapies (namixilab)<br>• More than 10 consecutive days oral or parenteral antibiotics within 4 weeks prior to study entry NOTE: subjects administered with antibiotics for more the 5 days prior to study entry would not be included in the stool and PBMC analysis)<br>• Variable dose of antilipidemic agents (3-hydroxy-3-methyl-glutaryl (HMG)-Co-A reductase inhibitors – “statins”) in the 3 months prior to study entry<br>15. The following laboratory abnormalities: |

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| | ○ Neutrophil count ≤1.0 x $10^9$/L<br>○ Platelets <100 x $10^9$/L<br>○ Hemoglobin <10g/dL<br>○ Albumin <3.5g/dL<br>○ International Normalized Ratio (INR) >1.5<br>○ Total bilirubin >1.5 x upper limit of reference range (unless Gilbert's syndrome or extrahepatic source as denoted by increased indirect bilirubin fraction)<br>○ Either creatinine clearance ≤60mL/minute calculated by Cockroft-Gault or creatinine >1.5x upper limit of reference range<br>16. Known substance abuse, including inhaled or injected drugs in the year prior to Screening<br>17. Cow milk allergy, lactose intolerance or any known or suspected hypersensitivity to study products |
| **Study procedures:** | Refer to Table 1 for the schedule of study procedures.<br><br>Subjects who provide voluntary written informed consent will be screened for eligibility. Subjects meeting all of the inclusion and none of the exclusion criteria will be eligible to participate.<br><br>Eligible subjects will be randomized at the Baseline visit to receive one of the three study treatments three times daily for a period of 24 weeks. Each subject will return to the study clinic for assessment and required study procedures on Day 7, 14 and 28 and every 4 weeks thereafter until Week 24. A final follow-up visit will be conducted at Week 28 if the subject ceases treatment at Week 24. Investigational product dispensing and accountability will be conducted on a 4 weekly basis.<br><br>Unscheduled or early withdrawal visits may be necessary with study related procedures defined. |
| **Safety parameters:** | Adverse events, vital signs, electrocardiogram (EKG) and safety laboratory assessments will be conducted at Screening, Baseline and 4 weekly throughout the study. |

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| **Sample size determination:** | Fat Liver Content Change from Baseline:<br>The current sample size of 40 subjects per group should yield approximately 85% power to detect a treatment effect of at least -5% with respect to the difference in the mean change from Baseline between one of the IMM-124E treatment groups and placebo assuming a standard deviation (SD) of 7.3% (α=0.05; two-tailed; unpaired t-test). The SD of 7.3% for the change from Baseline endpoint was derived assuming 1) SDs of 6% for each timepoint, 2) equal variances in each treatment group and 3) a low to moderate correlation of 0.25 between pre- and post-Baseline measurements.<br><br>Sample size and power estimates have not been adjusted for multiple comparisons or multiple endpoints, dropouts or stratification.<br><br>The analysis of each primary endpoint will be based on a linear model that incorporates the baseline measure as a covariate, the stratification factor and randomized treatment group. Pairwise comparisons of each active treatment group to control will be conducted via linear contrasts. |
| **Statistical analyses:** | The populations for analysis will be as follows:<br><br>Full Analysis Set (FAS) for Efficacy: includes all subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received may be excluded. In the event of treatment allocation errors, subjects will be analyzed for efficacy according to the treatment to which they were randomized.<br><br>Safety Analysis Set: includes all subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received may be excluded. In the event of treatment errors, subjects will be analyzed for safety according to the treatment received.<br><br>Per Protocol Analysis Set: includes all subjects in the FAS above who complete 24 weeks of study treatment and have MRI and ALT measured at Baseline and at Week 24 and no major protocol violations, determined prior to break of the treatment blind.<br><br>Intention-To-Treat (ITT) Analysis Set: The ITT analysis set includes all patients randomized to treatment. An ITT analysis will only be implemented in the event that one or more patients are randomized but do not receive any treatment.<br><br>When at least 30 subjects reach 24 weeks visit and have verified MRI results an interim analysis will be performed.<br>The interim analysis will include the full analysis of all primary and secondary un-blinded outcomes and will be used to determine the following:<br>1. Efficacy at time of analysis<br>2. Dose effect to determine the most effective dose for treatment |

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| | 3. Correct for the overall sample size for the primary endpoint<br>4. Overall Safety |

## Table 1: Table of Assessments

| Visit | Screening | Baseline | D3 | D7 | D14 | W4 | W8 | W12 | W16 | W20 | W24/WD | W28 | U/S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit Window (relative to Baseline)** | **-45 to 0** | **0** | **+/- 1D** | **+/- 1D** | **+/- 1D** | **+/- 1D** | **+/- 3D** | **+/- 3D** | **+/- 3D** | **+/- 3D** | **+/- 3D** | **+/- 3D** | |
| Written Informed Consent | X | | | | | | | | | | | | |
| Eligibility Criteria | X | X | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | | |
| Height | X | | | | | | | | | | | | |
| Randomization to study treatment [8] | | X | | | | | | | | | | | |
| Weight/ BMI | X | X | | | | X | X | X | X | X | X | X | |
| Waist circumference; waist hip ratio | | X | | | | X | X | X | X | X | X | | |
| HOMA-IR | | X | | X | X | X | X | X | X | X | X | | X |
| MELD score | | X | | | | | | X | | | X | | |
| Resting Pulse, Blood pressure, Resp., Temp. | X | X | | X | X | X | X | X | X | X | X | X | X |
| Physical examination | X | | | | | | | | | | | | |
| Targeted physical examination | | X | | X | X | X | X | X | X | X | X | X | X |
| 12 lead EKG | X | | | | | | | | | | | | |
| Diet and exercise counseling | | X | | | X | X | X | X | X | X | X | X | |
| Diet and exercise diary card issue | | X | | | | | | X | | | X | | |
| Diet and exercise diary card collection and review | | | | | X | | | | X | | | X | |
| AUDIT questionnaire administration | | X | | | | | | X | | | X | | |
| Concurrent Meds recording | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Adverse Events | | X | X | X | X | X | X | X | X | X | X | X | X |
| MRI of the liver[7] | | X | | | | | | | | | X | | |
| HIV, HCV & HBV serology[6] | X | | | | | | | | | | | | |
| Serum alpha feto protein (AFP) | X | | | | | | | | | | | | |
| Serum Pregnancy Test | X | | | | | | | | | | | | |
| Clinical Chemistry [1] | X | X | | X | X | X | X | X | X | X | X | X | X |
| Fasting lipid panel[2] | | X | | X | X | X | X | X | X | X | X | | |
| Hematology[3] | X | X | | X | X | X | X | X | X | X | X | X | X |
| Coagulation | X | X | | X | X | X | X | X | X | X | X | X | X |
| Pharmacokinetics | | X | | | | X | | X | | | X | | |
| HBA1C | X | | | | | | | X | | | X | | |
| Samples for serum biomarkers[4] | | X | | | | X | | X | | | X | | |
| Samples for PBMC (selected sites only)[5] | | X | | | | | | X | | | X | | |
| Serum metabolomics | | X | | | | | | | | | X | | |
| Urine safety | X | | | | | | | | | | | | |
| Urine pregnancy test | | X | | | | X | X | X | X | X | X | X | |
| Stool sample collection (selected sites only) | | X | | | | X | | X | | | X | | |
| Study Drug Dispensing and Accountability | | X | | | | X | X | X | X | X | X | | |

[1] Must be collected after subject has fasted for at least 8 hours (except for Screening and W28 assessment). Including: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatine phosphokinase, creatinine, total protein, albumin, serum amylase (fractionated if > greater than upper limited of reference range), serum lipase, phosphate, GGT, AST, ALT, alkaline phosphatase, total bilirubin, calcium, uric acid, glucose and insulin. In addition CRP and C-peptide will be included at the following visits: Baseline, W4, W12 and W24.
[2] Including: total cholesterol, triglycerides, HDL and LDL
[3]Including: hemoglobin, hematocrit, red blood cell (RBC) and RBC morphology, white blood cell (WBC) and differential WBC count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC).
[4]Serum biomarkers – CK-18, LPS, adiponectin, GLP-1 and cytokines (IL-1α, IL1β, IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-12, IL-17α, IL-23, IFNγ, TGF-β and TNFα)
[5] PBMC - FACS analysis for CD4 CD8 CD25 FoxP3 NKT CD62 CD69 T cells
[6] Serology (including HIV, HCV and HBV test) results will be acceptable for eligibility within range of 6 months prior to screening.
[7] MRI: Baseline MRI to be performed as soon as subject eligibility is determined. Week 24 MRI to be done within +/- 2 weeks week 24.
[8] Randomization is to be done within 2 weeks post baseline MRI, when the MRI result is available for stratification (hepatic fat fraction form – to be received by central imaging lab).

## TABLE OF CONTENTS

## LIST OF TABLES

## GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

| Term | Definition |
|---|---|
| $^{o}C$ | Degrees Celsius |
| µg | Microgram(s) |
| AE | Adverse Event |
| ALT | Alanine aminotransaminase |
| AST | Aspartate aminotransaminase |
| AUC | Area under the curve |
| AUDIT | Alcohol Use Disorders Identification Test |
| BCP | Bovine colostrum powder |
| BMI | Body mass index |
| BP | Blood pressure |
| CFR | Code of Federal Regulations |
| CK | Cytokeratin |
| cm | centimeter |
| $C_{max}$ | Maximum concentration |
| $C_{min}$ | Minimum concentration |
| CRF | Case report form |
| CRP | C-reactive protein |
| CTCAE | Common Terminology Criteria for Adverse Events |
| D | Day |
| dL | Deciliter |
| EKG | Electrocardiogram |
| ELISA | Enzyme-linked immunosorbent assay |
| ETEC | Enterotoxigenic Escherichia Coli |
| FDA | Food and Drug Administration, United States of America |
| g | Gram |
| GCP | Good Clinical Practice |
| GGT | Gamma glutamyl transpeptidase |
| GLP | Good Laboratory Practice(s) |
| GLP-1 | Glucagon-like peptide-1 |
| H&E | Hematoxylin & Eosin |
| HBA1C | Hemaglobin A1C |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| HDL | High density lipoprotein |
| HIV | Human immunodeficiency virus |
| HMG Co-A | 3-hydroxy 3-methyl glutaryl Co-A |
| HOMA-IR | Homeostatic Model of Insulin Resistance |
| HREC | Human Research Ethics Committee |
| IB | Investigator's Brochure |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IFNγ | Interferon gamma |
| IgG | Immunoglobulin G |
| IL | Interleukin |
| IND | Investigational New Drug |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| ISF | Investigator Site File |
| ITT | Intention-to-treat |

| | |
|---|---|
| kg | Kilograms |
| L | Liter |
| LDL | Low density lipoprotein |
| LPS | Lipopolysaccharide |
| mAb | Monoclonal antibody |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MELD | Model of End-stage Liver Disease |
| mg | Milligram(s) |
| min | Minute(s) |
| mL | Milliliter(s) |
| mmHg | Millimeter of mercury |
| MR | Magnetic Resonance |
| MRI | Magnetic Resonance Imaging |
| NAFL(D) | Non-alcoholic fatty liver (disease) |
| NAS | NASH activity score |
| NASH | Non-alcoholic steatohepatitis |
| PBMC | Peripheral blood mononuclear cells |
| PD | Pharmacodynamic(s) |
| pH | Measure of acidity |
| PK | Pharmacokinetic(s) |
| PT | Prothrombin time |
| PTT | Partial thromboplastin time |
| RBC | Red blood cells |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SOP | Standard Operating Procedure |
| $T_{1/2}$ | Half-life |
| TGS | Toxicity Grading Scale |
| TNFα | Tumor Necrosis Factor alpha |
| TK | Toxicokinetic(s) |
| $T_{regs}$ | Regulatory T cells |
| W | Week |
| WBC | White blood cells |

## 2. STUDY CONTACTS

Please refer to the Study Reference Manual for full study contacts.

# 3. INTRODUCTION

## 3.1 Epidemiology of Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in most of the Western world. Approximately 7.9% of the population in the United States has persistently elevated liver enzymes without any clear etiology [Clark *et al*., 2003] and it is hypothesized that over 80% of these cases are due to NAFLD. In those who have concomitant features of metabolic syndrome, the likelihood of NAFLD exceeds 90%. Based on Magnetic Resonance Imaging (MRI), it has been estimated that the overall prevalence of NAFLD in the United States is approximately 30% [Browning JD, 2004].

The more severe, progressive form of NAFLD, non-alcoholic steatohepatitis (NASH), is an increasingly common chronic liver disease with distribution that is closely associated with diabetes and obesity, which have both reached epidemic proportions. Approximately 6 million individuals in the USA are estimated to have progressed to NASH and some 600,000 to NASH-related cirrhosis [World Gastroenterology Organization Global Guidelines, Nonalcoholic Fatty Liver Disease, and Nonalcoholic Steatohepatitis, June 2012]. However, estimates of 5.7% of the general population [Ong *et al*., 2007] and in excess of 12% in a middle aged US population [Williams *et al* 2011] have been made.

## 3.2 Clinical Presentation

NAFLD is the hepatic manifestation of the metabolic syndrome, a condition characterized by the presence of at least three of five co-morbidities: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides and low high-density lipoprotein (HDL) levels [Kang *et al*, 2006].

The probability of having NAFLD rises with increasing body mass index (BMI) with over 80% of subjects with a BMI > 35 having NAFLD. The histological spectrum of NAFLD includes: isolated hepatic steatosis, characterized by a fatty liver with no other histological abnormalities and also referred to as nonalcoholic fatty liver (NAFL); and steatohepatitis, characterized by steatosis associated with histological abnormalities that include cytological ballooning, Mallory's hyaline, inflammation and pericellular fibrosis [Kleiner *et al*., 2005]. NASH is defined by the presence of predominantly macrovesicular hepatic steatosis or steatohepatitis in individuals who either do not consume alcohol or consume it in quantities that are not generally considered to be harmful to the liver [Sanyal, 2002]. There are currently no non-invasive ways to distinguish NAFLD from NASH.

NAFLD is associated with a benign clinical course and the majority of cases remain asymptomatic and free of fibrosis or development of steatohepatitis over a 5 to 10 year time frame from diagnosis [Teli *et al*., 1995]. The development of steatohepatitis requires both accumulation of fat and additional injurious processes in the liver. It is believed that oxidative stress plays an important role in this process. The impact of the gut microbiome on weight gain, fat accumulation and development of NAFLD is being actively investigated [Compare *et al*., 2012, Manco *et al*., 2010].

The immune system plays a role in the pathogenesis of NAFLD and its complications. Metabolic syndrome, obesity and NASH are all associated with a state of chronic inflammation. The immune system and the inflammatory cascade are common to the development of these conditions.

NASH progresses to cirrhosis in approximately 20% of cases [McCullough, 2002] and is also associated with an increased risk of cardiovascular mortality and type 2 diabetes mellitus. Cirrhosis due to NASH also increases the risk of hepatocellular carcinoma and NASH contributes substantially to the population burden of hepatocellular cancer [Vernon *et al*., 2011]. Twenty two to 33% of patients who have cirrhosis die of complications of liver failure or require liver transplantation [Ong *et al*., 2007].

## 3.3 Diagnostic Tools

Currently, abnormal liver function tests are the primary method of identifying patients for further investigation for NAFLD and NASH. Population based studies have utilized imaging modalities such as ultrasound and MRI to diagnose NAFLD but are limited by the absence of histologic confirmation. Recently, changes on MRI have been correlated with hepatic lipid content [Yokoo *et al* 2008]. The presence of NAFLD can be diagnosed with relative confidence using MRI [Schwenzer *et al*., 2009]. MR spectroscopy and imaging techniques have been shown to have utility and greater sensitivity for fat quantification compared with histology-determined steatosis. [Noureddin *et al* 2013].

NASH presents with variable presence of steatosis, inflammation and cytologic ballooning and is usually determined on an overall assessment of histologic findings on liver biopsy. In order to standardize the assessment, a NASH activity score (NAS) was developed by the National Institutes of Health (NIH) NASH Clinical Research Network [Kleiner *et al* 2005]. Steatohepatitis is diagnosed when scored 4 or more. MRI provides information about liver fat content but does not distinguish between NAFL and NASH [Saadeh *et al*., 2002].

### 3.3.1 Current Treatment for NASH

Currently, there is no specific treatment for NASH [Dixon *et al*., 2004; Neuschwender-Tetri *et al*., 2003]. Patients presenting with NASH are advised to reduce their weight (if obese or overweight), follow a balanced and healthy diet, increase physical activity, avoid alcohol and avoid unnecessary medications (http://digestive.niddk.nih.gov/ddiseases/pubs/nash/#treatment). Experimental approaches aimed at reducing oxidative stress have potential as treatments. It has been reported that vitamin E improved liver histology in 43% of patients with NASH compared with 19% in a placebo group [Sanyal *et al*., 2010].

Most patients with NASH have insulin resistance. Treatments for type-2 diabetes including metformin, rosiglitazone, and pioglitazone may have utility in this condition although these have been associated with weight gain. The metabolic syndrome and NASH can be treated with bariatric surgery in obese individuals to reduce dietary intake.

## 3.4 IMM-124E - Hyperimmune Bovine Colostrum

The following summarizes current information on IMM-124E.

### 3.4.1 Hyperimmune Bovine Colostrum as a Therapeutic

Mammals supply their newborn before birth, at birth or shortly after birth with antibodies, immunocytes and humoral constituents. This “borrowed immunity” is a form of passive immunization to protect the newborn against environmental pathogens until it establishes its own pathogen recognition and disposal systems. In cows, goats, horses and some other animal species, most immunoglobulins are obtained from the colostrum, the first milk after birth, via the gut but in humans the majority of immunoglobulins, and those of the Immunoglobulin(Ig)G-class in particular, are acquired from the mother by placental transport in the weeks prior to parturition [Struff & Sprotte].

Ingested proteins are normally degraded by proteases in the stomach and small intestine to form small peptides and amino acids which are subsequently absorbed. The proteolytic enzymes involved include pepsin, trypsin, chymotrypsin, carboxy-peptidase and elastase which initially degrade antibodies of the IgG type into F(ab’)2, Fab and Fc fragments. The F(ab’)2 and Fab fragments, however, retain some of their antigen neutralizing activity locally in the gastrointestinal tract [Reilly *et al*. 1997]. In addition the colostrum contains adjuvants which alter the systemic immune system (Adar *et al*. 2012).

### 3.4.2 IMM-124E Composition

IMM-124E is hyperimmune bovine colostrum that is lactose- and fat-reduced. IMM-124E is harvested from the colostrum of dairy cows which have been immunized against the outer antigens, mostly LPS, of the most common strains of Enterotoxigenic *Escherichia coli* (ETEC). This inoculation activates a generalized immune response in the host animal to produce antibody clones which recognize and bind with the bacterial cell-surface epitopes presented. These polyclonal antibodies can also cross-react with other similar bacterial cell surface antigens.

IMM-124E contains approximately 40% IgG in the drug substance, mainly IgG1 and IgA, with small amounts of IgM and IgE. The non-specific nature of the immunoglobulins in IMM-124E and high binding activity against the LPS moiety of ETEC and non-ETEC gram-negative bacteria facilitates the potential for broad protective coverage against these infective agents.

Immuron's IMM-124E-based product, Travelan, has been sold in Australia over-the-counter (for use by adults and children over 6 years of age) at a dose 200 mg three times a day since September 2004. Approximately 140,000 packets (30 tablets each packet) have been sold and no treatment-related adverse events have been reported.

For more information on IMM-124E, please refer to the current Investigator's Brochure

### 3.4.3 Pharmacology

#### 3.4.3.1 *In vitro* Pharmacology

IMM-124E contains anti-LPS antibodies to both the O polysaccharide and lipid A core region of all the serotypes included in the ETEC vaccine. These anti-LPS antibodies also cross-react with both lipid A-core and O-polysaccharide regions of eight other Gram negative bacteria. IMM-124E was able to bind and agglutinate ETEC bacteria *in vitro*. Flagella-specific antibodies present in IMM-124E Drug Substance were able to reduce bacterial motility and adherence to host-cells. For further information, please refer to the Investigator's Brochure.

#### 3.4.3.2 *In vivo* Pharmacology

##### 3.4.3.2.1 Effect of IMM-124E in Leptin Deficient Ob/Ob Mice

In leptin deficient Ob/Ob mice, oral administration of 1mg IgG-enhanced fraction of ETEC colostrum significantly decreased alanine aminotransferase (ALT) ($p<0.05$) and serum and hepatic triglycerides ($p<0.009$ and $p<0.05$, respectively) compared with control animals. Oral administration of the IgG-enhanced fraction of ETEC colostrum induced regulatory T cells and alleviated the chronic inflammatory state in the metabolic syndrome, alleviating insulin resistance and liver injury. For further details, please refer to the Investigator's Brochure.

### 3.4.4 Preclinical Safety

#### 3.4.4.1 *In vitro* Safety

No cytotoxicity or mutagenesis was shown in a Good Laboratory Practice (GLP) AMES study. For further information, please refer to the Investigator's Brochure.

#### 3.4.4.2 *In vivo* Safety

Two short-term, non-GLP, repeat-dose dietary studies were conducted in mice with hyperimmune bovine colostrum powder. There was no indication of treatment-related toxicity in either study.

Freeze-dried hyperimmune bovine 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). Mice were divided randomly into four groups (5/sex/group). After a 3-day acclimatization period on the diets, individual mice were

weighed each day and average food consumption was determined. All mice survived and the weight of mice in all four groups increased. Clinical observations did not reveal any adverse effects. There was no significant effect of diet, sex or diet-sex interaction on body weight changes between Days 3 and 10.

A second study was conducted using hyperimmune bovine colostrum powder at the Department of Microbiology, The University of Melbourne, Australia. Five female and five male mice per test group were given a bottle containing either bovine colostrum powder (50mg/mL) or skim milk or water for a total of eight days. All mice showed a steady increase in weight over the eight-day period with no adverse effects on health or behavior between the test and control groups. For further information, please refer to the Investigator's Brochure.

### 3.4.5 Metabolism and Pharmacokinetics

The pharmacokinetics (PK) and toxicokinetics (TK) of IMM-124E have not been established.

### 3.4.6 Clinical Trials of IMM-124E

IMM-124E has been studied for the prevention of traveler's diarrhea in three double-blind, randomized, placebo-controlled clinical trials in Europe and the USA, conducted to Good Clinical Practice (GCP) standard. A Phase 1 open label study in NASH has also been conducted.

#### 3.4.6.1 Study 1: Central Public University Hospital, Warsaw, Poland

This was a double-blind, randomized, placebo-controlled, challenge trial with 60 healthy volunteers divided randomly into 4 groups. Each group of 15 was given either one or two IMM-124E or matching placebo tablets 3 times a day (with or without bicarbonate) before meals for 7 days and were then challenged with a pathogenic dose of O78 ETEC (1-2 x $10^9$ bacteria). IMM-124E resulted in significant protection of the volunteers against the development of diarrhea after challenge with pathogenic doses of ETEC, compared with placebo ($p<0.01$). ETEC O78 was detected in the feces of most volunteers after challenge, independent of treatment, suggesting that the mode of action was blocking the bacteria from binding to the gut epithelium, rather than a bactericidal effect. There were no treatment related adverse events reported. For further information, please refer to the Investigator's Brochure.

#### 3.4.6.2 Study 2: Central Public University Hospital, Warsaw, Poland

This was a double-blind, randomized, placebo-controlled, challenge trial with 30 healthy volunteers randomly assigned to either 400mg IMM-124E or matching placebo tablets 3 times a day before meals for 7 days, before challenge with 1-2 x $10^9$ O78 ETEC. Continuing the results of study 1, IMM-124E was significantly more effective ($p<0.001$) than placebo treatment in protecting the volunteers against the development of diarrhea after challenge with pathogenic doses of ETEC. Bacterial output in stools was not significantly different between placebo and treatment groups. There were no treatment related adverse events reported. For further information, please refer to the Investigator's Brochure.

#### 3.4.6.3 Study 3: Center for Vaccine Development, University of Maryland, Baltimore, Maryland

This was a double-blind, randomized, two-group, placebo-controlled, challenge trial of 22 healthy volunteers. 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. Participants were closely observed by clinical staff. IMM-124E reduced the incidence of diarrhea by 44% following experimental challenge with ETEC strain O78. No treatment related adverse events were reported (Tacket *et al*., 1988).

#### 3.4.6.4 Phase 1 Clinical Trial of IMM-124E in Patients with NASH, Hadassah Medical Center, Jerusalem

This was an open-label investigator-initiated trial comprising 10 subjects with biopsy proven NASH and insulin resistance; subjects were treated for 30 days with IMM-124E 1800mg daily per orally. Response was determined by changes in liver function tests and metabolic syndrome. Comparison between Day 30 and Day 0 was performed for each patient.

Oral administration of IMM-124E was well tolerated and no treatment-related adverse events were reported. Improvement in markers of insulin resistance were observed though these changes were not statistically significant over 30 days of treatment. A downward trend in HbA1C was observed in a majority of patients. Increases in peripheral blood mononuclear cells (PBMCs) CD4+CD25+, CD4+CD25+Foxp3+ $T_{regs}$ and in CD4+CD62+ and CD4+CD25+ HLA-DR were also observed.

### 3.5 Rationale

A close interplay exists between the gut and liver. This is based on the evidence that more than 70% of the blood liver supply derives from the portal vein, the direct venous outflow of the intestine [Compare *et al*. 2012].

Disturbances in the homeostasis between bacteria- and host-derived signals at the epithelial level lead to a break in intestinal barrier function and may foster "bacterial translocation", defined as the migration of bacteria or bacterial products from the intestinal lumen to mesenteric lymph nodes or other extraintestinal organs and sites. While the full repertoire of gut-derived microbial products that reach the liver in health and disease is yet to be explored, the levels of bacterial LPS are increased in the portal and/or systemic circulation in several types of chronic liver diseases, including NASH [Manco *et al*., 2010]. Derangement of the gut flora, particularly small intestinal bacterial overgrowth, occurs in a large percentage (20-75%) of patients with chronic liver disease. It has been suggested that fatty diets promote gut microbiota changes and endotoxins produced by gram negative bacteria promote the development of NASH [Compare *et al*. 2012].

Hepatic steatosis is commonly associated with factors such as obesity and sub-clinical insulin resistance. Vulnerability to subsequent insults such as endogenous or exogenous toxins increases the production of pro-inflammatory cytokines. This further exacerbates insulin resistance, oxidative stress and organelle dysfunction within liver cells. In addition, circulating LPS has been found to trigger a molecular cascade which results in the release of pro-inflammatory cytokines. The latter eventually leads to hepatocyte death and the accumulation of inflammatory cells seen in NASH. In mice, LPS has also been associated in studies with increase in body weight and accumulated intrahepatic triglyceride [Manco *et al* 2010].

Bovine colostrum contains increased concentrations of immunoglobulins (mainly IgG) compared with normal cow's milk, presented with other physically protective milk proteins. IgGs are not absorbed into the blood and act in the lumen against bacterial antigens or interact at the mucosal surface with dendritic cells that sample antigens from gut contents, influencing populations of T regulatory lymphocytes.

IMM-124E has strong binding and neutralising activity against a wide range of LPS antigens. It is hypothesised that IMM-124E anti-LPS activity decreases the challenge of LPS to the liver and down-regulates the key T regulatory cell population associated with chronic inflammation and other metabolic defects associated with NASH.

In addition to its potential effect on bacterial translocation, IMM-124E contains adjuvants which promote regulatory T cells that suppress inflammation in target organs. The dual function of this product may be synergistic in alleviating NASH (Adar *et al*. 2012).

A clear unmet medical need remains in the treatment of NASH and an oral, well tolerated treatment for this condition is needed. Bovine colostrum, either as standard or hyperimmune colostrum, has an excellent safety profile [Sarker *et al*., 1998; Tacket *et al*., 1992; Mitra *et al*., 1995; Opekun *et al*., 1999].

### 3.5.1 Dose Rationale

#### 3.5.1.1 Pharmacodynamic Dose Modeling

Preclinical models of NASH do not provide reliably predictable data for allometric scaling. Further the open-label single-center investigator initiated trial conducted in a small number of subjects (n=10) with type 2 diabetes and NASH (1800mg daily for 30 days) was not of sufficient size or duration to answer whether the response was optimal. Empirically, the 1800mg/day dose will be included in this clinical trial and a higher dose evaluated to establish a dose response curve.

#### 3.5.1.2 Safety Dose Modeling

IMM-124E is well tolerated in clinical studies and in the market place. While the typical dose for traveler's diarrhea is 600mg/day, doses as high as 10g four times daily have been studied. Adverse reactions have been few in number and mild in severity. In most clinical studies of bovine colostrum, there have been no treatment related adverse events reported and no clear pattern in events [Tacket *et al*., 1992; Shield *et al*., 1993; Mitra *et al*., 1995; Sarker *et al*., 1998; Huppertz *et al*., 1999; Opekun *et al*.,1999].

Adverse events observed include:

- nausea, flatulence [Rump *et al*., 1992; Plettenberg *et al*., 1993]
- nausea and vomiting in 1/24 patients [Greenberg and Cello 1996]
- mild diarrhea in 1/8 adult volunteers [Lissner *et al*., 1998]

Tacket *et al*. (1988) reported no adverse clinical signs, but transient moderate elevations in serum levels of hepatic transaminases in volunteers who received bovine immunoglobulin concentrates prepared against E. coli (5/10) and rotavirus (2/10). This effect was attributed to diets high in protein provided at the hospital. Colostrum administration should be avoided in person allergic to cow's milk or with lactose intolerance.

The known safety profile of bovine colostrum gives a substantial therapeutic index. Therefore, the highest dose to be evaluated will be 3600mg/day.

### 3.5.2 Discussion of Study Design and Choice of Control Group(s)

A placebo controlled, randomized, double blind design represents the highest standard of clinical trial design available. The study will be multicenter to allow recruitment to occur in a reasonable timeframe. Given the known safety profile of IMM-124E, a parallel design with two dose levels is proposed.

# 4. OBJECTIVES AND ENDPOINTS

## 4.1 Objectives

The primary objectives of this study are to:

- Evaluate the safety and preliminary efficacy of two dose levels of IMM-124E in reducing liver fat evaluated on MRI compared with placebo in subjects with biopsy proven NASH.

The secondary objectives are to:

- Determine the pharmacokinetic profile of IMM-124E;
- Assess the impact of treatment with IMM-124E on markers of glucose metabolism and on serum lipid profile;
- Assess the impact of treatment on liver function over 24 weeks;
- Establish the recommended dose.

Exploratory objectives are to investigate the impact of treatment on potentially relevant immune and metabolic biomarkers associated with NASH.

## 4.2 Endpoints

Safety and efficacy measures for NASH subjects receiving IMM-124E compared with subjects receiving placebo:

**Primary endpoints:**

Safety:

- Incidence and severity of adverse events and changes in vital signs and clinical laboratory parameters

Efficacy:

- Mean change from Baseline in the percentage fat content of the liver measured by MRI at Week 24

**Secondary endpoints:**

Pharmacokinetics:

- Serum concentration of IMM-124E bovine antibodies over time (if measurable) including, but not limited to, maximum observed concentration (Cmax), minimum observed concentration (Cmin) and area under the concentration-time curve (AUC), and, if feasible, elimination half-life ($T_{½}$)

Metabolic markers:

- BMI, waist circumference, waist:hip ratio, HBA1C and HOMA-IR
- Serum lipid profile – total cholesterol, triglycerides, LDL and HDL fractions

Liver function:

- Mean change from Baseline in serum ALT, AST, bilirubin, albumin and GGT at Weeks 4, 8, 12, 16, 20 and 24
- Proportion of subjects whose ALT at Week 24 is within reference range defined a $\leq$19IU/L for women and $\leq$30IU/L for men
- The proportion of subjects demonstrating a decrease in serum ALT at 24 weeks compared to baseline equal or greater than 30% compared to placebo

MRI:

- The proportion of subjects demonstrating a decrease in Liver percent fat by MRI at 24 weeks compared to baseline equal greater than 5% compared to placebo
- The proportion of subjects demonstrating a decrease in Liver percent fat by MRI at 24 weeks compared to baseline equal greater than 10% compared to placebo

**Exploratory endpoints:**

- Mean serum concentrations of: LPS, CK-18 fragments and adiponectin and subsets of cytokines IL6, IL-1α, IL1β, IL-2, IL-3, IL-4 IL-10, IL-13, IL-12, IL-17α, IL-23, IFNγ TGFβ and TNFα
- Serum metabolomics
- Relative levels of regulatory T cells in PBMC samples, including CD4, CD8, CD25, FoxP3, NKT and CD62
- Characterization of the gut microbiome.
- Explore a dose effect

# 5. STUDY DESIGN

## 5.1 Study Design

This is a randomized, double blind, placebo controlled, 3-arm parallel group, multi-dose, multi-center study

## 5.2 Dosing Regimens

There are 3 regimens in this study as described in Table 2 below.

**Table 2: IMM-124E daily dose levels**

| Treatment arm | IMM-124E Dose |
|---|---|
| A | 600mg three times daily |
| B | 1200mg three times daily |
| C | matching placebo only, three times daily |

At least 40 subjects will be enrolled into each cohort. For each subject, the 24 week primary treatment period will consist of three times daily oral administration of the allocated IMM-124E dose or matching placebo.

## 5.3 Study Sites

This will be a multicenter study involving approximately 30 clinical study sites.

## 5.4 Estimated Duration of the Study

The recruitment period will be approximately 24 months. The Screening period will be up to 45 days and subjects will receive treatment for 24 weeks. Subjects who withdraw from treatment will be followed for 28 days after treatment cessation, where possible.

## 6. SUBJECT POPULATION

**6.1 Subject Selection and Numbers**The nature of the study and the potential risks will be explained to all candidates. Written informed consent will be obtained from each subject prior to performing any screening procedures. There will be no exemptions and subjects must satisfy all eligibility criteria in order to participate. At least 120 subjects will be enrolled.

Inclusion and exclusion criteria are to be determined at Screening unless otherwise indicated.

### 6.2 Inclusion Criteria

The criteria for entry into the study are:

1. Age ≥18 years
2. Provision of written informed consent
3. Diagnosis of NASH, histologically proven within 12 months of Screening with all of the following criteria met:
   - NASH activity score (NAS) of 4 or more
   - Cytologic ballooning score of at least 1
   - 10% or more macrovesicular steatosis
   - Hematoxylin & Eosin (H&E) stained slides and/or paraffin block available for independent assessment
4. HBA1C of ≤ 9.0
5. Agree to the use of effective contraceptive measures if either male or female of child bearing potential

### 6.3 Exclusion Criteria

The criteria for exclusion from the study are:

1. Presence of vascular liver disease or cirrhosis
2. Presence of liver disease from other cause (autoimmune, metabolic, medication induced)
3. BMI ≤25kg/$m^2$
4. Alcohol use ≥30g/day
5. Type 1 diabetes
6. History of major bariatric surgery (not including balloon / sleeve gastrectomy)
7. Weight loss or gain of 5kg or more in the past 6 months or ≥10% change in bodyweight in the past 12 months
8. Contraindication for MRI
9. Inadequate venous access
10. Lactating/breastfeeding/pregnant at Screening or Baseline
11. HIV antibody positive, hepatitis B surface antigen positive (HBsAg) or hepatitis C virus-RNA positive
12. Receiving an elemental diet or parenteral nutrition
13. Concurrent conditions:
    - Inflammatory bowel disease
    - Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of Screening
    - Ongoing infectious disease
    - Ongoing multi-systemic immune-mediated disease
    - Concurrent or past malignant disease
    - Any other concurrent condition which, in the opinion of the investigator, could impact adversely on the subject participating or on the interpretation of the study data

14. Concurrent medications including:
    - Anti-NASH therapy(s) taken for more than 10 continuous days in the last 3 months. These include S-adenosyl methionine (SAM-e), betaine, milk thistle, bacterial probiotic supplements (other than yoghurt or other natural food sources), vitamin E and gemfibrozil.

        - NOTE: If determinant biopsy is performed while on stable treatment – subject is eligible;
        - Wash out for any of the anti-NASH therapies is as follow: under 10 days no washout required, more than 10 days and up to 3 months treatment requires 6 weeks washout. Any treatment of over 3 months would require to re-biopsy to ensure histological eligibility
        - Thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or glucagon-like peptide-1 analogs in the last 6 months.
          NOTE: if determinant biopsy is performed while on stable treatment of at least 6 months – subject is eligible.
        - NOTE: Allowable anti-diabetic treatment includes metformin and/or sulfonylureas administered at constant dose for at least 2 months prior to study entry
        - NOTE: Subjects treated with Insulin are eligible if clinically stable on insulin treatment (i.e. no recurrent acute hypo-/hyperglycemic episodes diagnosed clinically and by Glucose serum levels of <50 mg/dL and >200 mg/dL respectively) for at least 2 months prior to study entry
    - immune modulatory agents including:
        - Within 3 months of study entry:
            - systemic steroids for more than 7 days
            - daily treatment with multiple non-steroidal anti-inflammatory drugs (such as aspirin >100mg/day, ibuprofen, naproxen, meloxicam, celecoxib) for more than 1 month
        - In the last 12 months:
            - azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, anti-TNFα therapies (infliximab, adalimumab, etanercept) or anti-integrin therapies (namixilab)
    - more than 10 consecutive days oral or parenteral antibiotics within 4 weeks prior to study entry NOTE: subjects administered with antibiotics for more the 5 days prior to study entry would not be included in the stool and PBMC analysis)
    - Variable dose of anti-lipidemic agents (HMG Co-A reductase inhibitors – "statins") in the 3 months prior to Screening
15. The following laboratory abnormalities:
    - Neutrophil count ≤ 1.0 x $10^9$/L
    - Platelets <100 x $10^9$/L
    - Hemoglobin <10g/dL
    - Albumin < 3.5g/dL
    - International Normalized Ratio (INR) >1.5
    - Total bilirubin >1.5 x upper limit of reference range (unless Gilbert's syndrome or extrahepatic source as denoted by increased indirect bilirubin fraction)
    - Either creatinine clearance ≤ 60 mL/minute calculated by Cockroft-Gault or creatinine >1.5x upper limit of reference range (see Section 6.4.2).
16. Known substance abuse, including inhaled or injected drugs in the year prior to Screening
17. Cow milk allergy, lactose intolerance or any known or suspected hypersensitivity to study products

## 6.4 Other Study Eligibility Criteria Considerations

In order to assess any potential impact on subject eligibility with regard to safety, the investigator must refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, adverse events, and other significant data pertaining to the investigational product(s) being used in this study. Such documents may include, but are not limited to, the Investigator's Brochure.

### 6.4.1 Contraception

All women of child bearing potential (defined as sexually mature women who have had menses within the preceding 24 months and have not undergone hysterectomy, bilateral oophorectomy or tubal ligation) must have a negative pregnancy test (with a sensitivity of at least 50IU/mL) performed at Screening and at Baseline.

Women of child bearing potential must agree not to attempt to become pregnant or undergo *in vitro* fertilization and, if participating in sexual activity that could lead to pregnancy, must use two reliable methods of contraception simultaneously while receiving protocol-specified medication and for 28 days after stopping the medication. Male subjects must agree to use two reliable methods of contraception simultaneously while receiving protocol-specified medication and for 28 days after stopping the medication if their partner is of child bearing potential.

A combination of two of the following methods must be used:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intra Uterine Device
- Hormonal-based contraception

Women who are not of reproductive potential (who have been postmenopausal for at least 24 consecutive months or have undergone hysterectomy, bilateral oophorectomy or tubal ligation) are not required to use contraception.

### 6.4.2 Renal Function

Renal function eligibility may be determined by either estimated creatinine clearance calculated by Cockroft-Gault formula or serum creatinine.

The Cockcroft-Gault Formula is:

$$\text{Creatinine clearance (mL/min)} = \frac{(140\text{-age}) \times (\text{Bodyweight in kg}) \times (0.85 \text{ if female})}{(72 \times \text{Serum creatinine (mg/dL)})}$$

## 6.5 Subject Enrollment

Before subjects may be entered into the study, Immuron requires a copy of the site's written institutional review board (IRB) or human research/independent ethics committee (HREC/IEC) approval of the protocol, informed consent form, and all other subject information, as applicable. All subjects or legally acceptable representatives must personally sign and date the consent form before enrolment.

All subjects who provide written informed consent will be sequentially assigned a Subject Number. This number will be used to identify the subject throughout the clinical study and must be used on all study documentation related to that subject. Subjects who meet the inclusion/exclusion criteria will be randomly assigned to treatment using a permuted block randomization.

All screening tests and procedures should be performed within the 45 days prior to enrolment in the study at Baseline, unless otherwise indicated. Randomization and enrolment will be conducted as described in Section 8.

# 7. SCHEDULE OF ASSESSMENTS AND PROCEDURES

## 7.1 Study Schedule of Evaluations

The schedule of assessments is presented in Table 1.

## 7.2 Study Procedures and Assessment Periods

The study procedures to be conducted for each subject enrolled in the study are listed below. Additional information on the study procedures is provided in Section 7.4. Any deviation from protocol procedures must be noted in the source notes and the Sponsor notified.

Additional visits and/or assessments may be conducted as clinically indicated. For these additional assessments, data may be collected on unscheduled Case Report Form (CRF) pages provided, together with all adverse events (AEs) and concurrent medication that must be recorded throughout the study period.

### 7.2.1 Screening Assessments

Subjects will be screened up to 45 days prior to treatment initiation to determine eligibility for participation in the study. Screening assessments may be conducted on different days during this 45 day period, if required.

The following will be performed and documented during Screening:

- Obtain written informed consent prior to any study related procedures
- Demographic data (see Section 7.3.1)
- Medical history (see Section 7.3.1)
- A complete physical examination including: (see Section 7.3.2)
  - vital signs (blood pressure, temperature, heart rate and respiratory rate: see Section 7.3.3)
  - bodyweight
  - height
  - assessment of all appropriate body systems to determine study eligibility
- 12-lead electrocardiogram (EKG) (see Section 7.3.4)
- Medication history and concurrent medication assessment (see Section 9.2)
- Collection of urine for dipstick urinary testing (see Section 7.3.6)
- Collection of blood samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Clinical chemistry (see Section 7.3.6)
  - Alfa-feto protein (see Section 7.3.6)
  - HBA1C (see Section 7.3.6)
  - HIV, HCV and HBV serology (see Section 7.3.7)
  - Pregnancy test for women of childbearing potential (see Section 7.3.7)

Results of all screening tests must be available and reviewed against the eligibility criteria (see Section 6) prior to the subject's Baseline visit.
Subjects meeting all the inclusion criteria and none of the exclusion criteria will return to the clinic within 45 days after commencement of Screening for MRI scan as part of the Baseline evaluations.

### 7.2.2 Randomization Process

Upon completion of screening assessments and confirmation that the subject meets all eligibility criteria, baseline MRI scan is to be performed and valid result from central imaging lab to be obtained. Upon receipt of scan results the subject will be randomized to study treatment at the Baseline visit (See Section 8.1 and the Study Reference Manual). The randomization number will be recorded in the subject's study documentation.

### 7.2.3 Day 0: Baseline Evaluations and Study Medication Administration

Upon completion of screening assessments and confirmation that the subject meets all eligibility criteria (up to 45 days), baseline MRI scan of the liver (Section 7.3.13) is to be performed. Valid result for baseline MRI scan by the central imaging lab (VirtualScopics) must be obtained before randomization.

Subjects meeting all of the inclusion and none of the exclusion criteria and have a valid baseline MRI result will attend the Baseline Visit (Day 0) within 14 days of the baseline MRI. At the Baseline Visit subjects will be randomized and receive study medication.
The following procedures performed and documented:

- Urine pregnancy test for women of childbearing potential (see Section 7.3.10)
- A targeted physical examination (see Section 7.3.2)
- Adverse event (AE) assessment (see Section 10.2)
- Measurement of vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Measurement of bodyweight and waist and hip circumferences (see Section 7.3.3)
- Concurrent medication recording (see Section 9)
- Diet and exercise counseling and 7-day diary issue (see Section 7.3.5)
- Administration of the Alcohol Use Disorders Identification Test (AUDIT) questionnaire
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Fasting clinical chemistry (see Section 7.3.6)
  - Fasting lipid analysis, including HOMA-IR (see Section 7.3.6)
  - Pharmacokinetics - prior to study drug administration (see Section 7.3.8)
  - PBMCs (see Section 7.3.9) – selected sites only
  - Biomarker analysis, including for CK-18 fragments, LPS, adiponectin, GLP-1 and cytokines (see Section 7.3.9)
  - Serum metabolomics
- Collection of a stool sample for microbiome assessment (see Section 7.3.11) – selected sites onlySubject randomization and dispensing of study medication (see Section 8). The first dose should be taken in the clinic where possible. Study treatment will be administered as described in Section 8.5.4

If a subject is screened and eligible for the study, but unable to participate immediately, they may be rescreened once, under a new Subject identification number. Rescreening of these subjects must be discussed and agreed with the Medical Monitor. Subjects with laboratory abnormalities that are exclusionary, but inconsistent with previous history, may be re-tested once.

### 7.2.4 Day 3

The following procedures will be performed by either phone call or clinic visit:

- AE assessment (see Section 10.2)
- Concurrent medication recording (see Section 9)

### 7.2.5 Day 7

Subjects will return to the clinic and the following procedures will be performed:

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)

- Concurrent medication recording (see Section 9)
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Fasting clinical chemistry (see Section 7.3.6)
  - Fasting lipid analysis, including HOMA-IR (see Section 7.3.6)

### 7.2.6 Day 14

Subjects will return to the clinic and the following procedures will be performed:

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Concurrent medication recording (see Section 9)
- 7-day diary review and counseling (see Section 7.3.5)
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Fasting clinical chemistry (see Section 7.3.6)
  - Fasting lipid analysis, including HOMA-IR (see Section 7.3.6)

### 7.2.7 Weeks 4, 8, 12, 16 and 20

Subjects will return to the clinic at Weeks 4, 8, 12, 16 and 20 and the following tests will be performed:

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Measurement of bodyweight and waist and hip circumferences (see Section 7.3.3)
- Diet and exercise counseling (see Section 7.3.5)
- Concurrent medication recording (see Section 9)
- Study medication accountability and dispensing (see Section 8)
- Urine pregnancy test
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Fasting clinical chemistry (see Section 7.3.6)
  - Fasting lipid analysis, including HOMA-IR (see Section 7.3.6)

Additional assessments at Week 4 and 12 are:

- Collection of blood and serum samples for:
  - Pharmacokinetics (see Section 7.3.8)
  - Biomarker analysis including CK-18 fragments, LPS, adiponectin, GLP-1 and cytokines (see Section 7.3.9)
- Collection of a stool sample for microbiome assessment (see Section 7.3.11) – selected sites only.

Additional assessments at Week 12 only are:

- Collection of blood sample for measurement of HBA1C and PBMCs (at selected sites only)
- Administration of the AUDIT questionnaire
- Diet and exercise 7-day diary issue (see Section 7.3.5)

Additional assessments at Week 16 only are:

- Diet and exercise 7-day diary review (see Section 7.3.5)

### 7.2.8 Unscheduled Visit

Subjects who require an unscheduled visit will have the following tests performed where possible:

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Concurrent medication recording (see Section 9)
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Clinical chemistry (see Section 7.3.6)

### 7.2.9 Week 24 (or early withdrawal from treatment)

All subjects will return to the clinic and the following procedures performed and documented

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Measurement of bodyweight and waist and hip circumferences (see Section 7.3.3)
- Concurrent medication recording (see Section 9)
- Study medication accountability (return of all unused medication) (see Section 8)
- Diet and exercise counseling and 7-day diary issue (see Section 7.3.5)
- Administration of the AUDIT questionnaire
- MRI scan (see Section 7.3.13)
- Urine pregnancy test for women of childbearing potential (see Section 7.3.10)
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)
  - Coagulation (see Section 7.3.6)
  - Fasting clinical chemistry (see Section 7.3.6)
  - Fasting lipid analysis, including HOMA-IR (see Section 7.3.6)
  - HBA1C
  - Pharmacokinetics (see Section 7.3.8)
  - PBMCs (see Section 7.3.9) – at selected sites only
  - Biomarker analysis including CK-18 fragments, LPS, adiponectin, GLP-1 and cytokines (see Section 7.3.9)
  - Serum metabolomics
- Collection of a stool sample for microbiome assessment (see Section 7.3.11) – selected sites only.

### 7.2.10 Week 28 (Post-treatment Follow-up)

Subjects will return to the clinic 4 weeks after completion of treatment at Week 24 or early withdrawal and the following assessments will be performed:

- A targeted physical examination (see Section 7.3.2)
- AE assessment (see Section 10.2)
- Vital signs (blood pressure, temperature, heart rate and respiratory rate) (see Section 7.3.3)
- Concurrent medication recording (see Section 9)
- Urine pregnancy test for women of childbearing potential (see Section 7.3.10)
- Collection of blood and serum samples for:
  - Hematology (see Section 7.3.6)

- Coagulation (see Section 7.3.6)
- Fasting clinical chemistry (see Section 7.3.6)
- Diet and exercise 7-day diary review (see Section 7.3.5)

## 7.3 Details of Study Assessments and Data Collection

### 7.3.1 Demographic Data, Medical History, Medication History and Concurrent Medication

Demographic data will include sex, ethnicity and date of birth. The medical history will include any diagnosed medical (including intermittent) conditions or surgical history. All NASH medication history, and concurrent medication, including non-prescription medication, will be collected and recorded in the CRF.

### 7.3.2 Physical Examination and Targeted Examination

A complete physical examination will include head, ears, eyes, nose, throat, heart, lungs, abdomen, lymph nodes, musculoskeletal and neurological assessment and skin. Genitourinary system will be examined only when clinically indicated.

A targeted physical examination will be performed throughout the study to determine physical findings of systems appropriate to assessing the clinical status of the subject relative to study treatment and their disease. In particular, subjects will be monitored throughout the study for gastrointestinal events. Other body systems should be examined as clinically indicated.

### 7.3.3 Vital Signs

Vital signs to be measured are:

- Body temperature (degrees Celsius [°C])
- Respiratory rate (breaths/min)
- Pulse rate (beats/min),
- Blood Pressure (millimeters mercury [mmHg])

BP and pulse are to be recorded after the subject has been resting semi-supine for at least 10 minutes. If there are abnormalities, at least two further repeat BP measurements will be performed to confirm results. Refer to the Study Reference Manual for more details of the methods to be used.

Body weight (kg, without shoes), waist circumference (centimeters, light clothing) and waist/hip ratio will be measured and recorded throughout the study.

Height (centimeters [cm], without shoes) will be measured and documented at the Screening visit.

### 7.3.4 Electrocardiograms

EKG recordings in this clinical trial will be performed at Screening in a standardized manner as outlined in the Study Reference Manual. Repeat measurements will be performed if there are any clinical abnormalities observed or artifacts are present. All EKG recordings will be reviewed by the Investigator or medically qualified nominee.

The EKG recordings will be performed once the subject has been resting semi-supine for at least 10 minutes and will be measured over approximately 3 minutes, in triplicate. The following parameters will be reported: QRS, QT, QTcB, RR and PR intervals and Heart Rate. Any clinically significant out of range or arrhythmia alarms will be printed out and retained in the subject's notes and reported as AEs.

### 7.3.5 Diet and Exercise Counseling and Assessment

Counseling of study participants regarding diet and exercise will be required during the study. Appendix E provides a standard set of questions to assist in holding an open discussion with goal setting and review at each monthly study visit.

Food and beverage consumption and physical activities will be collected through subject completion of a prospective 7-day diet and exercise diary card issued at Baseline, W12 and W24 visits (Appendix F). An AUDIT questionnaire (Appendix D) will be completed at these visits in order to confirm that alcohol consumption is low and does not change during the course of the study.

Written guidance on diary completion will be provided. Staff should ensure subjects understand how to complete the cards and understand the importance of recording the information. Food and drink consumed, including alcohol, and all exercise (including manual work) will be recorded in the diary each day for a week following the visit.

The completed diary cards will be returned and reviewed with the subject at the next scheduled study visit. Major changes in diet and exercise habits will be discussed and recorded in the CRF.

### 7.3.6 Safety Laboratory Assessments

Blood will be collected at Screening, Baseline, and scheduled clinic visits (except D3) and at the Withdrawal visit if applicable. These should be collected within the visit window period required for the visit date (see Section 7.4). Urine will be collected at Screening only.

Clinical chemistry will be performed at the Baseline visit and scheduled clinic visits (except D3) with the subject having fasted for at least 8 hours before sample collection. HOMA-IR score will be calculated when fasting glucose and insulin measurements have been reported, according to the HOMA-IR algorithm (Appendix C).

**Table 3: Safety Laboratory Parameters**

| Test | Parameters |
|---|---|
| **Hematology** (Screening, Baseline, D7, D14, W4 and 4 weekly thereafter) | Hemoglobin, hematocrit, red blood cell (RBC) and RBC morphology, white blood cell (WBC) and differential WBC count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and neutrophil count. |
| **Coagulation** (Screening, Baseline, D7, D14, W4 and 4 weekly thereafter) | Prothrombin time (PT), partial thromboplastin time (PTT), and International Normalized Ratio (INR). |
| **Clinical Chemistry** (Screening, then **fasting at** Baseline, D7, D14, W4 and 4 weekly thereafter) | Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatine phosphokinase, creatinine, total protein, albumin, serum amylase (fractionated if > upper limit of normal (ULN)), lipase, phosphate, GGT, AST, ALT, alkaline phosphatase, total bilirubin, calcium, uric acid, glucose and insulin.<br>CRP and C-peptide will be included at Baseline, W4, W12 and W24 only. |
| **Serum lipid panel** (Baseline, D7, D14, W4 and 4 weekly thereafter) | Total cholesterol, triglycerides, high and low density lipoproteins |
| **Blood glucose (average)** (Screening, W12 and W24) | HBA1c |
| **Urine** (Screening only) | Specific gravity, protein, glucose, blood, leukocytes, pH, ketones, bilirubin, urobilinogen, nitrites |

### 7.3.7 Screening & Serology Laboratory Parameters

The following laboratory assessments will be conducted at Screening to confirm the absence of excluded diagnoses:

**Table 4: Screening tests - serum**

| Test Parameters |
|---|
| Alfa-feto protein |
| HIV antibodies, hepatitis B surface antigen (HBsAg) and HCV-RNA<br>* Results will be acceptable for eligibility within range of 6 months prior to screening. |
| Pregnancy test (women of childbearing potential only) |

### 7.3.8 Blood Samples for Pharmacokinetics

Serum concentrations of IMM-124E will be quantified, where possible, at Baseline (prior to taking study medication), W4, W12 and W24. The data and actual times of blood sample collection will be recorded in the 24 hour format. At least 2 aliquots of 1mL of serum will be collected and stored at ≤ -70°C at each time-point, prior to analysis.

### 7.3.9 Biomarkers

Blood samples will be taken to prepare serum aliquots for measurement of biomarkers relevant to steatohepatitis. At least 3 aliquots of 1 mL of serum will be collected at Baseline, W4, W12 and W24 (see Table 5) and stored at ≤ -70°C for biomarker evaluation including serum cytokine concentrations and other markers of immunological response. C-peptide and C-reactive protein analysis will be included in addition to a standard clinical chemistry panel at Baseline, W4, W12 and W24.

In addition, PBMCs will be collected at Baseline, W12 and W24 from patients recruited at selected sites and for whom stool samples will also be collected for microbiome analysis. Details regarding the required method of collection, preparation and storage of PBMCs will be included in the Study Reference Manual.

**Table 5: Biomarkers for analysis**

| Test Parameters |
|---|
| CK-18 fragments, LPS fragments, adiponectin, GLP-1 and cytokines - (IL)-6, IL-1α, IL1β, IL-2, IL-3, IL-4 IL-10, IL-13, IL-12, IL-17α, IL-23, IFN-γ, TGF-β and TNFα<br>(CRP and C-peptide will be analyzed with clinical chemistry safety panel – see Table 3)<br><br>PBMCs –$T_{reg}$ population cell counts including CD4+, CD8+, CD25+,FoxP3+, NKT (CD3+ and CD56+), CD62+ and CD69+<br><br>Serum metabolomics |

Any unused biomarker samples will continue to be stored for up to 5 years for the future assessment of pharmacodynamic and/or markers of response in NASH, unless the study subject requests to have the sample(s) destroyed earlier.

### 7.3.10 Urine Pregnancy Test

Prior to administration of the first dose of study medication at Baseline and 4 weekly thereafter, women of childbearing potential will require confirmation of non-pregnancy by collection of a mid-stream urine specimen and testing with a standard commercially available urine pregnancy test kit. In case such test is not available or deemed unsatisfactory at specific site, a blood pregnancy test will be performed.

### 7.3.11 Fecal Samples for Microbiome Assessment (selected sites only)

Stool specimens should be collected in a clean, dry container at Baseline and on or before W4, W12 and W24. It should be stored prior to the visit according to directions until delivered to the clinic where the sample will be stored at -70°C or below. Please refer to the Study Reference Manual for details of the collection and storage of fecal samples.

### 7.3.12 Handling and Processing of Biological Specimens

Biological specimens collected during the trial may contain pathogens. All personnel involved in collecting and handling biological specimens should follow appropriate precautionary procedures for handling biohazardous materials as currently recommended by the national regulatory authority. The processing of all biological specimens will be in accordance with the site's standard practices.

Further details of the handling of biological samples can be found in the Study Reference Manual.

### 7.3.13 Disease Assessment

NAS score will be determined on the most recent biopsy not greater than 12 months before Screening. Liver biopsy slides will be made available for central confirmatory review by a pathologist expert in liver pathology.

Subjects will be staged using HOMA-IR which estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a reference population (see Appendix C)

Liver MRI will be performed according to a standard protocol at each center at Baseline and W24 and transmitted to a central radiological co-ordinating centre to be read by a radiologist blinded to clinical information and treatment allocation. Please refer to the Study Reference Manual for further details.

Measure of end-stage liver disease (MELD) score will be determined at intervals throughout the study according to the following formula:

3.78x ln[serum bilirubin (mg/dL)] + 11.2x ln[INR] + 9.57x ln[serum creatinine (mg/dL)] + 6.43

Refer to Study Reference Manual for further information.

## 7.4 Visit/Assessment Windows

Assessments should occur on the days and at the times specified in Section 7.3 and Table 1. The date of visits will be calculated from the date of the Baseline visit (D0). These should occur, wherever possible, on the exact day or number of weeks after the Baseline visit. The visit/assessment windows are specified in Table 6.

**Table 6: Visit/assessment windows**

| Assessments | Acceptable Window |
|---|---|
| Screening | -45 to Day 0 |
| Baseline | Day 0 |
| Up to and including Week 4 | ± 1 day |
| Week 8 to Week 24 | ± 3 days |
| Post-treatment Follow-up | ± 3 days |

# 8. STUDY DRUGS

## 8.1 Randomization

Subjects will be randomized to one of three treatment groups using a permuted block randomization algorithm supplied by a statistician blinded to patient data.

NASH without insulin resistance or diabetes may be clinically different to NASH with pre-diabetes or diabetes. Equal numbers of patients in each treatment arm is targeted. Randomization will be proportional (1:1:1) and stratified by Baseline HBA1C $\leq$ 6.0 or >6.0 – 9.0. (See Section 7.2.2 and the Study Reference Manual)

Additionally, to ensure baseline Hepatic Fat Fraction (HFF) would not be a confounding factor, subjects will be stratified for baseline HFF according to the following groups $\leq$10%, 10%<X$\leq$20%, 20%<X<30% and $\geq$30%.

If a subject discontinues from the study, the randomization code will not be reused, and the subject will not be allowed to re-enter the study.

## 8.2 Blinding

This is a double blind study. Therefore, subjects, investigational site staff and Sponsor clinical staff will remain blinded to treatment allocation.

## 8.3 Unblinding Procedures

In case of emergency and the need to know details of a subject's study treatment, an unblinding procedure to provide access to code-break information will be provided. Secure access to codebreak information will be provided to the Investigator at each study site. The Investigator will discuss with the Sponsor any unblinding prior to it taking place, unless immediate knowledge of the treatment allocated is required for the Subject's care.

Reason(s) for unblinding will be clearly documented and the details included in the study report.

## 8.4 Formulation

### 8.4.1 Investigational Products

Table 7 is a summary of the characteristics of the investigational products to be supplied in this study.

**Table 7: Investigational Products**

| **Name:** | **IMM-124E** |
|---|---|
| Characteristics & Physical State: | Off white *tablets* containing 600mg of IMM-124E |
| Formulated & Supplied by: | Immuron Ltd. |
| Storage Conditions: | Store at 2-8 degrees C |
| Package: | HDPE bottle |
| | |
| **Name:** | **Placebo to match active** |
| Characteristics & Physical State: | Off white *tablets* containing 600mg of milk powder (Pro-Milk 85®) |
| Supplied by: | Immuron Ltd. |
| Storage Conditions: | Store at 2-8 degrees C |
| Package: | HDPE bottle |

Table 8 a summary of the treatment regimens and tablet allocation for each of the three treatment cohorts.

**Table 8: Treatment regimen and tablet allocation**

| Treatment Arm | IMM-124E Dose (600mg tab) | Regimen | Tablet allocation per dose | |
|---|---|---|---|---|
| A | 600mg three times daily | 2 tablets (1 from each bottle) taken three times daily on an empty stomach | IMM-124E | Placebo |
| B | 1200mg three times daily | | IMM-124E | IMM-124E |
| C | matching placebo three times daily | | Placebo | Placebo |

### 8.4.2 Supply, Packaging and Labeling, Storage and Handling

IMM-124E must be stored in a secure area with access limited to the pharmacist and authorized staff. The bottles and outer packaging will be stored refrigerated between 2 and 8 degrees Celsius, prior to dispensing to patients, in a controlled and monitored facility.

At a minimum, the immediate packaging will include the following information:

- Sponsor name
- Protocol number
- Pack/treatment number
- Product name/drug code
- Number of tablets
- Directions for use, including route of administration
- Batch/lot number
- Storage conditions
- Period of use (use by, expiry or retest date, as applicable, in month/year format)

Additional information and specific cautionary statements will be included according to local law.

The packaging lot numbers will be recorded on the investigational product accountability record.

A Pharmacy reference manual containing detailed information regarding the storage, dispensing and accountability of IMM-124E will be provided as a separate document.

### 8.4.3 Dispensing and Accountability

The investigator will be responsible for ensuring accurate records are maintained for all study medications dispensed and returned. The inventory must be available for inspection by the study monitor. Study drug supplies, and the dispensing logs, must be accounted for by the study monitor and returned to the drug repository for destruction at the end of the study.

When requested in writing by the Sponsor, used and/or unused study medication supplies may be destroyed by the investigator provided such disposition can be performed safely. Records shall be maintained by the investigator of any such alternate disposition of the study medication. These records must show the identification and quantity of each unit disposed of, the method of destruction (taking into account the requirements of local law), and the person who disposed of the test substance. Such records shall be submitted to the sponsor.

For additional information, please refer to the Study Reference Manual for this study.

### 8.4.4 Dosage and Administration of Study Drugs

IMM-124E and matching placebo tablets may be either chewed before swallowing or swallowed whole three times daily per oral on an empty stomach. No intra-subject dose adjustment of IMM-124E will be allowed during this study.
Range of +/- 15% (85-115%) is the acceptable compliance range.
Any poor compliance out of this range will be considered as protocol deviation.
Any poor compliance should be escalated to Immuron and will potentially result in subject's termination from the study.

# 9. CONCURRENT MEDICATIONS AND TREATMENTS

At each study visit or contact, the Investigator should question the subject or their legal representative about any medication taken, including vitamin supplements and herbal remedies. Any concurrent medications will be recorded in the subject's records and the CRF. Any changes in doses or introduction of new medications during the course of the study will also be recorded.

## 9.1 Special Dietary Requirements

There are no special dietary requirements.

## 9.2 Concurrent Medications/Treatments Not Permitted

### 9.2.1 Prior to Study Entry

Refer to Exclusion Criteria in Section 6.3.

### 9.2.2 During the Study Dosing Period

Throughout the study medication dosing period, subjects may not receive any of the following concomitant medications:

- other anti-NASH therapy(s) including, SAM-e, betaine, milk thistle and probiotic supplements (not including yoghurt);
- thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or GLP-1 analogs, unless the subject was recruited on a stable dose.
- Allowable anti-diabetic treatment includes insulin, metformin and/or sulfonylureas administered at constant dose for at least 2 months prior to study entry;
- immune modulatory agents including
    - systemic steroids for more than 7 days
    - multiple non-steroidal anti-inflammatory drugs such as aspirin (>100mg/day), ibuprofen, naproxen, meloxicam, celecoxib;
    - azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, anti-TNFα therapies (infliximab, adalimumab, etanercept) or anti-integrin therapies (namixilab).

Treatment with antilipidemic agents (HMG Co-A reductase inhibitors – "statins"), vitamin E, gemfibrozil and allowable antidiabetic treatment (metformin and/or sulfonylurea) must be stable throughout the study.

Antibiotics should be taken if clinically indicated but avoided where possible.

# 10. ADVERSE EVENT REPORTING AND MANAGEMENT

## 10.1 Safety Parameters

Safety parameters will include adverse events, vital signs and clinical laboratory tests.

## 10.2 Adverse Events

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered an investigational product (whether it is the experimental product or the control) and which does not necessarily have a causal relationship with the investigational product. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Pre-existing events, which increase in frequency or severity or change in nature during or as a consequence of use of a drug in human clinical trials, will also be considered as AEs. AEs may also include pre- or post-treatment complications that occur as a result of protocol-mandated procedures (e.g. invasive procedures such as biopsies).

Any AE (i.e. a new event or an exacerbation of a pre-existing condition) with an onset date after study drug administration up to the last day on study (including the follow-up, off study medication period of the study), should be recorded on the appropriate CRF page(s).

An AE **does not** include:

- medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an adverse event
- pre-existing diseases or conditions present or detected prior to start of study drug administration, that do not worsen
- situations where an untoward medical occurrence has not occurred (e.g. hospitalization for elective surgery, social and/or convenience admissions)
- overdose of either study drug or concomitant medication without any signs or symptoms unless the subject is hospitalized for observation.

### 10.2.1 Assessment of AEs

All AEs will be assessed by an investigator and recorded in the subject medical record, including the date of onset and resolution, severity, relationship to study drug, outcome and action taken with study medication. See Appendix A for details of toxicity grade scales (TGS) relevant to vital signs, gastrointestinal and general system and clinical laboratory adverse events.

For all other reported adverse events, severity should be recorded and graded as:

**Table 9: Severity Grades**

| Grade | Severity | Comments |
|---|---|---|
| 1 | Mild | Aware of sign or symptom, but easily tolerated |
| 2 | Moderate | Discomfort enough to cause interference with usual activities |
| 3 | Severe | Incapacitating with inability to work or perform usual activities |
| 4 | Life-threatening | Participant is at immediate risk of death |
| 5 | Fatal | Any event causing death |

The relationship to study drug therapy should be assessed using the following definitions:

**Table 10: Adverse Event Causality Definitions**

| Causality | Comment |
|---|---|
| Unrelated | AE is clearly due to extraneous causes (e.g. underlying disease, environment, known effect of another drug) |
| Unlikely | The temporal association between the AE and study drug is such that study drug is not likely to have any reasonable association with the AE |
| Possible | The AE could have been produced by the subject's clinical state or study drug |
| Probable | The AE follows a reasonable temporal sequence from the time of study drug administration, abates upon discontinuation of the study drug and cannot be reasonably explained by the known characteristics of the subject's clinical state |
| Definite | The AE follows a reasonable temporal sequence from the time of study drug administration, abates upon discontinuation of the study drug and/or reappears when study drug is re-introduced |

These criteria, in addition to good clinical judgment, should be used as a guide for determining the causal assessment. If it is felt that the event is not related to study drug therapy, then an alternative explanation should be provided.

### 10.2.2 Adverse Event Reporting Period

All adverse events, regardless of severity, causality or seriousness must be reported from the date of informed consent until 28 days after the last dose of study medication. However, any adverse event that the investigator believes is at least possibly related to study medication should be reported regardless of time elapsed from the final dose.

## 10.3 Serious Adverse Events

### 10.3.1 Serious Adverse Event Definition

A serious adverse event (SAE) is defined as follows:

Any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death
- life-threatening situation (subject is at immediate risk of death)
- inpatient hospitalization or prolongation of existing hospitalization (excluding those for study therapy or placement of an indwelling catheter, unless associated with other serious events)
- persistent or significant disability/incapacity
- congenital anomaly/birth defect in the offspring of a subject who received study drug
- other: Important medical events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are:
    - intensive treatment in an emergency room or at home for allergic bronchospasm
    - blood dyscrasias or convulsions that do not result in hospitalization
    - development of drug dependency or drug abuse

### 10.3.2 Clarification of Serious Adverse Events

Death is an outcome of an AE, and not an AE in itself. In reports of death due to "Disease Progression", where no other information is provided, the death will be assumed to have resulted from progression of the disease being treated with the study drug.

All deaths, regardless of cause, must be reported to Immuron for subjects on study and within 28 days of last administration of study drug.

"Occurring at any dose" does not imply that the subject is receiving study drug at the time of the event. Dosing may have been given as treatment cycles or interrupted temporarily prior to the onset of the SAE, but may have contributed to the event.

"Life-threatening" means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death, if it had occurred with greater severity.

Complications that occur during hospitalizations are AEs. If a complication prolongs hospitalization, it is a SAE.

"In-patient hospitalization" means the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. It does not include presentation and care within an emergency department.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. When available, a diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

### 10.3.3 Serious Adverse Event Reporting Requirements

Immuron has requirements for expedited reporting of SAE's that meet specific criteria to worldwide regulatory authorities; therefore, all appropriate parties must be notified immediately of any SAE that occurs after the first dose of study drug has been administered. The procedures for reporting all SAEs, regardless of causal relationship, are as follows:

- Complete the "Serious Adverse Event Report" CRF page
- Send the SAE report to the Sponsor SAE hotline within 24 hours of the investigator's knowledge of the event:

  **Telephone:** +972 545 641464
  **Fax:** +972 15354 9277334
  **Email:** SAE@immuron.com

- For fatal or life-threatening events, also send copies of hospital case reports, autopsy reports, and other documents when requested and applicable

Immuron may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications necessary for treatment of the SAE must be recorded in the concomitant medication section of the subject's CRF.

### 10.3.4 Investigator Reporting Requirements for SAEs

A SAE may qualify for reporting to regulatory authorities if the SAE is considered to have a possible causal relationship to the study drug and is unexpected/unlisted based upon the current Investigator's Brochure. In this case, all investigators will receive a formal notification describing the SAE.

Where this is required by local regulatory authorities, and in accordance with the local institutional policy, the investigator should notify (in writing) the Institutional Review Board (IRB)/Ethics Committee (IEC) of SAEs according to local timelines.

## 10.4 Follow up of Serious and Non-serious Adverse Events

Follow-up of SAEs and non-serious AEs will continue through the last day on study (including follow-up) until the investigator and/or Immuron determine that the subject's condition is stable, whichever is longer. Immuron may request that all clinically significant AEs be followed until resolution or for a period of 30 days after.

## 10.5 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs or SAEs

All laboratory values must be reviewed by the investigator. Given that all laboratory data are collected and statistically analyzed with respect to relevant reference ranges, laboratory abnormalities that occur without related clinical symptoms and signs should generally not be recorded as adverse events unless they represent a clinically significant event. Where possible, the overall diagnosis rather than the laboratory abnormality should be recorded as the AE. This will avoid duplication of laboratory abnormalities in both the AE and laboratory reports.
Any laboratory test result that meets the criteria for a SAE (refer to Section 10.3.1) should be recorded as an AE, the AE page of the CRF completed and a SAE form also completed in order for the sponsor to collect additional information about that abnormality, including information regarding relationship to study product or other causes, any action taken and resolution.

## 10.6 Guidance for Discontinuation of Treatment

To date, no specific toxicities have been identified that result from IMM-124E treatment.
Any toxicities and/or abnormal laboratory findings should be investigated for etiology and graded according to Table 8 (Section 10.2.1) and Appendix A.

The following toxicity management will be followed:

**Grade 1 or 2:** Patients may continue study drug.

**Grade 3:** Patients with any Grade 3 toxicity considered to be at least possibly related to treatment (i.e. treatment related events) should be evaluated carefully by the investigator prior to continuing study drug. Investigators may discuss individual cases with the Medical Monitor.

**Grade 4:** Patients developing any Grade 4 toxicity should have treatment interrupted, unless the Grade 4 toxicity is clearly unrelated to study drug and continued treatment does not pose a serious risk to the patient. After the event has returned to normal or baseline levels, study drug may be re-started. If the toxicity re-appears, the investigator should contact the Medical Monitor to discuss the subject's withdrawal.

For any subject experiencing any event (irrespective of severity) which, in the opinion of the Investigator, contraindicates further dosing in that subject and the event is considered to be at least possibly related to treatment (or where causality to study treatment cannot be ruled out – see Section 10.2.1), continued dosing of the subject should be interrupted. In all cases, the final treatment decisions, made in response to toxicity, are the responsibility of the Principal Investigator. A careful evaluation of the potential risk/benefit will dictate the optimal therapeutic course. Should treatment be interrupted, re-initiation will follow review of the available safety data and in agreement with the Sponsor. There will be no dose reduction or modification.

If the investigator deems that management of the patient's medical condition requires knowledge of the study treatment regimen, then the Medical Monitor must be contacted for consultation and the procedures described in Section 8.2 followed. If a patient dies from an event that is considered to be at least possibly related to treatment, continued dosing of all study participants should be interrupted:

Clinically significant suspected adverse drug reactions, and serious adverse events considered to be related to study procedures will be followed until resolved or considered stable. All subjects who experience a study drug related AE should be followed until resolution of the AE, even if the subject has discontinued study drug.

If an unscheduled interruption of study drug occurs, the study site should notify the sponsor at the earliest possible time. A missed dose will not be made up. In the event that a subject requires an unscheduled interruption of study drug under conditions other than those associated with toxicity, the case will be reviewed by the Sponsor to determine whether such a subject will be allowed to resume study drug.

Subjects withdrawn from study drug will be treated as deemed appropriate by the investigator. Follow-up procedures should be performed and the appropriate CRFs should be completed.

## 10.7 Warnings and Precautions

The adverse event profile in humans is IMM-124E has not been fully characterized. In clinical trials and commercial use at doses equal or lower than those used in this study, IMM-124E was well tolerated with a safety profile expected of bovine colostrum. The most common adverse reactions expected include modified bowel movements and nausea.

For further information regarding warnings and precautions with the study drug, please refer to the Investigator's Brochure for Imm-124E.

## 10.8 Risks for Women of Childbearing Potential or During Pregnancy

The risks of treatment with IMM-124E during pregnancy have not been evaluated. Human IgG is known to cross the placental barrier. Therefore, IMM-124E has the potential to cause fetal harm when administered to pregnant women and pre-menopausal women of childbearing potential must follow a medically prescribed birth control regimen or agree to abstinence while participating in the study and for 30 days following the last dose of study drug.

## 10.9 Procedures to be Followed in the Event of Pregnancy

The subject must be instructed to inform the investigator IMMEDIATELY if she becomes pregnant during the study and seek advice regarding discontinuation of study medication. Whenever possible, treatment should be discontinued. The investigator should report all pregnancies to the Sponsor within 24 hours of becoming aware of the pregnancy. The outcome of pregnancies in a subject enrolled in the study must be reported.

In the event that the study drug is being used to treat an existing medical condition, prior to discontinuing therapy, the investigator should counsel the subject and discuss the risks of continuing study drug dosing and the possible effects on the fetus. Subjects should also be counseled regarding the potential for recurrence of their underlying disease if treatment is stopped and the availability of alternative treatment options.

Monitoring of the subject should continue until conclusion of the pregnancy. The outcome of the pregnancy should be reported to the Sponsor.

# 11. SUBJECT COMPLETION/WITHDRAWAL

## 11.1 Subject Completion

A subject will be deemed to have completed the study once all trial procedures have been conducted. Any AEs or SAEs still ongoing at the time of the Exit Evaluation will be followed in accordance with Section 10.4.

## 11.2 Criteria for Premature Withdrawal from Treatment or the Study

Subjects have the right to withdraw from treatment or the study at any time for any reason. The investigator must make every reasonable effort to keep each subject in the study except where termination or withdrawal is for reasons of safety. The investigator also has the right to withdraw subjects from treatment or the study in the event of intercurrent illness, AEs, pregnancy, treatment failure after a prescribed procedure, protocol violations, administrative reasons or other reasons.

The reasons for withdrawal of the subject must be recorded on the CRF.

The following are also considered justifiable reasons for subject withdrawal:

- The need to take medication which may interfere with study measurements
- Intolerable/unacceptable adverse experiences (see Section 10.7)
- Major violation or deviation of study protocol
- Non-compliance of subject with protocol
- Subject unwilling to proceed and/or consent is withdrawn
- Withdrawal from the study is, in the investigator's judgment, in the subject's best interest
- Pregnancy of female study subject at any time during the study period (if applicable)

It is understood by all concerned that an excessive rate of withdrawals from the study can render the study difficult to interpret. Therefore, unnecessary withdrawal of subjects from the study should be avoided.

Immuron may also decide to stop the study and withdraw all subjects for safety or other reasons.

## 11.3 Withdrawal of Subjects from Study drug

Section 10.6 provides guidance for dose discontinuation of Study drug in the event of AEs or abnormal laboratory values. If a subject permanently discontinues study drug, for example as a result of an AE, subjects should be encouraged to continue participation in the study in order to obtain ongoing measurement of study outcomes to minimize missing data. At a minimum, every attempt should be made to continue to follow the subject until resolution of the adverse event. All subjects who discontinue study medication dosing should be followed for at least 28 days after the last dose of Study drug in order to monitor subjects for possible post-treatment events which may occur after Study drug has been discontinued.

## 11.4 Withdrawal of Subjects from the Study

Should a subject decide to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible.

The investigator should contact the subject either by telephone or through a responsible relative to determine, if possible, the reason for withdrawal. A complete final evaluation at the time of the subject withdrawal should be made with an explanation of why the subject is withdrawing from the study.

If the reason for removal of a subject from the study is an AE or an abnormal laboratory test result, the principal reason will also be recorded on the CRF. Where possible, subjects should be followed until the AE is resolved or the abnormal laboratory test has returned to normal.

## 11.5 Replacement of Withdrawn Subjects

Any subjects who discontinue of their own volition or by a decision of the Investigator are defined as "withdrawals". Early withdrawals will be replaced to reach the predefined sample size of subjects completing the study.

# 12. STATISTICAL ANALYSIS

The study is a randomized, double-blind, placebo controlled, multicenter Phase 2 trial designed to describe the safety and preliminary efficacy of two IMM124-E dose groups as compared to placebo in a population of subjects diagnosed with NASH. The safety profile will be described by the incidence of treatment emergent adverse events (TEAE) as well as changes in vital signs, and laboratory measurements. Trends in efficacy will be examined primarily via reductions from baseline in liver fat content and serum ALT. Given the exploratory nature of the trial, adjustments for multiple endpoints and/or multiple comparisons will not be made.

The following section describes the general analytic approach to the analysis of the study. Details of the analysis will be provided in a separate Statistical Analysis Plan (SAP) which will be completed prior to any blind break.

## 12.1 Sample Size Determination

The power to detect a difference between placebo and active treatment with respect to changes from baseline in liver fat content and serum ALT has been determined for a sample size of 40 patients per arm. The sample size for the current study is based on a review of the literature to obtain estimates to inform sample size/power calculations. Estimates are based on published results of randomized placebo controlled treatment studies in populations of patients similar to those proposed for this study and in which one or both endpoints have been reported. Fat quantification by MRI, rather than other methods, have been included. (Belfort 2006; Promrat 2010; Ratziu 2008; Sanyal 2010 and Le 2012).

Further, since the endpoint is the *change from Baseline*, plausible estimates of the degree of correlation between pre and post-baseline measurements were also incorporated into the variance estimates. Given the wide range of results observed in the literature, several "what if" scenarios were examined. As such, the current sample size was considered sufficient for the exploratory objectives of the study for the given range of plausible treatment effects and variance estimates. There will be no adjustment for multiple comparisons between the 3 treatment groups.

Fat Liver Content Change from Baseline:
The current sample size of 40 subjects per group should yield approximately 85% power to detect a treatment effect of at least -5% with respect to the difference in the mean change from Baseline between one of the IMM-124E treatment groups and placebo assuming a standard deviation (SD) of 7.3% ($\alpha$=0.05; two-tailed; unpaired t-test). The SD of 7.3% for the change from Baseline endpoint was derived assuming 1) SDs of 6% for each timepoint, 2) equal variances in each treatment group and 3) a low to moderate correlation of 0.25 between pre- and post-Baseline measurements.

## 12.2 Analysis Populations

The populations for analysis will be as follows:

### 12.2.1 Full Analysis Set (FAS) for Primary Assessment of Efficacy

The FAS includes all subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received will be excluded. In the event of treatment allocation errors, subjects will be analyzed for efficacy according to the treatment to which they were randomized.

### 12.2.2 Safety Analysis Set

The Safety Analysis Set includes all randomized subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received may be excluded. In the event of treatment errors, subjects will be analyzed for safety according to the treatment received.

### 12.2.3 Per Protocol Analysis Set

The Per Protocol Analysis Set includes all subjects who complete 24 weeks of study treatment and have MRI and ALT measured at Baseline and at Week 24 and no major protocol violations, determined prior to break of the treatment blind.

### 12.2.4 Intention-To-Treat (ITT) Analysis Set

The ITT analysis set includes all patients randomized to treatment. An ITT analysis will only be implemented in the event that one or more patients are randomized but do not receive any treatment.

## 12.3 Subject Disposition, Baseline Demographics and Disease Status

Subject disposition will be tabulated by treatment arm and overall and will include the number of subjects randomized, received study drug, withdrawals etc. Subjects for whom treatment unblinding was required, including reason(s) will be included. In addition, demographic and baseline information, including Baseline NAS, diabetes status and concurrent medical conditions, will be summarized and tabulated by treatment group. The purpose of these summaries is to characterize the study population and to describe any baseline imbalances.

## 12.4 Statistical Analysis Plan

A detailed SAP in which all aspects of data analysis will be defined in detail and prepared prior to the blind break.

The SAP will include details of the following:

- Definitions of analysis populations
- Description of any data transformations and data derivations to be used together with rationale and references
- Details of the statistical methods to be conducted, together with methods used to estimate corresponding confidence intervals
- Details of methods for checking the appropriateness of the chosen statistical model
- Discussion of the use of covariates and subgroups
- Discussion of any multiplicity and adjustment procedures to be implemented.
- Methods of dealing with missing values and outliers together with details of any sensitivity analyses to be performed
- Specification of any subgroup or interaction analyses
- Example table and listing shells to indicate the proposed presentation of the data.

## 12.5 Efficacy Analyses

Primary determination of efficacy will be assessed by the measurement and comparison of the change in percentage liver fat by MRI at Week 24 for each treatment group. For each variable, summary tables will be produced by time point and by dose level. All data will be listed and outcomes by treatment, including pooled data for both active treatment groups, will be formally compared. The analysis will include determination of confidence intervals about the treatment group means.

### 12.5.1 Other Efficacy Analyses

Other efficacy analyses to investigate outcomes for secondary efficacy endpoints and including sensitivity analyses will be documented as part of the SAP.

Analysis of primary and secondary efficacy endpoints by diabetes status will be descriptive only.

### 12.5.2 Exploratory Analyses

Exploratory and/or data driven analyses will be identified as such and documented in the final Clinical Study Report.

### 12.5.3 Final Analysis

After the last study participant has completed Week 24 (and the subsequent 28 day follow up) the data will be analyzed and the primary clinical/statistical study report will be prepared.

## 12.6 Analysis of Safety

### 12.6.1 Incidence of Adverse events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized according to the number of subjects experiencing specific events, reported by system organ class, high level group term, high level term and preferred term. AEs will also be presented by severity and relationship to treatment. All data will be listed.

### 12.6.2 Clinical Laboratory Parameters

Routine samples are being collected for assessment of clinical chemistry and hematology. Summary tables at each time point and by toxicity grade will be presented by dose level. All data will be listed.

### 12.6.3 Vital Signs

Vital signs are being assessed frequently across the duration of the study. Summary tables will be produced for each sign (temperature, heart rate, respiratory rate, systolic and diastolic blood pressure) and by toxicity grade. All data will be listed.

## 12.7 Analysis of Pharmacokinetics

As serum concentrations of IMM-124E are not expected, concentrations of IMM-124E antibodies will be collected and simple concentrations of IMM-124E will be summarized by treatment visit. Actual dosing and sampling times will be captured. Serum concentrations of at least the lower limit of quantitation will be used in the analysis.

## 12.8 Interim Analysis

An un-blinded efficacy and safety interim analysis is scheduled
The interim analysis will be conducted on all primary and secondary outcome unblended data by a third party,
The interim analysis will be carried out when at least 22 eligible subjects complete the full 24 weeks of treatment and have verified baseline and 24 weeks MRI data.
The goals of the interim analysis are as follow:

1. Full efficacy analysis
2. Full safety analysis
3. Determining the best performing dose
4. To correct for sample size.

To account for multiple comparisons between the interim and final analyses, the critical alpha level for the interim analysis will be set at 0.0000147 while the critical alpha level for the final analysis will be set at 0.0499. These values are based on 25% of the study endpoints being available for the interim analysis and symmetric two-tailed tests being used within the O'Brien-Fleming spending function. The study will not be terminated for either success or failure. The purpose of this interim analysis will

be to verify, and if necessary re-calculate, the required study sample size and to potentially discontinue the worse performing dose arm.

The Interim-Analysis will be carried out by a third party so both sponsor and investigators would remain blinded to the allocation of subjects within the study groups.

# 13. GENERAL STUDY ADMINISTRATION

## 13.1 Ethical Aspects

### 13.1.1 Local Regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the protocol, the "Declaration of Helsinki and its amendments, and with the requirements of national drug and data protection laws of the countries in which the research is conducted.

The investigator will also ensure that the basic principles of "Good Clinical Practice" as outlined in 21 Code of Federal Regulations (CFR) 312, subpart D, and 21 CFR Parts 50 and 56 are adhered to, together with USA CFRs regarding Investigational New Drugs (INDs) and any local guidelines or regulations.

### 13.1.2 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from each subject participating in this study or legally acceptable representative after adequate explanation of the aims, methods, objectives and potential hazards of the study, prior to undertaking any study related procedures. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical study. The investigator must also explain to the subject that they are completely free to refuse to enter the study or to withdraw from it at any time for any reason.

A generic informed consent form template is provided in Appendix B for the investigator to prepare the informed consent document to be used at his or her site. The written informed consent document should be prepared in the language(s) of the potential subject population.

The investigator must utilize an IRB/IEC approved consent form for documenting written informed consent. The original signed informed consent form should be retained in accordance with institutional policy, and a copy of the signed consent form should be provided to the subject or legally acceptable representative.

### 13.1.3 Institutional Review Boards/Ethics Committees

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material must be submitted to the IRB/IEC for written approval. A copy of the written approval of the protocol and informed consent form must be received by Immuron before recruitment of subjects into the study and shipment of investigational product. Approval from the committee must be obtained before starting the study and should be documented in a letter to the investigator specifying the protocol number and version and the date on which the committee met and granted the approval.

Any modifications made to the protocol and/or informed consent form after receipt of IRB or IEC approval must also be submitted by the investigator to the committee in accordance with institutional procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, the Sponsor will assist the investigator in submitting the protocol to a central Ethics Review Committee.

### 13.1.4 Conditions for Modifying the Protocol

Protocol modifications which could potentially adversely affect the safety of participating subjects or which alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, duration of therapy, assessment variables, the number of subjects treated or subject selection

criteria, may be made only after consultation between an appropriate representative of the Sponsor and the investigator.

Protocol modifications (amendments) must be prepared by a representative of the Sponsor and initially reviewed and approved by the responsible medical monitor, scientist and (when applicable) the statistician.

All protocol modifications must be submitted to the IEC/IRB and responsible regulatory authority in accordance with local requirements. Approval must be awaited before significant changes can be implemented i.e. if the risk benefit ratio is affected and/or the modification represents a change in basic trial definitions such as objectives, design, sample size or outcome measures.

In the event of an emergency, the investigator may institute any medical procedures deemed appropriate. However, all such procedures must be promptly reported to Immuron, the Immuron Medical Monitor and the IEC/IRB.

Administrative changes of the protocol are defined as minor corrections and/or clarifications that have no effect on the way the study is to be conducted or on the safety of the subjects. These administrative changes will be agreed upon by Immuron and the investigator, and will be documented in a memorandum. The investigator will then notify the IEC/IRB of such administrative changes.

### 13.1.5 Conditions for Terminating the Study

The study may be prematurely terminated by the IEC/IRB or relevant regulatory authorities if the perception of the benefit/risk becomes unfavorable for continuation of the study.

Additionally, Immuron reserves the right to terminate the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative or commercial reasons.

Should this be necessary, the procedures will be arranged on an individual study basis after review and consultation by all parties. In terminating the study, Immuron and the investigator will ensure that adequate consideration is given to the protection of the subjects' interests. The Investigator should promptly inform the subjects and ensure appropriate therapy and follow-up, and inform the relevant regulatory authorities and the IRB/IEC. All delivered study materials must be collected and all CRFs completed to the extent possible.

## 13.2 Study Documentation, CRFs and Record Keeping

### 13.2.1 Investigator Files/Retention of Documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified.

These documents should be classified into two separate categories:

(1) Investigator Site File (ISF), and
(2) Subject clinical source documents.

The ISF will contain the protocol/amendments, CRFs, query forms, IRB/IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents and correspondence.

Subject clinical source documents include subject hospital/clinic records, physician's and nurse's notes, appointment books, original laboratory reports, EKG, EEG, X-ray, pathology and special assessment reports, and consultant letters. All clinical study documents must be retained by the

Investigator until at least 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region (i.e. USA, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. The investigator must notify Immuron prior to destroying any clinical study records.

Should the investigator wish to assign the study records to another party or move them to another location, Immuron must be notified in advance.

If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Immuron to store these in a sealed container(s) offsite so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the subject, appropriate copies should be made for storage offsite.

### 13.2.2 Background Data

The investigator shall supply the Sponsor, on request, with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that subject confidentiality is protected.

### 13.2.3 Audits and Inspections

An audit is a systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analyzed and accurately reported according to the protocol, Immuron' SOPs and those of their designees, GCP and the applicable regulatory requirement(s).

Authorized representatives of Immuron, its designee, a regulatory authority, or the IRB/IEC may visit the center to perform audits or inspections. The investigator should contact Immuron immediately if they are contacted by a regulatory agency about an inspection at their center. If an audit or inspection occurs, the PI and institution agree to allow the auditor/inspector direct access to all relevant documents and allocate their time and the time of their staff to the auditor/inspector to discuss findings and any relevant issues.

### 13.2.4 Case Report Forms

For each subject who provides consent, CRFs must be completed and be signed by the principal investigator or co-investigator. This also applies to records for those subjects who fail to complete the study (even during the screening period if a CRF was initiated). If a subject withdraws from the study, the reason must be recorded on the CRF. If a subject is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using a black ball-point pen, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The CRFs, as well as the protocol, are confidential. The CRFs remain the property of the sponsor at all times.

## 13.3 Monitoring the Study

In accordance with ICH GCP guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

It is understood that the responsible Sponsor monitor, as a Immuron representative, will contact and visit the investigator regularly and that he/she will be allowed, on request, to inspect the various records of the trial (CRFs and other pertinent data) provided that subject confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The PI agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

## 13.4 Confidentiality of Trial Documents and Subject Records

The investigator must assure the subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the Sponsor, subjects should not be identified by their names, but by the subject's initials and an identification code. The investigator should keep a subject identification log showing codes, names and addresses. Documents not for submission to Immuron (e.g. subject's written consent forms, identification log), should be maintained by the investigator in strict confidence.

All information concerning the study treatment and the Sponsor company and its operation, such as patent applications, formulae, manufacturing processes, basic scientific data and material not previously published are considered confidential and shall remain the sole property of the Sponsor. The investigator agrees to use this information only in accomplishing the study and will not use it for any other purposes without written consent from the Sponsor.

## 13.5 Publication of Data and Protection of Trade Secrets

In accord with standard editorial and ethical practice, Immuron will support publication of multicenter trials only in their entirety and not as individual site data.

The results of this study may be published or presented at scientific meetings. If this is envisaged, the investigator agrees to submit all manuscripts or abstracts to Immuron prior to submission. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

Any formal publication of the study in which input of Immuron personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Immuron personnel. Authorship will be determined by an mutual agreement prior to the start of the study. The number of subjects enrolled will be taken into consideration in the selection of authors. Additional authors will be agreed prior to the completion of the study. Investigators who do not enroll any subjects into the study will not be included in this list.

## 13.6 Anticipated Subject Accrual and Duration of the Study

It is anticipated that recruitment of subjects will take approximately 12 months form initiation of the first study site. The investigator should continually compare the actual and expected accrual rates, and make every effort to ensure that they are as closely matched as possible. If the investigator anticipates major problems with recruitment, or delay in the expected completion date, he/she should discuss this with Immuron as early as possible.

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World Gastroenterology Organisation Global Guidelines, Nonalcoholic Fatty Liver Disease, and Nonalcoholic Steatohepatitis, June 2012

## 15. APPENDICES

# APPENDIX A ADVERSE EVENT TOXICITY GRADING SCALES

The following toxicity grading scales (TGS) have been adapted from the *FDA Guidelines for Toxicity Grading Scales for healthy adult and adolescent volunteers involved in clinical studies of preventive vaccines*. The parameters described in the tables below provide further information on which to base assessment of the severity for AEs which may be reported in this study. These include vital signs, gastrointestinal and other systemic events and clinical laboratory tests. These guidelines should be reviewed in the context of the values measured at Screening and Baseline.

These TGS do not address all potential adverse events. All other adverse events reported during the study must be recorded, including an assessment of severity as described in Section 10.2.2 (Table 8) of this protocol.

## Clinical abnormalities

| **Vital Signs *** | **Mild (Grade 1)** | **Moderate(Grade 2)** | **Severe (Grade 3)** | **Potentially Life Threatening (Grade 4)** |
|---|---|---|---|---|
| Fever (°C) **<br>(°F) ** | 38.0 – 38.4<br>100.4 – 101.1 | 38.5 – 38.9<br>101.2 – 102.0 | 39.0 – 40<br>102.1 – 104 | > 40<br>> 104 |
| Tachycardia - beats per minute | 101 – 115 | 116 – 130 | > 130 | Emergency Room (ER) visit or hospitalization for arrhythmia |
| Bradycardia - beats per minute*** | 50 – 54 | 45 – 49 | < 45 | ER visit or hospitalization for arrhythmia |
| Hypertension (systolic) - mm Hg | 141 – 150 | 151 – 155 | > 155 | ER visit or hospitalization for malignant hypertension |
| Hypertension (diastolic) - mm Hg | 91 – 95 | 96 – 100 | > 100 | ER visit or hospitalization for malignant hypertension |
| Hypotension (systolic) – mm Hg | 85 – 89 | 80 – 84 | < 80 | ER visit or hospitalization for hypotensive shock |
| Respiratory Rate – breaths per minute | 17 – 20 | 21 – 25 | > 25 | Intubation |

- * Subject should be at rest for all vital sign measurements.
- **Oral temperature; no recent hot or cold beverages or smoking.
- *** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes

## APPENDIX A - Clinical abnormalities, continued

| Systemic General | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Nausea/vomiting | No interference with activity or 1 - 2 episodes/24 hours | Some interference with activity or > 2 episodes/24 hours | Prevents daily activity, requires outpatient IV hydration | ER visit or hospitalization for hypotensive shock |
| Diarrhea | 2 - 3 loose stools or < 400 gms/24 hours | 4 - 5 stools or 400 - 800 grams/24 hours | 6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration | ER visit or hospitalization |
| Headache | No interference with activity | Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity | Significant; any use of narcotic pain reliever or prevents daily activity | ER visit or hospitalization |
| Fatigue | No interference with activity | Some interference with activity | Significant; prevents daily activity | ER visit or hospitalization |
| Myalgia | No interference with activity | Some interference with activity | Significant; prevents daily activity | ER visit or hospitalization |

| Systemic Illness | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Illness or clinical adverse event (as defined according to applicable regulations) | No interference with activity | Some interference with activity not requiring medical intervention | Prevents daily activity and requires medical intervention | ER visit or hospitalization |

## APPENDIX A - Laboratory Abnormalities

| Serum | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4)** |
|---|---|---|---|---|
| Sodium – Hypernatremia mEq/L | 132 – 134 | 130 – 131 | 125 – 129 | < 125 |
| Sodium – Hypernatremia mEq/L | 144 – 145 | 146 – 147 | 148 – 150 | > 150 |
| Potassium – Hyperkalemia mEq/L | 5.1 – 5.2 | 5.3 – 5.4 | 5.5 – 5.6 | > 5.6 |
| Potassium – Hypokalemia mEq/L | 3.5 – 3.6 | 3.3 – 3.4 | 3.1 – 3.2 | < 3.1 |
| Glucose – Hypoglycemia mg/dL | 65 – 69 | 55 – 64 | 45 – 54 | < 45 |
| Glucose – Hyperglycemia<br>Fasting – mg/dL<br>Random – mg/dL | 100 – 110<br>110 – 125 | 111 – 125<br>126 – 200 | >125<br>>200 | Insulin requirements or hyperosmolar coma |
| Blood Urea Nitrogen<br>BUN mg/dL | 23-26 | 27 – 31 | > 31 | Requires dialysis |
| Creatinine – mg/dL | 1.5 – 1.7 | 1.8 – 2.0 | 2.1 – 2.5 | > 2.5 or requires dialysis |
| Calcium – hypocalcemia mg/dL | 8.0 – 8.4 | 7.5 – 7.9 | 7.0 – 7.4 | < 7.0 |
| Calcium – hypercalcemia mg/dL | 10.5 – 11.0 | 11.1 – 11.5 | 11.6 – 12.0 | > 12.0 |
| Magnesium – hypomagnesemia mg/dL | 1.3 – 1.5 | 1.1 – 1.2 | 0.9 – 1.0 | < 0.9 |
| Phosphorous – hypophosphatemia mg/dL | 2.3 – 2.5 | 2.0 – 2.2 | 1.6 – 1.9 | < 1.6 |
| CPK – mg/dL | 1.25 – 1.5 x ULN | 1.6 – 3.0 x ULN | 3.1 –10 x ULN | > 10 x ULN |
| Albumin – Hypoalbuminemia g/dL | 2.8 – 3.1 | 2.5 – 2.7 | < 2.5 | -- |

**APPENDIX A – Laboratory Abnormalities, continued**

| Serum | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4)** |
|---|---|---|---|---|
| Total Protein – Hypoproteinemia g/dL | 5.5 – 6.0 | 5.0 – 5.4 | < 5.0 | -- |
| Alkaline phosphate – increase by factor | 1.1 – 2.0 x ULN | 2.1 – 3.0 x ULN | 3.1 – 10 x ULN | > 10 x ULN |
| Liver Function Tests –ALT, AST increase by factor | 1.1 – 2.5 x ULN | 2.6 – 5.0 x ULN | 5.1 – 10 x ULN | > 10 x ULN |
| Bilirubin – when accompanied by any increase in Liver Function Test increase by factor | 1.1 – 1.25 x ULN | 1.26 – 1.5 x ULN | 1.51 – 1.75 x ULN | > 1.75 x ULN |
| Bilirubin – when Liver Function Test is normal; increase by factor | 1.1 – 1.5 x ULN | 1.6 – 2.0 x ULN | 2.0 – 3.0 x ULN | > 3.0 x ULN |
| Cholesterol | 201 – 210 | 211 – 225 | > 226 | --- |
| Pancreatic enzymes – amylase, lipase | 1.1 – 1.5 x ULN | 1.6 – 2.0 x ULN | 2.1 – 5.0 x ULN | > 5.0 x ULN |

# APPENDIX B MODEL PATIENT INFORMATION SHEET

| | |
|---|---|
| **Title** | A phase II, randomized, double-blind, placebo-controlled study of IMM-124E for patients with non-alcoholic steatohepatitis. |
| **Protocol Number** | IMM-124E-2001 |
| **Project Sponsor** | Immuron Limited |
| **Principal Investigator** | *[Insert name]* |
| **Associate Investigator(s)** | *[Insert name, if required or delete]* |
| **Location** | *[Insert site location]* |

## Part 1 What does my participation involve?

### 1 Introduction

You are invited to take part in this research project. This is because you have a condition called non-alcoholic steatohepatitis (NASH). The research project is a clinical trial, also known as a 'study', to test a new treatment for NASH called IMM-124E.

This Participant Information Sheet and Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent form.

By signing it you are telling us that you:

- understand what you have read;
- consent to take part in the research project;
- consent to have the tests and treatments that are described;
- consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

## 2 What is the purpose of this research?

The aim of this clinical trial is to test the safety and effectiveness of IMM-124E for treatment of NASH.

IMM-124E is made from the first milk of dairy cows that have been vaccinated against bacteria that can cause diarrhea and vomiting in humans. These types of bacteria are also thought to contribute to the symptoms of NASH. IMM-124E does not contain the bacteria. IMM-124E contains proteins from the cows milk that may protect against the effects of the bacteria.

Tablets with a lower dose of the active ingredient in IMM-124E is available without prescription in Australia and other countries for the prevention of traveler's diarrhea (for people 6 years and over). It is not approved for the treatment of NASH in any country. This means it must be tested to see if it is an effective treatment for NASH.

IMM-124E has only been investigated in one other 4 week study of people with NASH. Further research is needed to learn more about how IMM-124E works, what dose to give and its safety and effectiveness for treatment of this condition. This study will provide important information which may be reviewed in the future by regulatory authorities and used to plan further research.

This research has been funded by Immuron Limited.

## 3 What does participation in this research involve?

If you agree to take part, you will be asked to sign the consent form before any study assessments are performed. You will visit the clinic to see if you are eligible to take part. If you are eligible, there will be a phone call visit and 10 more visits to the clinic over approximately 32 weeks. Additional visits may be necessary if you experience any health problems or difficulties during the study. Your study doctor can advise you if these are needed.

Physical assessments and blood tests will be required at the screening visit to find out whether you are eligible to take part. These assessments will confirm that you don't have any other conditions which would exclude you and that you are well enough to participate. Assessments throughout the study will continue to monitor your health and provide valuable information about changes that occur while you are on treatment.

You will be participating in a randomized controlled research project. Sometimes we do not know which treatment is best for treating a condition. To find out, we need to compare different treatments. Groups of participants receive the treatment and information is collected. The results for each group are compared to see if one is better. To make sure the groups are the same, each participant is put into a group by chance (random). This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

If you are eligible for this study, you will be randomly put into a group to receive one of the 3 different treatments being studied: either IMM-124E at a dose of 600 milligrams (mg) or 1200mg or matching placebo tablets, three times a day. A placebo is a medication with no active ingredients. It looks like the active treatment, but it is not. In this study you have a 2 in 3 chance of receiving one of the active treatments. Neither you nor the study staff will know which treatment you are on, although they are able to find out if necessary.

You will be given the first dose of treatment in the clinic and enough tablets to take at home for 4 weeks treatment. You will be asked to take 2 tablets each morning, 2 in the middle of the day and 2 each night, preferably with a minimum of 1 hour fasting before and 1/2 hour after. At each 4 weekly clinic visit you will be given more tablets to continue taking at home, for a total of 24 weeks treatment.

The following is a detailed summary of how often the clinic visits occur, the procedures and tests which will be done and the time commitment involved in taking part in the study:

| Visit Day or Week (from Baseline visit) | Procedure | Approximate Visit Duration |
|---|---|---|
| Screening, up to 45 days before study drug dosing | • Answer your questions about the study and sign the consent form, if you have not already done so.<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, breathing rate, weight, height and waist and take an electronic trace of your heart rhythm (EKG).<br>• You will be asked questions about your medical history, your current health and any medicines you are taking, including herbal preparations.<br>• Blood samples will be collected using a needle and syringe (55mL or about 11 teaspoons of blood) and prepared for testing for:<br>o HIV, hepatitis B and hepatitis C virus exposure;<br>o alpha-feto protein (a marker of liver cancer).<br>o hematology and clinical chemistry (tests of general health and wellbeing also known as 'safety blood tests);<br>o HBA1C (test for average blood glucose levels);<br>o blood clotting time;<br>o pregnancy, if you are a woman able to have children (pregnancy can be also tested by urine).<br>• A urine sample for safety screening. | 2 hours |
| Day 0 visit (Baseline) | • Before the baseline visit you will be sent for a magnetic resonance (MR) scan of your abdomen to measure the fat levels in your liver.<br>• *(Selected sites only)* You will be asked to collect a sample of your feces in the 24 hours before you come to the clinic. You will be given instructions on how to collect and store it. This will be used to test the types of bacteria in your digestive system.<br>• You will be asked to fast for at least 8 hours before you come to the clinic. You will not be allowed to drink alcohol, coffee, tea and other drinks while fasting. However, you can drink as much water as you would like.<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, breathing rate, weight, waist and hips.<br>• You will be asked questions about your current health and any new medicines you have taken since the screening visit.<br>• Your completed 7-day diary will be collected and reviewed and you will be asked detailed questions about your diet and physical exercise. | 3 hours |

| Visit Day or Week (from Baseline visit) | Procedure | Approximate Visit Duration |
|---|---|---|
| | • If you are a woman able to have children, a urine sample will be collected and tested to confirm you have not become pregnant.<br><br>• Blood samples will be collected (up to 75mL or about 15 teaspoons of blood) and prepared for testing:<br>o hematology and clinical chemistry<br>o blood lipid (fat) levels,<br>o blood clotting time<br>o serum biomarkers of NASH 'activity' - CK-18 (fibrosis) and LPS (fragments from gut bacteria)<br>o levels of immune markers including cytokines (cell signaling molecules) and *(selected sites only)* white blood cells<br>o levels of IMM-124E<br>o metabolomics – blood testing for small molecules that can potentially identify disease state<br>• You will be allocated and dispensed study medication and will take the first dose in the clinic. | |
| Day 3 | • A telephone call will be made to you to find out how you are feeling and what medications you are taking. | Telephone call |
| Day 7<br>and<br>Day 14 visits | • You will be asked to fast for at least 8 hours before you come to the clinic (drink water only).<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, and breathing rate.<br>• You will be asked questions about your current health and medicines you have been taking.<br>• Blood samples will be collected (30mL or about 6 teaspoons of blood) and prepared for testing:<br>o hematology and clinical chemistry<br>o blood lipid (fat) levels,<br>o blood clotting time | 1 hour |
| Week 4, 8,12, 16 and 20 visits | • You will be asked to fast for at least 8 hours before you come to the clinic (drink water only).<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, breathing rate, weight, waist and hips.<br>• You will be asked questions about your current health and any new medicines you have taken since your last visit.<br>• You will be asked detailed questions about your diet and physical exercise.<br>• Blood samples will be collected (30mL or about 6 teaspoons of blood) and prepared for testing:<br>o hematology and clinical chemistry<br>o lipid (fat) levels, | 1 hour |

| Visit Day or Week (from Baseline visit) | Procedure | Approximate Visit Duration |
|---|---|---|
| | ○ clotting time<br>You will be allocated and dispensed enough study medication for 4 weeks treatment.<br>• If you are a woman able to have children, a urine sample will be collected and tested to confirm you have not become pregnant.<br><u>At Week 4 only:</u><br>Additional blood samples (20mL or about 4 teaspoons of blood) will also be collected and prepared for testing:<br>○ biomarkers of NASH 'activity'<br>○ levels of immune markers (cytokines)<br>○ levels of IMM-124E<br>• *(Selected sites only)* You will be asked to collect a sample of your feces in the 24 hours before you come to the clinic.<br><u>At Week 12 only:</u><br>Additional blood samples (up to 55mL or about 11 teaspoons of blood) will also be collected and prepared for testing:<br>○ biomarkers of NASH 'activity'<br>○ levels of immune markers including cytokines and *(selected sites only)* white blood cells<br>○ levels of IMM-124E<br>• You will be issued with a 7-day diet and exercise diary card to complete during the next week.<br>• *(Selected sites only)* You will be asked to collect a sample of your feces in the 24 hours before you come to the clinic. | +1 hour |
| Week 24 | • You will be asked to fast for at least 8 hours before you come to the clinic (drink water only).<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, breathing rate, weight, waist and hips.<br>• You will be asked questions about your current health and medicines you have been taking<br>• You will be asked detailed questions about your diet and physical exercise.<br>• You will be sent for a magnetic resonance (MR) scan of your abdomen to measure the fat levels in your liver.<br>• Blood samples will be collected (up to 75mL or about 15 teaspoons of blood) and prepared for testing:<br>○ hematology and clinical chemistry<br>○ lipid (fat) levels,<br>○ clotting time<br>○ biomarkers of NASH 'activity'<br>○ levels of immune markers including cytokines and *(selected sites only)* white blood cells<br>○ levels of IMM-124E<br>○ Metabolomics – blood testing for small molecules that can potentially identify disease state | 2 hours |

| Visit Day or Week (from Baseline visit) | Procedure | Approximate Visit Duration |
|---|---|---|
| | • If you are a woman able to have children, a urine sample will be collected and tested to confirm you have not become pregnant.<br>• You will be asked to return any unused medication to the clinic.<br>• *(Selected sites only)* You will be asked to collect a sample of your feces in the 24 hours before you come to the clinic. | |
| Week 28 or early withdrawal | • This final visit after completing the study treatment is to review how you are feeling and what medications you are taking.<br>• As previously, you will be asked to fast for at least 8 hours before you come to the clinic.<br>• The study doctor will examine you and measure your blood pressure, temperature, heart rate, breathing rate, weight and waist.<br>• Blood samples will be collected (15mL or about 3 teaspoons of blood) and prepared for testing:<br>  ○ hematology and clinical chemistry<br>  ○ clotting time<br>• You will be asked questions about your current health and medicines you have been taking<br>• You will be asked detailed questions about your diet and physical exercise. | 1 hour |

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge.

You may be reimbursed for any reasonable travel, parking, meals and other expenses associated with the research project visits.

If you decide to participate in this research project, the study doctor will inform your primary care doctor.

## 4 What do I have to do?

You will need to attend each of the clinic visits on the study visit days specified.

You may need to follow a diet and exercise program that has been recommended to help manage your NASH. It is important that you continue to follow your program throughout the study. You will be asked regularly to report what you have had to eat and drink and what type and how much exercise you have had.

It is important that you agree to take the study medication you are allocated three times each day according to the instructions you will be given. If you miss a dose, you should take the next dose as planned and let the study staff know at your next visit.

The study doctor will ask about medications you are taking at the time you are screened. You are also asked to tell the study doctor if you take any prescribed, over-the-counter, herbal or other medicines during the course of the study. This is to be sure that you are not taking anything that could interact with the study medication or affect the study results.

You are not allowed to take some types of medication for a period of time before the Screening visit and while you are on the study.

The medications you are not allowed are:

- SAM-e (also known as S-adenosyl methionine), betaine (trimethylglycine or TMG), milk thistle and probiotic supplements (but you are allowed to eat yoghurt or other natural food sources of probiotics) for more than 10 days in the 3 months before and during the study; you could be eligible if you haven't used such drugs for less than 3 months and if you have stopped taking them 6 weeks before the screening visit.
- corticosteroid tablets or injections (for example dexamethasone and cortisone) for more than 7 consecutive days in the 6 months before the Screening visit and during the study;
- daily treatment with more than one non-steroidal anti-inflammatory drugs, NSAIDs - for example aspirin (except if a very low dose for heart health), ibuprofen, Nurofen®); naproxen Naprosyn®, meloxicam, Mobic® and celecoxib , Celebrex®) for more than 1 month within 3 months before the Screening visit and during the study;
- azathioprine (Imuran®); 6-mercaptopurine (Purinethol®); methotrexate (Rheumatrex® and Trexall®), cyclosporine (Neoral, Cicloral, Sandimmun), anti-TNF alpha therapies (infliximab, adalimumab, etanercept) and anti-integrin therapies (namixilab) in the 12 months before the Screening visit and during the study.

You may not be eligible for this study if you have not had a liver biopsy in the last 12 months to confirm that you have NASH. You may also not be eligible if you have been diagnosed with certain other medical conditions. You will not be allowed to start treatment if your screening test results show you are not well enough to do so. If you are allergic to cow's milk or have lactose intolerance you will also not be able to take part, because the study drug is prepared from cow's milk and contains lactose.

## 5 Other relevant information about the research project

It is anticipated that at least 120 people with NASH will be included in this study at an estimated 30 clinical trial sites. These include sites located in the United States, Australia and other potential locations.

## 6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with this institution.

## 7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options may be available to you. There are currently no approved medicines specifically for treatment of NASH. Your study doctor will discuss options for your treatment with you before you decide whether or not to take part in this research project. You can also discuss options with your local doctor.

## 8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research. However, possible benefits may include reducing the amount of fat in your liver and improving measurements of your liver function.

Even if you do not benefit from the treatment you receive in this study, the information collected may help researchers to learn more about NASH and how to treat it. Other people may benefit from this knowledge in the future.

## 9 What are the possible risks and disadvantages of taking part?

Side effects

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

To date, possible side effects reported in clinical trials of IMM-124E have been few, mild to moderate and not permanent. There have been no severe or life threatening events. There has been no clear pattern to events observed. These events include:

- nausea
- vomiting
- flatulence (bloating or wind)
- mild diarrhea
- raised levels of some liver enzymes

There is a possibility that you will experience an allergic reaction. Allergic reactions may be mild, such as skin rashes or hives, or severe or life-threatening such as difficulty breathing or swallowing. If this occurs immediately it is called anaphylaxis.

For your safety, the first dose of study drug will be given when you are still at the clinic.

Pregnancy

The effects of IMM-124E on an unborn child and newborn baby are not known. Because of this, it is important that research project participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in the research if you are pregnant or trying to become pregnant, or breast-feeding. If you are female and child-bearing is a possibility, you will be required to undergo a pregnancy test prior to commencing the research project. If you are male, you should not father a child or donate sperm for at least 3 months after the last dose of study medication.

Both male and female participants are strongly advised to use effective contraception during the course of the research and for a period of 3 months after completion of the research project. You should discuss methods of effective contraception with your study doctor.

If you are a female participant and you do become pregnant whilst participating in the research project, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

If you are a male participant you should advise your study doctor if you father a child while participating in the research project. Your study doctor will advise on medical attention for your partner should this be necessary.

Blood Tests

Having blood samples taken may cause some discomfort or bruising. Sometimes the blood vessel may swell or blood may clot in the blood vessel or the place where the blood was taken from becomes inflamed. Rarely, some people have fainted. Infection or bleeding might occur.

When taking larger quantities of blood, people may become anemic (low red blood cell count); this will be monitored in the study.

Scans using Magnetic Resonance Imaging

MRI stands for magnetic resonance imaging. A MRI scanner is a machine that uses electromagnetic radiation (radio waves) in a strong magnetic field to take clear pictures of the inside of the body. Electromagnetic radiation is not the same as ionising radiation used, for example, in X-rays. The pictures taken by the machine are called MRI scans.
We will ask you to lie on a table inside the MRI scanner. The scanner will record information about your liver. It is very important that you keep very still during the scanning. When you lie on the table, we will make sure you are in a comfortable position so that you can keep still. The scanner is very noisy and we can give you some earphones to reduce the noise. Some people may experience symptoms of claustrophobia from lying in a confined space. If you do experience discomfort at any time during the scan, you will be able to alert staff by pressing on a call button provided to you.

There are no proven long-term risks related to MRI scans as used in this research project. MRI is considered to be safe when performed at a center with appropriate procedures. However, the magnetic attraction for some metal objects can pose a safety risk, so it is important that metal objects are not taken into the scanner room.

We will thoroughly examine you to make sure there is no reason you should not have the scan. You must tell us if you have metal implanted in your body, such as a pacemaker or metal pins.

The scans we are taking are for research purposes. They are not intended to be used like scans taken for a full clinical examination. The scans will not be used to help diagnose, treat or manage a particular condition. A specialist will look at your MRI scans for features relevant to the research project. On rare occasions, the specialist may find an unusual feature that could

have a significant risk to your health. If this happens, we will contact you to talk about the findings. We cannot guarantee that we will find any/all unusual features.

## 10 What will happen to my test samples?

By consenting to take part in this study, you also consent to the collection, storage and use of your blood samples and urine samples. During the course of the study blood samples will be taken on 11 occasions. The total volume of blood taken for the entire study will be about 600mL (about 1.4 cups). In comparison, one standard blood donation is 470mL.

Collected blood samples will be used to check your general health at screening and throughout the study. The proposed blood tests also include a screening test for HIV (also called the 'AIDS' virus) and hepatitis. This is because the study doctors need to know whether you have any other condition that affects your liver. You will receive information and counselling before the test. If a test shows you have HIV or Hepatitis, you will have follow-up counselling and medical advice. If your test results are positive, the study doctors are required by law to notify government health authorities. Signing the consent form means that you agree to have this testing; it will not be done without your consent.

Your blood will also be tested to ensure that clotting time is normal and, if you are a woman able to have children, to check you are not pregnant. Urine will be collected to confirm the health of your kidneys and, if you are a woman able to become pregnant, to do a final check that you are not pregnant before starting treatment with the study medication and at the end of the study.

Serum will be prepared from some of the blood you give (serum is the clear liquid that carries red and white blood cells). It will be tested for the presence of the study medication, for a bi-product of gut bacteria (lipopolysaccharide) and for fats, glucose and insulin. Some of the serum will also be stored to test for the levels of biomarkers which are associated with NASH. White blood cells which relate to infection and immunity will be also be collected for analysis. Some of these samples may be stored for testing at a later stage. All other urine and blood samples will be destroyed after they have been tested.

Your blood samples will be labelled with your unique study number (but not your name).

Samples of your blood or tissue obtained for the purpose of this research project may be transferred to Immuron Limited (the Sponsor) or an organisation working on its behalf. Your tissue will not be sold by *[Name of institution]*, however *[Name of institution]* may charge study doctors a fee to recover some of the costs of storing and administering the samples.

Once your blood or tissue samples are transferred to Immuron, *[Name of institution]* will not be able to control whether Immuron transfers or sells your samples at some future date, however *[Name of institution]* will not knowingly transfer your samples to anyone who has expressed intent to sell the samples.

## 11 What if new information arises during this research project?

Sometimes during the course of a research project, important new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.
Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

### 12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain which treatments or medications need to be stopped for the time you are involved in the research project.

### 13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the Sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

### 14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:
• Unacceptable side effects being reported
• The drug being shown not to be effective
• The drug being shown to work and not needing further testing
• The decision is made in the commercial interests of the sponsor or by local regulatory/health authorities.

### 15 What happens when the research project ends?

Once you have completed 24 weeks of treatment you will return to the clinic 4 weeks later for a final check-up. Your doctor will discuss with you options available for the continued treatment of your NASH.

Once all the participants have completed treatment in the study, the treatment blind will be broken. This means that the treatment each participant was taking and the results of the testing done can be analysed together. The results of the analysis will be published. Your doctor may/will provide you with a lay summary of the overall results of the study.

## Part 2 How is the research project being conducted?

### 16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project may be inspected by relevant regulatory authorities, including the US Food and Drug Administration (FDA) and the Australian Therapeutics Goods Administration (TGA), authorised representatives of the Sponsor, Immuron, the institution relevant to this Participant Information Sheet, *[Name of institution]*, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

Information about your participation in this research project may be recorded in your health records.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Your individual data will only ever be linked with your unique study identification number, not your name. In most cases, your data will be combined with others who received the same study medication and compared with the results for the other treatment groups.

In accordance with relevant Australian *and/or [Name of state/territory]* privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

A description of this clinical trial will be available on http://www.ClinicalTrials. gov, as required by U.S. Law and on the Australian clinical trials registry website http://www.anzctr.gov.au. These websites will not include information that can identify you. At most, the Web site will include a summary of the results. You can search these websites at any time.

## 17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

There are two avenues that may be available to you for seeking compensation if you suffer an injury as a result of your participation in this research project:

- The Sponsor of this research project, Immuron Limited, has agreed to comply with guidelines for compensation in for injury resulting from participation in a Company-sponsored clinical trial as applicable in *[Country].* Details of the process and conditions are set out in the *[Reference document]*. In accordance with these Guidelines, the sponsor will determine whether to pay compensation to you, and, if so, how much. The research staff will give you a copy of the Guidelines together with this Participant Information and Consent Form. If you have any

questions about the Guidelines, please ask to speak to *[Name of designated person, associated with the research trial or in the organization, capable of explaining the Guidelines]*.

• You may be able to seek compensation through the courts.

## 18 Who is organizing and funding the research?

This research project is being conducted, sponsored and funded by Immuron Limited.

You will not benefit financially from your involvement in this research project even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to Immuron Limited.

In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to Immuron Limited the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

*[Name of institution]* will receive a payment from Immuron for undertaking this research project.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages). *Add any declarations of interest of study doctors, sponsors and institutions*

## 19 Who has reviewed the research project?

All research involving humans is reviewed by an independent group of people called an Independent Ethics Committee (IEC), also known as a *[Institutional Review Board (IRB) or* Human Research Ethics Committee (HREC)]. The ethical aspects of this research project have been approved by *[Name of IEC]*.

This project will be carried out according to the ICH Harmonised Tripartite Guideline For Good Clinical Practice (E6) and *[ list other relevant documents e.g.Australian National Statement on Ethical Conduct in Human Research (2007)].* This documents have been developed to protect the interests of people who agree to participate in human research studies.

Approval has also been granted by *[Name of institution]*.

## 20 Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on *[phone number]* or any of the following people:

**Clinical contact person**

| | |
|---|---|
| Name | *[Name]* |
| Position | *[Position]* |
| Telephone | *[Phone number]* |
| Email | *[Email address]* |

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

**Complaints contact person**

| | |
|---|---|
| Name | *[Name]* |
| Position | *[Position]* |

| | |
|---|---|
| Telephone | *[Phone number]* |
| Email | *[Email address]* |

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

**Reviewing HREC/IRB approving this research and HREC/IRB Executive Officer details**

| | |
|---|---|
| Reviewing HREC name | *[Name]* |
| Executive Officer | *[Name]* |
| Telephone | *[Executive Officer Phone number]* |
| Email | *[Executive Officer Email address]* |

**Local HREC/IRB Office contact (Single Site -Research Governance Officer)**

| | |
|---|---|
| Name | *[Name]* |
| Position | *[Position]* |
| Telephone | *[Phone number]* |
| Email | *[Email address]* |

# APPENDIX C HOMEOSTATIC MODEL ASSESSMENT OF INSULIN RESISTANCE SCORE

The following formula is to be used to calculate the HOMA-IR score based on laboratory reported measurements of serum fasting glucose and insulin as required:

$$\text{HOMA-IR} = \frac{\text{glucose (mmol/L*) x insulin (mU/mL)}}{22.5}$$

* To convert mg/dL to mmol/L, divide by 18

## APPENDIX D ALCOHOL USE DISORDERS IDENTIFICATION TEST (AUDIT) QUESTIONNAIRE

Please circle the answer that is correct for you:

1. How often do you have a drink containing alcohol?
   - · Never
   - · Monthly or less
   - · 2-4 times a month
   - · 2-3 times a week
   - · 4 or more times a week

2. How many standard drinks containing alcohol do you have on a typical day when drinking?
   - · 1 or 2
   - · 3 or 4
   - · 5 or 6
   - · 7 to 9
   - · 10 or more

3. How often do you have six or more drinks on one occasion?
   - · Never
   - · Less than monthly
   - · Monthly
   - · Weekly
   - · Daily or almost daily

4. During the past year, how often have you found that you were not able to stop drinking once you had started?
   - · Never
   - · Less than monthly
   - · Monthly
   - · Weekly
   - · Daily or almost daily

5. During the past year, how often have you failed to do what was normally expected of you because of drinking?
   - · Never
   - · Less than monthly
   - · Monthly
   - · Weekly
   - · Daily or almost daily

6. During the past year, how often have you needed a drink in the morning to get yourself going after a heavy drinking session?
   - · Never
   - · Less than monthly
   - · Monthly
   - · Weekly
   - · Daily or almost daily

7. During the past year, how often have you had a feeling of guilt or remorse after drinking?
- · Never
- · Less than monthly
- · Monthly
- · Weekly
- · Daily or almost daily

8. During the past year, have you been unable to remember what happened the night before because you had been drinking?
- · Never
- · Less than monthly
- · Monthly
- · Weekly
- · Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?
- · No
- · Yes, but not in the past year
- · Yes, during the past year

10. Has a relative or friend, doctor or other health worker been concerned about your drinking or suggested you cut down?
- · No
- · Yes, but not in the past year
- · Yes, during the past year

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**Scoring the audit**
Scores for each question range from 0 to 4, with the first response for each question (eg never) scoring 0, the second (eg less than monthly) scoring 1, the third (eg monthly) scoring 2, the fourth (eg weekly) scoring 3, and the last response (eg. daily or almost daily) scoring 4. For questions 9 and 10, which only have three responses, the scoring is 0, 2 and 4 (from left to right).

A score of 8 or more is associated with harmful or hazardous drinking, a score of 13 or more in women, and 15 or more in men, is likely to indicate alcohol dependence.

[1]Saunders JB, Aasland OG, Babor TF *et al*. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption II. Addiction 1993, 88: 791–803.

As the alcohol content of a "standard drink or unit" varies by country, responses will be converted to grams of alcohol. The calculated quantity of alcohol will be recorded in the subject's CRF.

Examples of standard alcohol units are as follows:

US – 1 standard drink is equivalent to 14g of alcohol
Australia: - 1 standard drink is equivalent to 10g of alcohol
UK – 1 standard drink is equivalent to 7.9g (10ml) of alcohol

Country specific "standard drink/ unit" charts will be provided to participating clinical trial sites to assist patients and site staff in estimating alcohol consumption.

# APPENDIX E STANDARDIZED DIET AND EXERCISE COUNSELING

At each visit, it is recommended that patients be engaged in a discussion about diet and exercise in the management of their NASH and relevant factors associated with the metabolic syndrome. It is suggested that the discussion follow a 'counseling style' which includes clear and easy-to-manage recommendations which align with a patient's preferences (Bellantini *et al* 2008).

The following is a simple five step procedure which may assist in conducting the discussion*:

1. Open the discussion "What you eat and how much you exercise is very important for your health and for the management of your NASH (diabetes, high cholesterol, etc)."
   a) Visit 1 invitation – "I recommend that we review your current habits and try to make some improvements."
   b) Subsequent visit invitation – "Let's review progress with your plan."

2. Using open questions, assess the patient's motivation, past diet experience and current diet.

3. Focus on problem areas: "Based on your current diet, I recommend that we focus on making some changes to your to e.g. high fat intake, excess calories, inadequate intake of fruits and vegetables."

4. Discuss and negotiate a plan, including two or three simple and specific dietary and physical activity goals, addressing possible barriers and ways to handle them.

Determine whether the patient needs additional information or help; refer to a dietician as needed.

5. Follow-up at each return visit to assess progress (see step 1b).

*Adapted from recommendations presented at the "Innovative Teaching Strategies for Training Physicians in Clinical Nutrition: The Nutrition Academic Award (NAA) Medical Schools" symposium given at the 2002 Experimental Biology meeting on April 20,2002, New Orleans, LA. The symposium was sponsored by The American Society for Nutritional Sciences. The proceedings are published as a supplement to The Journal of Nutrition.

# APPENDIX F DIET AND EXERCISE DIARY CARD

7-Day food Diary

| Name: | Date: |
|---|---|

| Day | Breakfast | Mid-Morning | Lunch | Mid-Afternoon | Dinner | Exercise | Alcohol |
|---|---|---|---|---|---|---|---|
| Monday | | | | | | | |
| Tuesday | | | | | | | |
| Wednesday | | | | | | | |
| Thursday | | | | | | | |
| Friday | | | | | | | |
| Saturday | | | | | | | |
| Sunday | | | | | | | |

www.checkmydiet.co.uk

# APPENDIX G PROTOCOL AMENDMENT #3 – SUMMARY OF CHANGES

RATIONALE FOR PROTOCOL AMENDMENT No. # 3

This protocol amendment has been generated to achieve the following goals:

1. to improve recruitment by eliminating the ALT eligibility requirement and using ALT as a secondary endpoint instead of a primary endpoint
2. to allow for a planned interim analysis
3. to allow for MRI-based stratification
4. extend screening period from 28 days to 45 days to allow for better operational streamlining
5. to better clarify existing wording and phrasing issues within the protocol
6. correct typo and add administrative changes

**The following sections of the protocol have been amended to reflect changes in study procedure.**

1. Administrative Protocol Amendments

| Section | Original Text | Revised to Read | Rationale for Change |
|---|---|---|---|
| Cover page and footer | Version 1.2 (Incorporating Amendment 1 and 2) 24 June 2015 | Version 1.3 (Incorporating Amendment 3) 08 May 2016 | Version number updated to indicate protocol amendment throughout document |
| Cover page | Level 1, 18 Kavanagh street | Unit 10, 25 – 37 Chapman Street, Blackburn North VIC 3130 | Change to the Immuron office address |
| Table of contents | Changes in page numbering | Changes in page numbering | Changes to reflect the actual contents and applicable page numbering |

2. Formal Protocol Amendments

| Section | Original Text | Revised to Read | Rationale for Change |
|---|---|---|---|
| Study synopsis and main body of the protocol | To evaluate the safety and preliminary efficacy of two dose levels of IMM-124E in reducing liver fat and/or serum ALT compared with placebo in subjects with biopsy proven NASH. | To evaluate the safety and preliminary efficacy of two dose levels of IMM-124E in reducing liver fat evaluated by MRI compared with placebo. | To achieve a better study design with a single primary endpoint and to improve recruitment due to the ALT requirement, ALT was removed from the primary endpoints |
| Study synopsis and main body of the protocol | Efficacy:<br>Mean change from Baseline in the percentage fat content of the liver measured by MRI at Week 24;<br>Mean change from baseline in serum ALT at week 24. | Efficacy:<br>Mean change from Baseline in the percentage fat content of the liver measured by Magnetic Resonance Imaging (MRI) at Week 24. | To achieve a better study design with a single primary endpoint and to improve recruitment due to the ALT requirement, ALT was removed from the primary endpoints |
| Study synopsis and main body of the protocol | • Mean serum concentrations of: LPS, CRP, CK-18 fragments, C-peptide and adiponectin and subsets of cytokines IL6, IL12, IFNγ and TNFα;<br>Relative levels of regulatory T cells in PBMC samples, including CD4, CD8, CD25, FoxP3, NKT and CD62;<br>Characterization of the gut microbiome. | • Mean serum concentrations of: lipopolysaccharide (LPS), C-reactive protein (CRP), cytokeratin (CK)-18 fragments, C-peptide , glucogon-like peptide (GLP)-1 and adiponectin and subsets of inflammatory cytokines: interleukin (IL)-6, IL-1α, IL1β, IL-2, IL-4 IL-10, IL-13, IL-12, IL-17, IL-23 interferon gamma (IFNγ), Transforming Growth Factor Beta )TGF-β and tumor necrosis factor alpha (TNFα);<br>• Metabolomics;<br>• Relative levels of regulatory T cells in peripheral blood mononuclear cells (PBMC) samples, including CD4, CD8, CD25, FoxP3, NKT, CD62 and CD69 T cells;<br>• Characterization of the gut microbiome. | To get a better perspective on the immunological effect additional interleukins and other immunological markers had been added to the panel as per the existing literature. |

| | | | |
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| | | • Explore a dose effect. | |
| Study synopsis and main body of the protocol | Up to 28 days for Screening and 24 weeks of treatment. | Up to 45 days for Screening and 24 weeks of treatment. | Change to study duration participant |
| Study synopsis and main body of the protocol | Approximately 120, randomized 1:1:1<br><br>Treatment allocation will be stratified by status defined as being normal (HBA1C $\leq$6.0) or diabetic (having a diagnosis of Type II diabetes and/or HBA1C >6.0 and $\leq$ 9.0 whether or not receiving allowable medication). | at least 120, randomized 1:1:1<br><br>Treatment allocation will be stratified by the following:<br>• Baseline MRI Liver Fat, according to the following scale: $\leq$10%, 10%<X$\leq$20%, 20%<X<30% and $\geq$30%<br>• Diabetic status defined as being normal (HBA1C $\leq$6.0) or diabetic (having a diagnosis of Type II diabetes and/or HBA1C >6.0 and $\leq$ 9.0 whether or not receiving allowable medication). | To avoid bias created by baseline MRI, stratification was added at baseline. |
| Study synopsis and main body of the protocol | IMM-124E tablet | IMM-124E 600mg tablet | Study Drug – dose specification |
| Exclusion criteria 6 (in study synopsis and main body of the protocol) | History of bariatric surgery | History of major bariatric surgery (not including balloon / sleeve gastrectomy); | To better define the excluded population. |
| Exclusion criteria 13 (in study synopsis and main body of the protocol) | Concurrent conditions<br>• Inflammatory bowel disease;<br>• Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of Screening;<br>• Ongoing infectious, ongoing multi-systemic immune-mediated and/or concurrent or past malignant disease;<br>• Any other concurrent condition which, in the opinion of the investigator, could impact adversely on the subject | Concurrent conditions<br>• Inflammatory bowel disease;<br>• Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of Screening;<br>• Ongoing infectious disease<br>• ongoing multi-systemic immune-mediated disease<br>• concurrent or past malignant disease;<br>• Any other concurrent condition which, in the opinion of the investigator, could | The changes made here are to better clarify the eligibility criteria as per questions coming from clinical sites |

| | | | |
|---|---|---|---|
| | participating or on the interpretation of the study data; | impact adversely on the subject participating or on the interpretation of the study data; | |

| | | | |
|---|---|---|---|
| Exclusion criteria 14 (in study synopsis and main body of the protocol) | Concurrent medications including:<br>o anti-NASH therapy(s) taken for more than 10 continuous days in the last 3 months.<br>These include S-adenosyl methionine (SAM-e), betaine, milk thistle, bacterial<br>probiotic supplements (other than yoghurt or other natural food sources), vitamin E<br>and gemfibrozil.<br>• NB: If vitamin E or gemfibrozil are used, the dose must be stable and liver<br>biopsy confirming diagnosis of NASH subsequent to commencing treatment;<br>• Wash out for any of the anti-NASH therapies is as follow: under 10 days no<br>washout required, more than 10 days and up to 3 months treatment requires 6<br>weeks washout. Any treatment of over 3 months would require to re-biopsy to<br>ensure histological eligibility<br>o thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or<br>glucagon-like peptide-1 analogs in the last 6 months. If treatment commenced and is<br>stable for more than 6 month prior to the determinant biopsy and the dose is still<br>stable at time of study entry, subjects will be eligible for recruitment.<br>o Allowable anti-diabetic treatment includes metformin and/or sulfonylureas<br>administered at constant dose for at least 2 months prior to study entry; | Concurrent medications including:<br>o anti-NASH therapy(s) taken for more than 10 continuous days in the last 3 months. These include S-adenosyl methionine (SAM-e), betaine, milk thistle, probiotic supplements (other than yoghurt), vitamin E and gemfibrozil.<br>▪ NOTE: if determinant biopsy is performed while on stable treatment – subject is eligible;<br>▪ Wash out for any of the anti-NASH therapies is as follows: under 10 days no washout required, more than 10 days and up to 3 months treatment requires 6 weeks washout. Any treatment of over 3 months would require to re-biopsy to ensure histological eligibility<br>o thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or glucagon-like peptide-1 analogs in the last 6 months. NOTE: if determinant biopsy is performed while on stable treatment of at least 6 months – subject is eligible.<br>o NOTE: Allowable anti-diabetic treatment includes metformin and/or sulfonylureas administered at constant dose for at least 2 months prior to study entry.<br>o NOTE: Subjects treated with Insulin are eligible if clinically stable on insulin treatment (i.e. no recurrent acute hypo-/hyperglycemic episodes diagnosed clinically and by Glucose serum levels of <50 mg/dL and >200 mg/dL respectively) for at least 2 months prior to study entry. | Clarifying Exclusion criteria 14 |

| | | | |
|---|---|---|---|
| | o Subjects treated with Insulin are eligible if clinically stable on insulin treatment (i.e.<br>no recurrent acute hypo-/hyperglycemic episodes diagnosed clinically and by Glucose<br>serum levels of <50 mg/dL and >200 mg/dL respectively) for at least 2 months prior<br>to study entry.<br>o immune modulatory agents including:<br>In the last 3 months<br>• systemic steroids for more than 7 days<br>• daily treatment with multiple non-steroidal anti-inflammatory drugs<br>(such as aspirin >100mg/day, ibuprofen, naproxen, meloxicam,<br>celecoxib) for more than 1 month within 3 months prior to study<br>entry;<br>In the last 12 months<br>• azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, anti-<br>TNF $\alpha$ therapies (infliximab, adalimumab, etanercept) or anti-integrin<br>therapies (namixilab) ;<br>o more than 10 consecutive days oral or parenteral antibiotics within 4 weeks prior to<br>study entry (Note: such subjects would not be included in the stool and PBMC<br>analysis);<br>o variable dose of anti-lipidemic agents (HMG Co-A reductase inhibitors –a"statins") in<br>the 3 months prior to Screening. | ○ immune modulatory agents including<br>▪ within 3 months of study entry;<br>• systemic steroids for more than 7 days<br>• daily treatment with multiple non-steroidal anti-inflammatory drugs (such as aspirin (>100mg/day), ibuprofen, naproxen, meloxicam, celecoxib) for more than 1 month;<br>▪ In the last 12 months:<br>• azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, anti-TNFα therapies (infliximab, adalimumab, etanercept) or anti-integrin therapies (namixilab) ;<br>○ More than 10 consecutive days oral or parenteral antibiotics within 4 weeks prior to study entry NOTE: subjects administered with antibiotics for more the 5 days prior to study entry would not be included in the stool and PBMC analysis).<br>○ Variable dose of antilipidemic agents (3-hydroxy-3-methyl-glutaryl (HMG)-Co-A reductase inhibitors – "statins") in the 3 months prior to study entry. | |

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| Study Synopsis and main body of the protocol<br>Sample size determination | Further, since the endpoint is the *change from Baseline*, plausible estimates of the degree of<br>correlation between pre and post-baseline measurements were also incorporated into the variance estimates. Given the wide range of results observed in the literature, several "what if" scenarios were examined. As such, the current sample size was considered sufficient for the exploratory objectives of<br>the study for the given range of plausible treatment effects and variance estimates. Due to the exploratory objectives of the protocol, no adjustment for the two co-primary endpoints was made. Additionally, there will be no adjustment for multiple comparisons between the 3 treatment groups. | Further, since the endpoint is the *change from Baseline*, plausible estimates of the degree of correlation between pre and post-baseline measurements were also incorporated into the variance estimates. Given the wide range of results observed in the literature, several "what if" scenarios were examined. As such, the current sample size was considered sufficient for the exploratory objectives of the study for the given range of plausible treatment effects and variance estimates. There will be no adjustment for multiple comparisons between the 3 treatment groups. | Sample size determination |
| Study Synopsis and main body of the protocol<br>Interim analysis section 12.8 | *Not applicable* | Interim analysis<br>An un-blinded efficacy and safety interim analysis is scheduled<br>The interim analysis will be conducted on all primary and secondary outcome unblended data by a third party,<br>The interim analysis will be carried out when at least 22 eligible subjects complete the full 24 weeks of treatment and have verified baseline and 24 weeks MRI data.<br>The goals of the interim analysis are as follow:<br>5. Full efficacy analysis<br>6. Full safety analysis<br>7. Determining the best performing dose<br>8. To correct for sample size. | Allowing for an interim analysis during the course of the clinical study |

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| | | To account for multiple comparisons between the interim and final analyses, the critical alpha level for the interim analysis will be set at 0.0000147 while the critical alpha level for the final analysis will be set at 0.0499. These values are based on 25% of the study endpoints being available for the interim analysis and symmetric two-tailed tests being used within the O'Brien-Fleming spending function. The study will not be terminated for either success or failure. The purpose of this interim analysis will be to verify, and if necessary re-calculate, the required study sample size and to potentially discontinue the worse performing dose arm.<br>The Interim-Analysis will be carried out by a third party so both sponsor and investigators would remain blinded to the allocation of subjects within the study groups. | |
| Table 1. Table of assessments And Baseline evaluation and study medication administration section 7.2.3<br>And Week 24 section 7.2.9<br>And Table 5 | *Not applicable* | Serum Metabolomics | Addition of serum metabolomics to the procedures of baseline and week 24 visits to evaluate the effect by other means |
| Table 1. Table of assessments<br>Comment 1 | [1] Must be collected after subject has fasted for at least 8 hours (except for Screening and W28 assessment). Including: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatine phosphokinase, creatinine, total protein, albumin, serum amylase (fractionated if > greater than upper limited of reference range), serum lipase, phosphate, GGT, AST, ALT, | [1] Must be collected after subject has fasted for at least 8 hours (except for Screening and W28 assessment). Including: sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatine phosphokinase, creatinine, total protein, albumin, serum amylase (fractionated if > greater than upper limited of reference range), serum lipase, phosphate, GGT, AST, ALT, alkaline phosphatase, total bilirubin, | Clarification for the chemistry parameters to be assessed. |

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| | alkaline phosphatase, total bilirubin, calcium, uric acid, glucose and insulin. | calcium, uric acid, glucose and insulin. In addition CRP and C-peptide will be included at the following visits: Baseline, W4, W12 and W24. | |
| Table 1. Table of assessments Comment 4 | [4]Serum biomarkers – CK-18, CRP, C-peptide, LPS, adiponectin, GLP-1 and cytokines | [4]Serum biomarkers – CK-18, LPS, adiponectin, GLP-1 and cytokines (IL-1α, IL1β, IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-12, IL-17α, IL-23, IFNγ, TGF-β and TNFα) | Removing CRP and C-peptide as these parameters are being assessed in clinical chemistry.<br>Specifying all cytokines to be assessed – see rationale above |
| Table 1. Table of assessments Comment 6<br>Table 4 screening tests – serum | No comment 6 | [6] (including HIV, HCV and HBV test) results will be acceptable for eligibility within range of 6 months prior to screening. | Additional comment to clarify the timeline of serology requirement for screening |
| Table 1. Table of assessments Comment 7 | No comment 7 | [7] MRI: Baseline MRI to be performed as soon as subject eligibility is determined. Week 24 MRI to be done within +/- 2 weeks week 24. | Additional comment to clarify the timeline of MRI in screening/baseline visit and week 24. |
| Table 1. Table of assessments Comment 8 | No comment 8 | [8] Randomization is to be done within 2 weeks post baseline MRI, when the MRI result is available for stratification (hepatic fat fraction form – to be received by central imaging lab). | Additional comment to clarify the timeline for randomization. The 2 weeks are required to obtain the MRI result from central imaging lab. |
| Endpoint section 4.2 Secondary endpoint | <u>Liver function:</u><br>• Mean change from Baseline in serum ALT, AST, bilirubin, albumin and GGT at Weeks 4, 8, 12, 16, 20 and 24.<br>• Proportion of subjects whose ALT at Week 24 is within reference range defined a ≤19IU/L for women and ≤30IU/L for men. | <u>Liver function:</u><br>• Mean change from Baseline in serum ALT, AST, bilirubin, albumin and GGT at Weeks 4, 8, 12, 16, 20 and 24.<br>• Proportion of subjects whose ALT at Week 24 is within reference range defined a ≤19IU/L for women and ≤30IU/L for men. | Definition of endpoints |

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| | | • The proportion of subjects demonstrating a decrease in serum ALT at 24 weeks compared to baseline equal or greater than 30% compared to placebo. | |
| Endpoint<br>section 4.2<br>Secondary endpoint | | MRI:<br>• The proportion of subjects demonstrating a decrease in Liver percent fat by MRI at 24 weeks compared to baseline equal greater than 5% compared to placebo.<br>• The proportion of subjects demonstrating a decrease in Liver percent fat by MRI at 24 weeks compared to baseline equal greater than 10% compared to placebo. | Definition of endpoints |
| Endpoint<br>section 4.2 Exploratory endpoints | • Mean serum concentrations of: LPS, CRP, CK-18 fragments, C-peptide and adiponectin and subsets of cytokines IL6, IFNγ and TNFα;<br>• Relative levels of regulatory T cells in PBMC samples, including CD4, CD8, CD25, FoxP3, NKT and CD62;<br>• Characterization of the gut microbiome. | • Mean serum concentrations of: LPS, CK-18 fragments and adiponectin and subsets of cytokines IL6, IL-1α, IL1β, IL-2, IL-3, IL-4 IL-10, IL-13, IL-12, IL-17α, IL-23, IFNγ TGFβ and TNFα;<br>• Serum metabolomics<br>• Relative levels of regulatory T cells in PBMC samples, including CD4, CD8, CD25, FoxP3, NKT and CD62;<br>• Characterization of the gut microbiome. | Definition of endpoints with the addition of serum immunological and metabolomics markers |
| Randomization Process<br>section 7.2.2 | Upon completion of screening assessments and confirmation that the subject meets all eligibility criteria, the subject will be randomized to study treatment at the Baseline visit (See Section 8.1 and the Study Reference Manual). The randomization number will be recorded in the subject's study documentation. | Upon completion of screening assessments and confirmation that the subject meets all eligibility criteria (up to 45 days):<br>• Baseline MRI scan of the liver (Section 7.3.13) is to be done.<br>Valid result for baseline MRI scan by the central imaging lab (VirtualScopics) must be obtained before randomization. | After determining eligibility, the subject should undergo liver MRI scan. The MRI result (hepatic fat fraction %) to be available before randomization. The hepatic fat fraction will be utilized as stratification factor. |

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| | | Randomization (Day 0) should take place within 14 days after baseline MRI scan was done.<br>Once a subject is eligible to be randomized and to receive study medication, the following procedures are to be performed and documented: | |
| Baseline evaluation and study medication administration section 7.2.3 | Prior to randomization, final confirmation of eligibility will be performed through the review of the inclusion/exclusion criteria. Subjects meeting all of the inclusion and none of the exclusion criteria<br>will be eligible to be randomized and to receive study medication and the following procedures performed and documented: | Prior to randomization, final confirmation of eligibility will be performed through the review of the inclusion/exclusion criteria. Then subjects will undergo MRI scan. Subjects meeting all of the inclusion and none of the exclusion criteria and have a valid result for their baseline MRI scan by the central imaging lab (VirtualScopics) will be eligible to be randomized and to receive study medication and the following procedures performed and documented: | |
| Urine pregnancy test<br>Section 7.3.10 | Prior to administration of the first dose of study medication at Baseline and 4 weekly thereafter, women of childbearing potential will require confirmation of non-pregnancy by collection of a mid-stream urine specimen and testing with a standard commercially available urine pregnancy test kit. | Prior to administration of the first dose of study medication at Baseline and 4 weekly thereafter, women of childbearing potential will require confirmation of non-pregnancy by collection of a mid-stream urine specimen and testing with a standard commercially available urine pregnancy test kit. In case such test is not available or deemed unsatisfactory at specific site, a blood pregnancy test will be performed. | Adding the possibility to perform urine pregnancy test by blood test, instead of urine kit - in case this is not available. |
| Randomization section 8.1 | Not applicable | Additionally, to ensure baseline Hepatic Fat Fraction (HFF) would not be a confounding factor, subjects will be stratified for baseline HFF according to the following groups $\leq 10\%$, $10\% < X \leq 20\%$, $20\% < X < 30\%$ and $\geq 30\%$. | Defining the way subjects are to be randomized by baseline MRI fat fraction |
| Dosage and Administration of study drugs section 8.4.4 | IMM-124E and matching placebo tablets may be either chewed before swallowing or swallowed whole three times daily per oral on | IMM-124E and matching placebo tablets may be either chewed before swallowing or swallowed whole three times daily per oral on an empty | Clarification of allowed IMP compliance range. |

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| | an empty stomach. No intra-subject dose adjustment of IMM-124E will be allowed during this study. | stomach. No intra-subject dose adjustment of IMM-124E will be allowed during this study.<br>Range of +/- 15% (85-115%) is the acceptable compliance range.<br>Any poor compliance out of this range will be considered as protocol deviation.<br>Any poor compliance should be escalated to Immuron and will potentially result in subject's termination from the study. | |
| Appendix B Model patient information sheet<br>Section 3 - What does participation in this research involve? | You will be given the first dose of treatment in the clinic and enough tablets to take at home for 4 weeks treatment. You will be asked to take 2 tablets each morning, 2 in the middle of the day and 2 each night on an empty stomach, preferably 1 hour before or 3 hours after eating. At each 4 weekly clinic visit you will be given more tablets to continue taking at home, for a total of 24 weeks treatment. | You will be given the first dose of treatment in the clinic and enough tablets to take at home for 4 weeks treatment. You will be asked to take 2 tablets each morning, 2 in the middle of the day and 2 each night on an empty stomach, preferably with a minimum of 1 hour fasting before and 1/2 hour after. At each 4 weekly clinic visit you will be given more tablets to continue taking at home, for a total of 24 weeks treatment. | Clarifying the timing of drug administration and the recommendation to administer on an empty stomach. |
| Appendix B Model patient information sheet<br>Screening visit | Blood samples will be collected using a needle and syringe (30mL or about 6 teaspoons of blood) | Blood samples will be collected using a needle and syringe (55mL or about 11 teaspoons of blood) | Correction of the total amount of blood to be withdrawn during the visit. |
| Appendix B Model patient information sheet<br>Day 0 visit (Baseline) and Week 24 | Not applicable | metabolomics – blood testing for small molecules that can potentially identify disease state | Adding metabolomics to baseline and week 24 visits and explaining what metabolomics is. |
| Appendix B Model patient information sheet<br>Day 7 and Day 14 visits | Blood samples will be collected (20mL or about 4 teaspoons of blood) | Blood samples will be collected (30mL or about 6 teaspoons of blood) | Correction of the total amount of blood to be withdrawn during the visit. |
| Appendix B Model patient information sheet<br>Week 4, 8,12, 16 and 20 visits | Blood samples will be collected (20mL or about 4 teaspoons of blood) | Blood samples will be collected (30mL or about 6 teaspoons of blood) | Correction of the total amount of blood to be withdrawn during the visit. |