IRB00084686

NCT03042767

Anti-LPS Antibody in Pediatric Nonalcoholic Fatty Liver Disease

Short Name: Hyperimmune Milk in Pediatric NAFLD

Funding Source: National Institutes of Health & Immuron Ltd

Sponsor-Investigator: [redacted]

Version Number: 5.0

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Responsible Persons and Institution

Principal Investigator:

Name: [redacted]
Institution: Emory University School of Medicine
[redacted]
[redacted]

Research Pharmacist:

[redacted]
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[redacted]
[redacted]

Contact person in case of serious adverse event:

[redacted]

Protocol Synopsis

Title:	Anti-LPS Antibody in Pediatric Nonalcoholic Fatty Liver Disease
Version/Date:	Version 5 – April 3, 2017
Study Phase:	II
Study Design:	12 week, randomized, double-blind, placebo-controlled, pilot study
Indication:	NAFLD
Number of Sites:	One, Emory University/Children's Healthcare of Atlanta
Name of study drug:	IMM-124E
Drug Dosage:	3 x 600 mg daily
Route of administration:	Oral
Conditions of administration:	Inclusion criteria: • Age 6 – 19 years at time of initial screening • NAFLD diagnosis (confirmed by biopsy, MRI, or Ultrasound) • ALT ≥ 2 x ULN at screening (girls ≥ 46, boys ≥ 54) • Written informed parent consent and child assent • Willingness to consume IMM-24E 3 x daily for 12 weeks • At least 2 months of attempted lifestyle changes after diagnosis Exclusion criteria: • Disease or condition deemed by physician to interfere with absorption, digestion, or mechanism of intervention of drug • Diagnosis of diabetes and an HbA1c of > 9% • Change in supplement or anti-oxidant therapy within past 90 days (must be on a stable dose and willing to continue it throughout the trial or not on any vitamin or supplement, includes SAMe, vitatmin E, betaine, Milk thistle etc) • Use of probiotics or antibiotics in the past 30 days • Use of anti-NAFLD medications (metformin, thiazolidinediones, UDCA) in the 30 days prior to randomization • Acute illness within past 2 weeks prior to enrollment (defined as fever > 100.4ºF) • Planned pregnancy, nursing an infant, confirmed or suspected to be pregnant between screening and time of study enrollment • Evidence of other chronic liver disease other than NAFLD (Hepatitis B and C, Alpha-1 antitrypsin, Wilson's disease) • Intolerance to lactose or dairy-based products • Unable to have blood drawn at study visits • Unwillingness to provide and/or collect stool samples • Current GI bleeding or inflammatory bowel disease (IBD, colitis) • Current enrollment in another therapeutic clinical trial or receipt of an investigational study drug within 6 months prior to study enrollment • Participants who are not able or willing to comply with the protocol or have any other condition that would impede compliance or hinder completion of the study, in the opinion of the investigator
Number of participants:	40
Randomization:	Administered by research pharmacy and blinded to study staff and participants

Primary Endpoints:	Percent change in ALT from Week 0 to Week 12 in treatment compared to placebo
Secondary and Exploratory Endpoints:	• Mean change in ALT in treatment group compared to placebo • Fasting glucose and insulin • Hemoglobin A1C • Adipo-IR • Oral glucose tolerance test • NAFLD pediatric diagnostic panel • Fasting plasma lipids • Stool microbiome • Blood transcriptomics • Systemic inflammation (cytokines) • Metabolomics • Visceral Adiposity (AMRA MRI scan) • Hepatic fat percent (AMRA MRI scan) • BMI z-score • Waist circumference • Depression • Quality of life • Composite endpoint of "metabolic improvement" specified as >10% improvement in TG/HDL ratio, improvement in insulin resistance, and >10% improvement in ALT

Study Duration (per participant):	• Up to 19 weeks total
	  ○ Up to 6 weeks screening
	  ○ 12 week treatment

Study Duration (calendar time):	• Study start-up: 0 – 4 months • Recruitment phase: 5 -14 months • Follow-up phase: 15 - 18 months • Analysis and reporting: 18 - 24 months
Visit Schedule:	• Screening Visit • Baseline visit • Follow up visits ○ Week 6 and Week 12 visits
Statistical Analysis:	Descriptive variables of the cohort and subgroups will be analyzed using means and standard deviations or medians and ranges, as appropriate. Percent change in primary and secondary outcomes will be compared using two sample t-test or Mann-Whitney test. Absolute change over time will be compared using mixed model or analysis of covariance.
Sample Size:	A sample size of 11 participant per group is required to achieve a least 90% power to detect a 15% point difference in the average % change in ALT from baseline to week 12 between the two groups. Sample size was calculated assuming common standard deviation of 10% using a two-sided Mann-Whitney tests with a 0.05 significance level. We will include 20 participants per group to allow for drop out, intra-individual variability and multiple hypothesis testing.
Safety Monitoring:	A DSMB will monitor the data for safety and efficacy for outcomes such as nausea, abdominal pain, flatus, diarrhea, pregnancy, hepatotoxicity and any other outcomes or events identified as safety related.

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1. Objectives

We hypothesize that IMM-124E, a bovine colostrum enriched with anti-LPS antibodies, will improve insulin resistance and decrease systemic and hepatic inflammation through modulation of bacterial products and the microbiome in pediatric NAFLD.

1.1 Primary Objective: The principal objective of this blinded, placebo-controlled, pilot study is to evaluate whether 12 weeks of IMM-124E in children with NAFLD in combination with standard of care (SOC) will decrease inflammation in the liver as measured by ALT. Specifically, percent change in ALT from Week 0 to Week 12 in treatment compared to placebo.

1.2 Secondary Objectives:

- To assess the safety and tolerability of IMM-124E in children with NAFLD
- To assess the effects of IMM-124E on secondary and exploratory endpoints:
    - Mean change in ALT in treatment group compared to placebo
    - Fasting glucose and insulin
    - Hemoglobin A1C
    - Adipo-IR
    - Oral glucose tolerance test
    - NAFLD pediatric diagnostic panel
    - Fasting plasma lipids
    - Stool microbiome
    - Blood transcriptomics
    - Systemic inflammation (cytokines)
    - Metabolomics
    - Visceral Adiposity (AMRA MRI scan)
    - Hepatic fat percent (AMRA MRI scan)
    - BMI z-score
    - Waist circumference
    - Depression
    - Quality of life
    - Composite endpoint of “metabolic improvement” specified as > 10% improvement in TG/HDL ratio, improvement in insulin resistance, and >10% improvement in ALT

2. Background and Significance

Nonalcoholic fatty liver disease (NAFLD) is an aggressive, obesity-associated chronic liver disease that affects ~7 million children in the US (10% of all children) and is one of the top 3 indications for liver transplant in adults. Currently, the only treatment available for NAFLD is lifestyle advice to decrease adiposity and improve insulin sensitivity but this is rarely effective. It is our long term goal to improve the health of children by understanding how to prevent, treat and cure NAFLD. In our previous studies, we demonstrated that children with NAFLD have endotoxemia as well as systemic inflammation. Further, individuals with NAFLD have a disturbed microbiome (dysbiosis) and increased gut permeability. Thus, the mechanism of NAFLD is thought to include chronic inflammation from intestinal microbiome-derived

products (lipopolysaccharide (LPS) and metabolites) which pass through the "leaky gut" to the liver where they activate the innate immune response.

IMM-124E is a hyperimmune colostrum-derived product from cows inoculated with bacterial products from 13 strains of *E. coli*. Preliminary data suggests that IMM-124E improves insulin sensitivity, reduces liver inflammation and promotes regulatory T cells that suppress chronic inflammation. IMM-124E acts at the level of the mucosa in the gut by improving barrier function and decreasing systemic exposure to microbial products (LPS). It could also alter the gut microbial populations by targeting pathogenic bacteria (*E. coli*). IMM-124E is being tested in adults for treatment of NAFLD and alcoholic hepatitis under 2 separate FDA approved INDs. A similar product is sold over-the-counter in Canada and Australia. IMM-124E is considered a medical food and *little is known regarding its potential use in children.* It is important to study in children because the NAFLD histology is different compared to adults. Waiting for the results of adult trials will have little benefit for children. The objective of this proposal is to evaluate the preliminary effectiveness, safety and mechanisms of action of IMM-124E in children with NAFLD.

In this proposal, we will test the following central hypothesis: IMM-124E, a bovine colostrum enriched with anti-LPS antibodies, will improve insulin resistance and decrease systemic and hepatic inflammation through modulation of bacterial products and the microbiome in pediatric NAFLD. The aims of this study are the following:

1. To determine if a 3-month treatment with IMM-124E in combination with standard of care lifestyle advice (SOC) is safe and results in greater improvement in 1) hepatic inflammation (alanine amino transferase - ALT) 2) insulin sensitivity 3) blood lipids in children with NAFLD compared to placebo with SOC as well as safety related endpoints.

2. To define mechanism of action related endpoints in response to 3 months of IMM-124E in children with reduction in the above outcomes including: stool microbiome (16S rRNA), metabolomics, intestinal inflammation (stool calprotectin), systemic inflammation (cytokines), transcriptomics (immune response) and correlate these with changes in clinical measurements.

We will test our hypothesis and accomplish the aims through a randomized, double-blind, 3 month treatment trial of children age 6-19 years with a diagnosis of NAFLD by biopsy or MRI. IMM-124E and placebo will be provided by Immuron. Patients will be recruited from the Children's Healthcare of Atlanta pediatric liver clinical practice which cares for over 400 pediatric NAFLD patients and has an extensive record of successful clinical trials in children. The sample size of 40 participants is powered for change in ALT as the primary outcome and allows sufficient power for subgroup analysis and secondary outcomes. High resolution, high throughput metabolomics will be combined with transcriptomics of PBMCs, 16S rRNA characterization of the stool microbiome to determine gut-level and system-level response to IMM-124E.

These studies are designed to inform decision making about the design of a future large, multicenter trial testing IMM-124E. We will leverage our position as members of the NIH supported clinical research network (NASH CRN) in which pediatric investigators are eager to conduct a phase III trial of IMM-124E if

the data shows promise. These studies have great potential to advance progress towards a new therapeutic for NAFLD, thus improving health for millions of children.

Why study pediatric NAFLD? NAFLD is a chronic liver disease defined by accumulation of fat in the liver in the absence of alcohol or other secondary causes of steatosis[1]. In the US, over 7 million children are estimated to have it[2]. NAFLD occurs in the setting of insulin resistance (IR) and it is histologically categorized into nonalcoholic fatty liver (NAFL), a milder form without hepatocellular injury and nonalcoholic steatohepatitis (NASH), defined as hepatic fat plus hepatocellular injury with or without fibrosis[1]. In children, NAFLD often presents as NASH with fibrosis at diagnosis[3, 4]. It is crucial to study pediatric NAFLD in children because it is commonly severe and there is not yet an effective treatment for it. Further, pediatric NAFLD differs from adult NAFLD because in children the histology frequently has zone 1 accentuation (portal based) chronic inflammation and fibrosis as opposed to zone 3 (central venule), which is classic in adults. Zone 1 predominance of inflammation suggests that toxins are delivered by the portal venous blood from the gut to the liver. Previous therapeutics for NAFLD have included antioxidants (vitamin E) and metformin; however neither sufficiently improved histopathology[5, 6]. Finding a treatment for NAFLD will prove most beneficial to children by avoiding a lifetime of increased risk of cirrhosis, cardiovascular disease and type II diabetes.

Why target the gut to treat NAFLD? The cause of NAFLD is multifactorial, resulting from a combination of genetic suceptibility and environmental hits modified by individual factors such as the microbiome. Among Hispanic children, the rs738409 polymorphism of PNPLA3 is associated with increased hepatic fat in children as young as 8-10 years of age[7] only in the setting of a high sugar diet[8], suggesting a strong gene-environment interaction. Our group previously showed that children with NAFLD are less tolerant to fructose and have increased post prandial hypertriglyceridemia compared to those without[9]. Further, we studied the response to diet in these same children and found that endotoxin levels rise immediately postprandially in NAFLD while in obese and normal weight children they do not[10]. Increased endotoxin results from a combination of altered microbiota composition (dysbiosis) and increased gut permeability from disruption of tight junctions, thus allowing increased movement of microbial products from the lumen into the portal blood stream into the liver (reviewed in[11] Figure 1). Supporting this, pediatric NAFLD studies have shown increased Gammaproteobacteria and *Prevotella* as well as increased energy production by the microbiota[12]. There is growing consensus that treatment for NAFLD at the intraluminal level is needed[12-16].

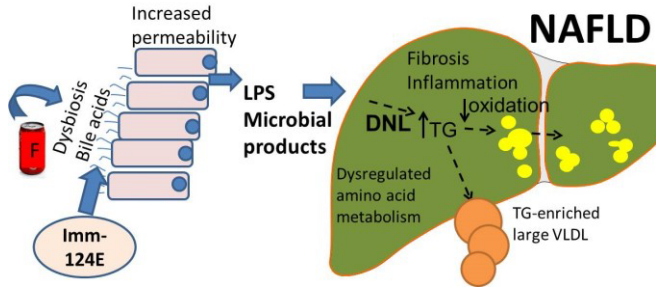

Figure 1. Proposed mechanisms in NAFLD include increased lipopolysaccharide (LPS) released into the portal blood by carbohydrate-stimulation of gut microbiome. Increased LPS in combination with increased fructose leads to upregulated de novo lipogenesis (DNL), overload of oxidation and results in increased fat storage (steatosis) and triglyceride (TG) export on very low density lipoproteins (VLDL). IMM-124E works at the mucosal surface to prevent transference of LPS and moderate the innate immune response.

What is IMM-124E (Anti-LPS antibody)? [redacted]

Why use IMM-124E to treat pediatric NAFLD? In a mouse model of extreme obesity, treatment with anti-LPS enhanced bovine colostrum (similar to IMM-124E) led to decreases in IR, alanine amino transferase (ALT) levels and serum triglycerides when compared to control mice, and decreases in serum TNFα levels[19]. IMM-124E was used to treat 10 adults with biopsy-proven NASH for 30 days in an open-label pilot study[18]. IR improved in all participants and an improved lipid profile and liver enzymes was seen in 5 out of 10. There are two active trials using IMM-124E reported on clinicaltrials.gov. One is a 30 day RCT treating patients with alcoholic hepatitis sponsored by the NIH and the other is an industry sponsored phase II study of IMM-124E for patients with NASH. Bovine colostrum has been well-tolerated in previous studies. The most common adverse events are nausea, abdominal pain, flatus and/or diarrhea and all are rare.

What is known about the mechanisms of IMM-124E in NAFLD and what needs to be tested? IMM-124E acts through targeting bacterial products (LPS) at the mucosal surface, before transference into the portal blood. LPS is thought to activate TLR signaling which then induces upregulation of inflammatory pathways such as activation of NFκB pathway and subsequent altered expression of adipocytokines known to be important in NAFLD including tumor necrosis factor alpha (TNFα) and interleukin-6. In animal models, LPS has been shown to induce hepatic steatosis as well as hepatic inflammation and fibrosis progression[20]. In adults with NAFLD, several studies show elevated LPS[21] or anti-LPS antibodies[22], while NAFLD treatment is associated with improvement in LPS levels and liver enzymes[23] after 6 months. Thus, the theoretical basis for utilization of IMM-124E as a therapy of NAFLD in children is that IMM-124E taken 3 x daily will block LPS in the gut, decreasing the transfer of LPS to the liver and plasma, thus alleviating chronic inflammation, improving insulin sensitivity and leading to decreased inflammation.

In summary, pediatric NAFLD affects many children and is often severe. The pathophysiology of NAFLD involves increased microbial products (LPS) entering the liver through the portal blood causing chronic inflammation. There is no effective treatment available yet. IMM-124E is a medical food approved for over-the-counter sales in the US and may decrease endotoxin in the setting of NAFLD. It has not yet been tested in children and preliminary data is needed. It is thought to be highly safe; although further confirmation of this is prudent. Thus, exploratory studies of IMM-124E in pediatric NAFLD are needed.

3. Study Design

3.1 Design Overview:

This is a 12 week randomized, double-blind, placebo-controlled "proof of concept" trial (RCT) testing the effect of IMM-124E on change in ALT in children with NAFLD. A placebo-controlled trial was selected because this is the highest quality method and pediatric NAFLD has a substantial placebo response rate (typically ranges from no change to a 30 U/L improvement in ALT at 12 weeks after the initial diagnosis by liver biopsy). Further, a parallel arm trial is necessary because the sustainability of IMM-124E influence is not known and thus the appropriate length of a wash-out period was unclear. ALT is an accepted surrogate marker of hepatic inflammation in children and improvements can be seen within several weeks in pediatric NAFLD[6].

3.2 Inclusion criteria:

- Age 6 – 19 years at time of initial screening
- NAFLD diagnosis (confirmed by liver biopsy, MRI, or Ultrasound)
- ALT ≥ 2 x ULN at screening (girls ≥ 46, boys ≥ 54)
- Written informed parent consent and child assent
- Willingness to consume the investigational product 3 x daily for 12 weeks
- At least 2 months of attempted lifestyle changes after diagnosis

3.3 Exclusion criteria:

- Disease or condition deemed by physician to interfere with absorption, digestion, or mechanism of intervention of drug
- Diagnosis of diabetes and an HbA1c of > 9%
- Change in supplement or anti-oxidant therapy within past 90 days (must be on a stable dose and willing to continue it throughout the trial or not on any vitamin or supplement, includes SAMe, vitamin E, betaine, Milk thistle etc)
- Use of probiotics or antibiotics in the past 30 days
- Use of anti-NAFLD medications (metformin, thiazolidinediones, UDCA) in the 30 days prior to randomization
- Acute illness within past 2 weeks prior to enrollment (defined as fever > 100.4ºF)
- Planned pregnancy, nursing an infant, confirmed or suspected to be pregnant between screening and time of study enrollment
- Evidence of other chronic liver disease other than NAFLD (Hepatitis B and C, Alpha-1 antitrypsin, Wilson's disease)
- Intolerance to lactose or dairy-based products
- Unable to have blood drawn at study visits
- Unwillingness to provide and/or collect stool samples
- Current GI bleeding or inflammatory bowel disease (IBD, colitis)
- Current enrollment in another therapeutic clinical trial or receipt of an investigational study drug within 6 months prior to study enrollment
- Participants who are not able or willing to comply with the protocol or have any other condition that would impede compliance or hinder completion of the study, in the opinion of the investigator

3.4 Rescreening

If a potential participant fails screening due to a changeable criteria such as acute illness, use of a supplement or anti-oxidant, and ALT < 2 x ULN, the participant may rescreen a second time if they desire to after a reasonable amount of time has passed and if the investigator thinks the participant is likely to qualify the second time.

3.5 Treatment Groups:

Characteristics: 40 children age 6-19 years from any ethnic and racial categories will be included. Males and females will be recruited equally, however because of the increased prevalence of NAFLD in adolescent boys compared to girls, we may enroll more boys. In order for 40 children to complete the protocol, we will over-enroll at baseline by approximately 5 additional participants to allow for dropout.

NAFLD Patient Eligibility: Children ages 6-19 will be included because NAFLD typically presents around age 10, although sometimes at a young age. Young adults, age >19 years, will not be included because their travel for school and work makes participation more difficult. For NAFLD, each patient must meet the typical criteria for diagnosis including typical clinical and laboratory changes of NAFLD and liver biopsy or MRI consistent with NAFLD. If the subject only has had an ultrasound they may have their baseline MRI conducted up to 2 weeks prior to confirm the diagnosis. Subjects with 5% or more liver fat on their MRI will meet entry criteria and can proceed with the study. Other chronic liver disease must have been ruled out using a serologic work-up.

3.6 IMM-124-E Administration:

Dose and Frequency: IMM-124E is a medical food and has a wide range of potential dosing. The dose recommended by Immuron is 20 mg/kg. Previous trials for adults have ranged from 600 – 3,600 mg/day and the tablets range from 200 – 450 mg each. Because IMM-124E is very safe and well-tolerated, our goal is to provide the most effective dose without the major burden of taking pills. In our previous pediatric NAFLD trials, the weights have ranged from 50 kg to 120 kg. Because of this, we will use the recommended adult dose of 600 mg 3 x daily. To facilitate compliance the IMM-124E is provided as a dried powder with flavoring. At the time of consuming, the powder is mixed with a small amount of water and then the child/teen drinks it. Administration instructions will be provided and standardized for the trial. An example of this is participants will be asked to take the product (or placebo) before breakfast (preferably on an empty stomach and about 30 minutes before eating), afterschool or in the afternoon (before snacking) and at bedtime (corresponds to 3 times per day).

Storage and record keeping of the IMM-124E and placebo will be performed by the research pharmacy of the ACTSI and Children's Healthcare of Atlanta.

Manufacturing of Placebo: Placebo will be prepared from cow's milk protein concentrate (PROMILK 85) purchased by Immuron from Tatura Industries in Australia. The milk protein is certified for human consumption and is typically used to make ice cream, yogurt, cheese, and other milk products. All of the milk sourced for PROMILK 85 is from Australian dairy cattle which is similar to the source of IMM-124E. The placebo packets will look and taste similar to IMM-124E and have similar dairy fat and micronutrient content. The lactose content of the placebo is higher but this is unavoidable. Because of this, lactose

intolerant children will be excluded. Lactose content will be standardized across all the placebo packets used in the study.

3.7 Recruitment and Retention Strategies:

Our research team has years of experience recruiting and retaining children into research studies at Emory University and Children's Healthcare of Atlanta. We have a large network of recruitment sites and many families that have expressed interest in participating in future studies. From our current database of participants who have previously participated in our protocols, we have already identified over 50 eligible individuals who could be approached to participate in this study. In addition, new patients with evidence of NAFLD from Children's Healthcare of Atlanta hepatology clinics, and referring centers/practices may be considered as candidates. It is our experience that approximately 50 new cases of children who have liver-biopsied NAFLD can be identified each year from the Children's liver clinics. We will continue to use our proven retention strategies, including scheduling children on weekends and school holidays (to minimize missing school), completing visits early in the morning (to allow return to school), contact after visits to ensure participant satisfaction with visits, child-friendly facilities and providing entertainment during longer visits (movies, video games, etc).

3.8 Sources of Materials:

The sources of research material obtained will include blood specimens, medical records, imaging results and data collection forms used to record data during the study. Participants will complete the Block Physical Activity Questionnaire to record current activity level during the study. The survey will be performed at baseline. Participants will be asked to maintain their current level of activity. Nutrition data will be collected by telephone or in person in the form of triple pass 24-hour recalls prior to randomization and again before the final study visit. Phone calls will be used to assess tolerability and compliance with the product. Specimens and data will be obtained and recorded both as part of routine medical care and for research purposes. Only study personnel (PI, co-Is, study coordinator, research assistant and nutritionist) will have access to identifiable data. Data will be de-identified before analysis. Data will be stored in locked research material cabinets and on password-secured databases on the Emory network.

3.9 Potential Risks

There is minimal risk in these studies. Potential risks are related to 1) blood draws, 2) NPO status, 3) cumulative blood loss 4) IMM-124E side effects and 5) confidentiality.

1) The placement of an intravenous catheter and drawing of blood specimens has minimal risk of discomfort, bruising, or bleeding. There is minimal risk of infection or extravasation. Experienced staff with pediatric expertise will place all catheters and draw blood.
2) Patients will remain NPO from the midnight of the morning of the study until the fasting portion of the study is concluded. Fasting will be done before the Week 0 (Baseline) and Week 12 visits. Some children may become agitated with NPO status. Water will be encouraged to maintain hydration. Each participant will be asked to drink one glass of

water at bedtime prior to the study and one glass in the morning before leaving home for the research center.

3) Blood loss: To minimize risk to the patient, we will carefully monitor the total blood required for the 4 study visits and stay well within the NIH clinical center guidelines recommending less than 3ml/kg at a single research visit and no more than 7ml/kg over any 6 week period. See Table 2 for blood collection schedule and amounts.
4) Patients will be taking a concentrated milk protein product. It is possible that it could cause GI symptoms including nausea, diarrhea, gas, bloating or pain. Participants will be monitored for incidence and severity of gastrointestinal events.
5) There is risk of loss of confidentiality. Efforts will be made to keep all personal information confidential. All data will be stored in locked offices and password-protected computers. Personal identity will be protected in any publication.

Alternative treatments and procedures: New information on NAFLD diagnosis or new treatments for NAFLD in children may become available over the study period. This information will be incorporated into these studies and alternate diagnosis or treatment methods may be substituted if more appropriate. They will continue in the study if they desire and lab information from the study will be made available to their physician if requested.

3.10 Protection Against Risks:

Recruitment and Informed Consent

Recruitment will be through posted flyers and discussions of the study in the Children's subspecialty and general pediatric clinics and Health 4 Life clinic, by phone calls and letters to previous research participants who have previously consented to be contacted regarding future studies, letters to known NAFLD patients in the hospital medical record at Children's and through flyers and contacts at community pediatric offices and organizations.

Permission to perform this study will be sought from both the Emory University IRB and the Children's Healthcare of Atlanta IRB. Informed consent will be obtained from the parent or legal guardian and verbal or written assent will be obtained from the minor (depending on age). Informed consents and HIPAA waivers will be obtained by the parents or legal guardian prior to initiating any study procedure. Participants and their parents or legal guardian will be approached to participate in the study. The research coordinators and/or PI/Co-I will discuss the study with them and give them all the information listed above in language understandable at the level of the parent/guardian and all information needed to make an informed choice about participation, including information about NAFLD, the study medication, possible side effects, IMM-124E, study procedures, study visits/contacts and potential benefits to the participant. Consent will be documented by signature of the parent/guardian. Oral consent will be obtained for 6-10 year old participants. Written assent will be documented by signature in age 11-17 year old participants and written consent in 18 and 19 year old participants. Verbal consent will be used for fasting procedures and 24-hour recalls. The screening visit requires that the patient is

fasting at the time of the blood draw. Verbal consent will be used to document that the participant agrees to fast and possibly answer questions about their diet over the phone before coming in for their first visit where they will then sign the informed consent document. They will be made aware of the risks of fasting during this discussion.

Other Protections Against Risk

Risk will be minimized to the children by using nurses and research coordinators experienced in pediatrics for the research studies. Risk to confidentiality will be reduced by using non identifying participant numbers and removing all identifiers as early as feasible. Only study personnel will have access to identifying data. Laboratory personnel will only have access to numeric identifiers and the key will be kept in a password protected database.

Additional protection for children: all children must provide verbal and written assent or consent. In our studies, we allow children to decline to continue to participate at any time before and during a study or study visit. During study visits, if blood cannot be drawn and the child does not wish to have it attempted again, we encourage them to tell us this and we assess willingness to complete other study procedures not requiring venipuncture. Study stipends are provided at a standardized pro-rated level appropriate for the time/study procedures completed.

Side effects of IMM-124E will be monitored and tracked carefully. We will report Serious, Unexpected Suspected Adverse Reactions (SUSAR) to the FDA, and IRB within 15 calendar days after determining reportability. Any unexpected fatal or life threatening suspected adverse reaction will be reported within 7 calendar days of initially receiving information on the event. Unanticipated problems (UPs) are reportable to the IRB within 10 days according to IRB policy. Serious adverse events or SUSARs that are not UPs and for which there is a reasonable possibility that the drug and/or research procedures caused the event or reaction are reportable to the IRB at continuing review.

3.11 Potential Benefits:

Potential Benefits to Participants:

The proposed research has substantial potential benefits for children but may not benefit the individual participant. All children will receive standard of care. The establishment of a drug that is safe and has high efficacy in treating NAFLD in children will allow for prevention of secondary diseases associated with NAFLD as well as improved quality of life in the affected children.

Participants in the study will receive copies of their liver transaminases results and these will be discussed with them at the end of the study. Education will be provided as appropriate regarding the results. At the conclusion of the study, participants will meet with a member of the study team and the families will be provided important long term healthy diet and physical activity education as well as information for the parent on methods to prevent/reduce childhood obesity.

Importance of Knowledge to be Gained:

NAFLD is the most common liver disease in children today and the prevalence of the disease has more than doubled over the past 2 decades to include > 7 million children in the US. Liver disease is more

common among Hispanics and is responsible for increased morbidity and mortality. NAFLD is a contributor to this health disparity. There is evidence to suggest that once a child has NAFLD, it may be difficult to completely reverse. Further, there is substantial evidence to show that NAFLD is a key event in the global dysmetabolism of the metabolic syndrome and acquiring NAFLD predicts future morbidity and mortality from type II diabetes, strokes, heart attacks, cirrhosis and liver cancer. It is critical to develop prevention and treatment for NAFLD. At this time, it is not known when NAFLD typically starts and standard of care is to test all obese children periodically starting at age 10 to see if and when they have developed NAFLD. This study may provide data to inform clinical practice regarding younger children in high-risk groups. This study will improve patient care, provide potential treatment, support public health policy regarding NAFLD, and decrease health disparity in the affected children.

3.12 Data and Safety Monitoring:

Data Management: Data for each participant will be collected in individual folders kept in a secure filing cabinet in the offices of the Division of Pediatric Gastroenterology/Hepatology/Nutrition. Data will be entered into a secure database in the same division.

Adverse Events: The principal investigator will monitor side effects and unexpected occurrences through the course of the study. Side effects of IMM-124E will to be monitored closely.

A status report will be provided to the Institutional Review Board of Emory University at the time of continuing review. In the event of a serious and unexpected adverse event, the A-CTSI, and the NIH program officer will be notified within 3 working days of the event. The Emory and Children's IRBs will be notified according to IRB policies & procedures.

The PI will determine if any of these events which occur, is due to the study medication or study procedure or is an expected event given the disease state of the participants. The PI is an experienced pediatric hepatologist and pediatrician and will be responsible for determining serious adverse events (SAE) and whether or not they are due to the study medication.

A SAE will be reported to the DSMB within 2 days of occurrence. The DSMB will review all potential SAEs to make the final decision.

Data and Safety Monitoring Board: Medical monitoring will also be provided by the DSMB by Emory faculty who are not involved with this study. All SAE will be reported to the DSMB within 2 days of the occurrence and all side effects recorded will be reported at the end of the study to allow independent assessment of the likelihood of them being from the study mediation.

The DSMB will meet bi-annually to review progress, SAEs, and outcome data.

4. **Study Plan**

This study will be conducted in accordance with current US FDA regulations and guidelines, ICH guidelines on GCP, as well as all other applicable research regulations set forth by Emory University and Children's Healthcare of Atlanta. Informed consent and assent will be obtained for this study by the principal investigator or her designees from all study participants prior to any protocol-specific procedures are performed.

4.1 Screening and baseline data collection:

Candidates who appear eligible will be screened by reviewing their medical records to determine whether they meet the eligibility criteria. Physicians of eligible candidates will be contacted and the patients may be visited in clinic by the study coordinator directly.

Subjects may be approached by the research team about the study either in person in clinic or over the telephone. A verbal consent will be obtained at this time if the subject is interested in scheduling a screening visit. The patient will be instructed to not have anything to eat or drink (except plenty of water) for 12 hours prior to the scheduled research visit. They will also be made aware of the risks associated with fasting (feeling lightheaded or agitated). If the screening window is anticipated to be shorter than usual (based upon the patients schedule etc) then the study team may need to collect a 24-hour dietary recall during the screening period prior to the initial screening visit (where the patient first signs the informed consent document). This procedure is also detailed in the verbal consent. A member of the study team will call the subject on the telephone and will ask them what they have had to eat and drink in the last 24 hours.

If the patient is approached about the study in clinic and chooses to screen during their appointment then the verbal consent will not be required if the subject is already fasting for intended SOC labs.

Participants who sign consent/assent documents will be registered in study and assigned a study number. If the participant does not meet eligibility criteria or meets all the eligibility but decides not to continue in the study before randomization at Week 0 he or she will be deemed a screen failure. A screening log will be kept to track all screening activities and reasons for screen failures. As described above, a patient may be screened a second time if they desire this and the reason for failure is likely to have resolved in the opinion of the investigator.

Screening procedures will be performed as indicated in the Study Schedule of Events (Attachment 1). Recording of any patient information will not begin until informed consent and assent has been obtained. The screening visit will take place within six weeks of the randomization date, Week 0.

Screening visit (up to - 6 weeks)

Pre-screening activities such as chart review of standard of care labs or referral from a colleague will identify potential participants for screening. If the participant is deemed eligible he or she along with a legal guardian will sign the consent/assent documents before or during the screening visit.

After informed consent is obtained the following procedures will be conducted at the screening visit:

- Review inclusion and exclusion criteria
- Collection of demographics
- Collection of vitals (including blood pressure, respiratory rate, heart rate, and body temperature)
- Anthropometric assessments (including height, weight, waist and hip circumference)
- Medical history review
- Review of concomitant medications
- Administration of 3, 24-hour recalls (NDS-R) during the screening period
- Administration of the Alcohol Use Disorders Identification Test (AUDIT)
- laboratory assessments (see Blood Collection Schedule – Attachment 2 for more detail)
- If the subject has had an ultrasound, but not an MRI or liver biopsy prior to screening:
    - The Baseline MRI will be conducted prior to the baseline visit. If the liver fat from this MRI is great or equal to 5% then the subject may continue in the study. These subjects will have their Baseline visit within 2 weeks of the MRI.

Baseline Visit (Week 0)*:*

In order for a participant to be randomized all screening procedures must be completed and participants must meet all inclusion and meet no exclusion criteria prior to randomization. The randomization visit will take place up to 6 weeks after the screening visit.

All participants will be randomized at Week 0 to receive one of two arms of therapy (drug or placebo). All participants will receive a supply of study drug/placebo at Week 0. These bottles will be issued to the patient by the research pharmacist based on the randomization assignment generated at the Week 0 visit.

The following procedures will be conducted at the randomization visit:

- Reaffirmation of consent
- Physical Examination
- Concomitant medication and adverse event review
- Confirmation that participant is feeling well
- PROMIS Questionnaires (patient reported outcomes)
- Collection of vitals (including blood pressure, respiratory rate, heart rate, and body temperature)
- Anthropometric assessments (including height, weight, waist and hip circumference)
- Education on drug administration and adverse effects monitoring
- 2-hour oral glucose tolerance test
- Standardized education on an appropriate diet (participant-specific) and living a healthy lifestyle:
    - A goal of 60 minutes of exercise every day
    - Reduce sugar-sweetened beverage intake
    - Increase vegetable intake
    - Reduced carbohydrate intake
- Drug dispensing and instruction from research pharmacy
- Fasting laboratory assessments (see Blood Collection Schedule – Attachment 2 for more detail)
- Stool sample collection
- Urine pregnancy test for females of childbearing potential
- Blood spot collection
- MRI (Brief scan for full body and liver fat assessment)

- Optional Sub-study: Accelerometer data collection for 1 week

4.2 Randomization Procedures:

After consent and eligibility for the trial is confirmed, participants will be randomly assigned to either IMM-124E or placebo. The randomization will be assigned by the research pharmacist and will be blocked by age group (6-12 years versus 13-19 years) to ensure balanced groups. The investigators, lab personnel, research coordinators and participants will be blinded to the assignment. The research pharmacy will dispense the similarly appearing IMM-124E and placebo at baseline and again at Week 6.

4.3 Follow-up Visits

Participants will be required to return to the study site at Weeks 6 and 12. Each study visit will have a visit window of ±5 days, although the ideal visit date will fall on the exact anniversary date from randomization. Procedures to be performed at these visits can be seen in Attachment 1 (Schedule of Events).

The study assessments to be completed at each visit are outlined below:

Week 6 visit:

- Collection of vitals (including blood pressure, respiratory rate, heart rate, and body temperature)
- Anthropometric assessments (including height, weight, waist and hip circumference)
- Review of concomitant medications and adverse events
- Drug accountability assessment
- Drug dispense from research pharmacy
- laboratory assessments (see Blood Collection Schedule- Attachment 2 for more detail)
- Stool sample collection
- Blood spot collection

Week 12 visit:

- Administration of 3, 24-hour recalls (NDS-R) prior to visit
- PROMIS Questionnaires
- Physical Examination
- Collection of vitals (including blood pressure, respiratory rate, heart rate, and body temperature)
- Anthropometric assessments (including height, weight, waist and hip circumference)
- Review of concomitant medications and adverse events
- Drug accountability assessment
- 2-hour oral glucose tolerance test
- Participant counseling regarding follow-up care
- Fasting laboratory assessments (see Blood Collection Schedule- Attachment 2 for more detail)
- Stool sample collection
- Blood spot collection
- MRI
- Optional Sub-study: Accelerometer data collection for 1 week (prior to Week 12 visit)

Optional Sub-study: Accelerometers

An optional sub-study will be presented to subjects at the time of consent. Participation in this sub-study will be optional and will not be required if subjects are participating in the main study. Actigraph GT3X accelerometers will be given to subjects at the Baseline visit and prior to the Week 12 visit. Study staff will place the accelerometer around the subject's waist over the right hip and instruct them on how to properly replace the equipment after showering. Phone calls will be made to remind the subjects as needed. Subjects will collect 7 consecutive days of data and will bring the device back with them to their next appointment or mail the equipment back in prepaid mailers. A phone interview will be performed at the end of each monitoring period to record any unusual activity or episodes of removal.

4.4 Standardized Questionnaires

A number of standardized questionnaires will be administered at screening and throughout the duration of the study.

Alcohol Use Disorders Identification Test (AUDIT): This 10-item self-report questionnaire will be used at screening in order to determine that no significant alcohol consumption is present among participants.

24 hour food recall (NDSR 2016) - A triple pass, evaluation of current diet. The NDSR will be collected from all participants at the visits indicated in the Schedule of Events (Attachment 1).

Patient Reported Outcomes Measurement Information System (PROMIS) – A highly reliable tool for measuring patient-reported health statues for physical, mental, and social well-being. Selected questionnaires include: pediatric fatigue, depression, and anxiety.

Block Physical Activity Survey (NutritionQuest) – A 9 item screening tool that asks about frequency and duration of activities in the past 7 days.

4.5 Specimens

Plasma samples are all collected in EDTA tubes and plasma is separated within two hours of the blood draw. The samples are aliquoted into tubes and immediately frozen and stored at -80 C. Stool will be stored at -80 C. We will use the Nautilus software, by Thermo Scientific, which is a central laboratory information management system (LIMS) service provided by the ACTSI and Emory research laboratories. The systems facilities the tracking and management of specimens, laboratory inventories, and reagent ordering; the management of lab and QC data; the acquisition of data directly from instruments; and the exchange of laboratory results data. This will allow us to easily store and retrieve samples for future analyses.

4.6 Drug Accountability

In compliance with local regulatory requirements the Investigator or designated staff will document the amount of study drug administered to study participants as well as the amount returned by study participants. The study coordinator and research pharmacist will count all tablets returned by the participants at the visits indicated in the Schedule of Events (Attachment 1). Study drug accountability

logs will be maintained throughout the course of the study. In addition, the study coordinator will call participants in between visits to assess compliance. If study product compliance becomes an issue the study coordinator will discuss the importance of maintaining a proper dosing schedule and will try to identify any problems or barriers that exist in taking the study product. If doses are missed repeatedly, the Investigator will use her clinical judgment to determine the participant's ability to comply with the protocol and whether the participant should continue in the study.

4.7 Participant Retention:

Resources will be utilized to remove barriers to participation such as child or elder care, transportation and parking expenses. These resources may be provided as cash, transportation cost coverage and/or parking passes. An honorarium will be paid to participants in recognition of their time and effort when scheduled visits are completed successfully.

4.8 Management of Concurrent Conditions

During the screening period the PI will review concomitant medications on a case by case basis to determine if there are any potential drug interactions. If there is a potential drug interaction, the participant will not be randomized. Of note, there are no known product/drug interactions for IMM-124E.

4.9 Definitions:

Adverse event is any untoward medical occurrence in a participant participating in a clinical study that does not necessarily have a causal relationship with the study drug. It can also mean any unfavorable and unintended sign (including laboratory findings, symptom, or disease) temporarily associated with the use of IMM-124E or placebo, whether or not directly related to IMME-124E.

Suspected Adverse Reaction: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. All gastrointestinal adverse events that begin within a pre-defined period of administration of the product will be presumptively considered possibly related to the investigational product per FDA recommendation.

Serious adverse event or serious suspected adverse reaction

Any adverse event that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity (i.e. a substantial disruption in a participant's ability to conduct normal activities of daily living)
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Is an important medical event that, while it may not result in death or be immediately left-threatening or require/prolong hospitalization, may jeopardize the participant and/or may require medical or surgical intervention to prevent one of the outcomes listed above

Note that hospitalization due to disease progression is considered an SAE. Hospitalization for an elective or planned procedure to treat a pre-existing condition is not considered an SAE unless it results in one of the other outcomes listed above.

4.10 Safety Monitoring and Reporting

The investigators and site staff for this study will monitor and report adverse events to ensure patient safety. All untoward events occurring between the time of obtaining informed consent and following the last visit must be documented, regardless of whether they are considered study-related. The only exception is for participants who have withdrawn informed consent. Any AE that worsens in intensity, or becomes serious, should be recorded as a new event.

The DSMB will have semi-annual meetings where summary data on adverse events will be analyzed. Using Version 3.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE).81 signs and symptoms associated with adverse events will undergo assessment of intensity and will be graded by the clinical site staff as:

- Mild: Awareness of symptoms that are easily tolerated causing minimal discomfort and not interfering with everyday activities
- Moderate: Sufficient discomfort is present to cause interference with normal activity
- Severe: Extreme distress causing significant impairment of functioning or incapacitation. Prevents normal everyday activities.

The PI will submit a summary of study data findings to the IRB at the time of continuing review. Adverse event aggregate reports will not be submitted to the IRB, except those that involve unanticipated problems that might put participants at risk or protocol deviations.

The Investigator will coordinate with Emory Research Compliance office for the reporting of any and all IND safety reports to the FDA as *per* the requirements outlined in 21 CFR 312.32. A summary of all non-expedited safety reports will be submitted in the annual report.

Adverse events must be reported to the Emory University IRB according to IRB policy. SAEs will also be reported to the DSMB within 2 days or the event of notification of the event.

Protocol Deviations:

The PI and research team intend to not deviate from the protocol except in the case of a medical emergency. However, humans are by nature not perfect and sometimes protocol deviations occur. The Vos research team has instituted internal procedures to minimize protocol deviations. Emory University's IRB will be informed of any protocol deviations that occur according to applicable regulations and the IRB's established procedures.

A summary of adverse events will be reported to the FDA as part of the IND annual report.

Monitoring:

The research team will comply with their Monitoring Plan. This will involve monitoring by a compliance coordinator for the Center for Clinical and Translation Research at Emory University. Informed consents, protocol versions, source documents, CRFs, and AE reporting will be monitored. This will occur after the first two subjects have been enrolled and then will continue minimally once a year, if not more. A minimum of 10% of the subjects will be reviewed at each of the monitoring visits and a report will be provided to the Investigator at the conclusion of each monitoring visit.

4.11 Unblinding Procedures

Under normal circumstances, so as to not compromise the integrity of the study, the blind should not be broken until all participants have completed the study and the database in finalized. Otherwise, the blind should be broken only if specific emergency treatment would be dictated by knowing the treatment status of the participant.

Unblinding and disclosure to the participant, PI, and PCP may occur if any of the following conditions occur:

- Pregnancy (study product discontinued)
- Development of hepatotoxicity (study drug discontinued, unblinding may occur at the conclusion of the study)

If the treatment is unblinded for any participant, the participant will not be withdrawn from the study. The DSMB will review all instances of unblinding. Unblinded subjects will continue to be followed in the trial to allow for an intent-to-treat analysis.

5. Statistical Design and Analysis:

5.1 Primary endpoint

There is debate regarding utility of various surrogate markers of NAFLD in clinical trials[28]. Consensus opinion is that histology-based outcomes should be reserved for longer trials because of risks of liver biopsy. ALT is a "reasonably likely to predict clinical outcome surrogate marker" frequently used in phase I and II trials of NAFLD because it reflects inflammation in the liver. CK-18 detects hepatocellular death, however it is less useful in children[24]. Finally, while MRI is highly accurate for measuring steatosis and has been used extensively by our group[9, 29-31], change in steatosis is less predictive of longer term clinical benefit. Thus, we have selected the percent change in ALT as the primary efficacy related outcome. The amount of change in ALT that meets the surrogate marker standard is debated and depends on 1) the baseline ALT, 2) the length of time of treatment and 3) the mechanism of the product/drug. In this study, all participants will have at least ALT ≥ 2 x ULN at screening (girls ≥ 46, boys ≥ 54). Furthermore, a clinically relevant outcome would be at least a 25% reduction in ALT from baseline to week 12. Because this is a proof of concept study, if the primary outcome is not met but other important secondary markers are

met, this will support the design of future studies. In addition, other potential primary outcomes are being tested as secondary outcomes in this proof of concept study.

5.2 Secondary Endpoints

Secondary efficacy-related outcomes will include change in: GGT, fasting glucose and insulin, total body insulin sensitivity (OGTT), BMI z-score, waist circumference, and quality of life. Safety-related outcomes will include: sex hormones, proportion of participants with nausea, emesis, diarrhea, and any other adverse event, PROMIS, body fat by MRI, hepatic fat by MRI, and change in metabolic state.

5.3 Power Analysis:

A typical group of patients with NAFLD have a mean ALT level of 130 ± 63 U/L[10]. In the TONIC treatment study of children with NAFLD, there was a -20 ± 10 U/L and -30 ± 8 U/L change (average of 15% – 23% change) in ALT in treatment groups respectively and no change in ALT with placebo at 12 weeks[6]. A sample size of 11 participant per group is required to achieve a least 90% power to detect a 15% point difference in the average % change in ALT from baseline to week 12 between the two groups. Sample size was calculated assuming common standard deviation of 10% using a two-sided Mann-Whitney tests with a 0.05 significance level.

5.4 Statistical Analysis:

Primary Outcome Analysis:

Change in ALT from baseline to week 12 will be converted to a % change from baseline to account for the wide range of ALT values expected at baseline. A Mann-Whitney U test will be used to compare the percentage change in ALT among the two intervention groups. Data will be described as mean or median percent change, as appropriate.

General Statistical Considerations:

Analysis of primary and secondary outcomes will be performed using an intention to treat analysis. Descriptive variables of the cohort and subgroups will be analyzed using means and standard deviations, medians and ranges, or counts and percentages, as appropriate. For secondary measures, absolute change over time will be compared using mixed models or analysis of covariance. We will test the data to evaluate underlying model assumptions (i.e., normality, homoscedasticity). If these assumptions are not met, then we will attempt to transform the data. Standard transformations of the response variable such as the log, square root and Box-Cox transformations will be examined. If data transformation is inadequate to meet the analysis assumptions, then rank transformation of the data will be performed and the rank transformed response variables will be analyzed and reported. In situations where covariates could potentially have an effect on the response variable, we will add covariates to adjust for subgroup effects. Underlying model assumptions will be tested (such as homogeneity of slopes across exposure groups). Standard regression criteria will be used to assess appropriateness of including particular covariates. Collinearities will be reduced in multivariable modeling through the careful initial assessment of correlations and the avoidance of redundancies in candidate covariates.

With multiple follow-ups, longitudinal data will be available, and these repeated measures are likely to be correlated (i.e., weight, PROMIS measures, BMI-score, etc.). The analysis of repeated measures data is needed that properly accounts for the correlation between multiple observations from the same participant. Mixed effects models, accounting for the longitudinal measures, will be summarized with adjusted means and 95% confidence intervals for continuous outcomes. For categorical outcomes, we will apply generalized linear mixed models with appropriate distribution and link functions. In these models, the statistical test for interaction between time on study and intervention group will be the primary overall hypothesis to test to determine whether an outcomes in the two study groups changed in significantly different ways during follow-up (i.e., different temporal patterns over time). For significant interactions, post-hoc tests will be used to compare differences in the least square means at each time point in each intervention arm. All statistical tests will be two-sided and unadjusted for multiple comparisons due to the pilot nature of this trial.

Missing Data: A potential problem in any of the proposed analyses is missing data, which can cause the usual statistical analysis of complete available data to be subject to bias. Every effort will be made to minimize missing data on outcomes and covariates, and we will encourage patients that drop out of a study to return for final follow-up testing even if they missed previous scheduled evaluations.

Sensitivity Analysis: A sensitivity analysis will be performed by examining the effect of compliance (i.e., number of doses taken) on the primary and secondary endpoints.

5.5 Data Storage & Sharing:

Hard copy data will be entered by the study coordinators. All data will be stored in a REDCap database. Only study personnel (PI, Co-I's study coordinators, research assistants etc.) will have access to identifiable data. Data will be de-identified before analysis. Data will be stored in locked research material cabinets and in password-secured on the Emory network.

De-identified data will be shared with Immuron Ltd. and a sub-set of de-identified data will further be shared with Advanced MR Analytics AB (AMRA) who is providing imaging analysis services for the study.

5.6. Sample storage:

Because so much remains to be understood in pediatric NAFLD for mechanism, surrogate endpoints, treatment opportunities, the samples from this study have great potential use in further study with Emory investigators and investigators outside Emory. We will include a statement in the consent allowing indefinite use of de-identified samples until they are gone for studying questions related to improving the health of children.

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7.1 Attachment 1: Study Schedule of Events

	Screening (-6 weeks)	Baseline (Week 0)	Week 6	Week 12
Informed Consent and HIPAA Authorization	X			
Review Inclusion/Exclusion Criteria	X	X		
Randomization		X		
Demographics & Medical History	X			
Physical Exam		X		X
Vital Signs	X	X	X	X
Standardized lifestyle counseling		X		
Height	X	X	X	X
Weight	X	X	X	X
Waist and hip measurements	X	X	X	X
Alcohol Use Disorders Identification Test (AUDIT)	X			
24-hour food recalls (NDS-R) x 3	X			X
MRI	(X[1])	X		X
PROMIS Questionnaires (fatigue, depression, anxiety)		X		X
Physical Activity Assessment		X		X
Urine Pregnancy Test		X		
Oral Glucose Tolerance Test (2-hr)		X		X
Blood Draw (see Attachment 2)	X	X	X	X
Blood Spot		X	X	X
Stool Sample		X	X	X
Instructions for Drug Compliance		X		
Drug Dispense		X	X	
Discuss follow-up with primary hepatologist				X
Adverse Events Review		X	X	X
Concomitant Medication Review	X	X	X	X
Assess Drug Accountability			X	X
Sub-study: Accelerometer		X		X

[1] The Baseline MRI will be conducted at the Baseline visit for those subjects with a historic liver biopsy or MRI. Subjects with just a historic ultrasound may have their baseline MRI conducted up to two weeks prior to the baseline visit (during the screening period).

7.2 Attachment 2: Blood Collection Schedule (amounts in mL)

	Screening (-6 weeks)	Baseline (Week 0)	Week 6	Week 12	Total
Comprehensive Metabolic Panel	4	4	4	4	**16**
Serum	-	6	6	6	**18**
Plasma	-	12	6	12	**30**
Oral Glucose Tolerance Test (includes 4 mL 30 min serum tube)	-	9	-	9	**18**
PBMCs	8				**8**
Total	**12**	**31**	**16**	**31**	**90**