Omegaven™ and Parenteral Nutrition Associated Cholestasis (PNAC)

Kara Calkins, MD, Principle Investigator
Assistant Professor
Department of Pediatrics
Division of Developmental Biology and Neonatology
UCLA

James Dunn, MD, Co-investigator
Professor
Department of Surgery
Stanford University

Stephen Shew, MD, Co-Investigator
Associate Professor
Department of Surgery
Stanford University

Robert Venick, MD, Co-investigator
Associate Professor
Department of Pediatrics
Division of Pediatric Gastroenterology
UCLA

CFR 312.23, 12. 2-9.
Revisions: January 12, 2011, June 9, 2011, January 11, 2012, May 31, 2012, December 7, 2012, August 2013, November 21, 2013, November 13, 2014, March 6, 2015, May 6, 2015, June 02, 2015, March 5, 2016, February 12, 2018
IND 105, 326

Table of Contents

Introductory Statement

Short Bowel Syndrome (SBS) results from congenital intestinal disorders or intestinal resections that lead to significant malabsorption. SBS remains the most common cause of pediatric intestinal failure (IF) which is defined as the inability of the gastrointestinal tract to maintain adequate nutrient, fluid and electrolyte absorption to sustain life without total parenteral nutrition (PN) supplementation for > 60 days. Prior to the advent of PN, children unable to absorb adequate enteral nutrition (EN) due to insufficient bowel length or function would die of malnutrition and associated complications. **Today, > 20,000 patients with IF require PN for survival. The actual cost of maintaining a PN patient is estimated to be $100-150,000 per year. More importantly, 67% of SBS patients develop PNAC, and 25% progress to liver failure[8].** Infants with IF dependent on PN for > 1 year who develop severe PNAC will universally face mortality unless they receive a timely liver and/or small bowel transplant. In North America, due to the shortage of available donor organs, the highest death rates of all patients awaiting solid-organ transplantation occur in children with IF and PNAC who are awaiting combined intestine/liver transplants. **Transplants in and of themselves are not a panacea given that the five year survival rates post-transplant remain low (50-60%)[9]. Transplants and post-transplant care pose a large economic burden to society and the healthcare system.** The estimated cost of a transplant is ~$150,000 - $250,000 and post-transplant medications cost ~$12,000/year per child. Children on immunosuppressive drugs after transplant, are at higher risk for malignancy, life-threatening complications, and repeated, lengthy hospitalizations.

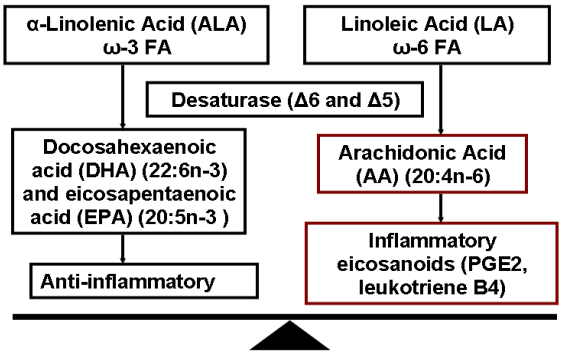

Fig.1 The metabolic pathway of ALA producing anti-inflammatory mediators and LA towards inflammatory mediators production is shown. DHA and EPA replacement from FO are expected to tip the balance towards anti-inflammatory mediators (CRP, TNFα, IL6).

Table1. Fatty Acid Composition of of Fish Oil and Soybean Oil per 10 gram of fat

	Linoleic (ω-6FA)	Linolenic (ω-3FA)	EPA (ω-3FA)	DHA (ω-3FA)
FO lipid emulsion	0.01-0.07	0.2	1.3-2.8	1.4-3.1
SO lipid emulsion	50	0.9	----	----

Prevention and treatment of PNAC is paramount, but lacking. The pathogenesis of PNAC is multifactorial. There is now cumulating evidence that suggests soybean oil (SO) is a major culprit in PNAC. SO is used to supplement PN to provide additional calories and prevent essential fatty acid deficiency. However, with a high content of phytosterols, SO impairs biliary secretion and promotes lipid accumulation. Moreover, SO contains a large amount omega-6 fatty acids and small amount of omega-3 fatty acids. **In animal and human studies, fish oil (FO), an alternate lipid emulsion that is rich in omega-3 fatty acids and lacks phytosterols (Table1), has been shown to ameliorate PNAC and improve morbidity and mortality[11,24,30-32].** By down-regulating

the production of inflammatory and thrombotic mediators and promoting hepatic protection by inducing an anti-inflammatory and anti-thrombotic state, FO could potentially reverse the development and progression of PNAC (Figure 1). To date, there is no randomized controlled study comparing FO to standard SO**.**

General Investigational Plan

The specific aim of this pilot study is to investigate if intravenous (IV) FO, commercially available as Omegaven™ (Fresenius Kabi), at 1 gm/kg/d, to children > 2 weeks, but < 18 years of age, will safely reverse or ameliorate liver disease in 80 subjects with PNAC. Study subjects will be compared to historical controls with PNAC who received standard SO . The following outcomes will be measured:

1) Reversal of PNAC in infants receiving FO
2) Growth of infants receiving FO
3) Safety of FO in infants with PNAC
4) Mechanism of FO

To address the above specific aims the following will be performed:
1) Serial laboratory tests to evaluate cholestasis and hepatic dysfunction (total and direct bilirbuin, ALT, AST, alkaline phosphatase, and GGT),
2) Serial anthropometric measurements (weight, length, and head circumference),
3) Serial laboratory tests to evaluate for safety (coagulation parameters, platelets and essential fatty acid profile)
4). Serial serum proteomic, inflammatory markers, phytosterols, microRNA and microarray analysis and fatty acid analysis of red blood cell (RBC) membranes
5) Serial fecal specimens will be collected to evaluate the intestinal microbiome in children at risk for PNAC.

PNAC is defined as a direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements. Reversal of PNAC will be defined as direct bilirubin < 2 mg/dL. on 2 occasions. Essential fatty acid deficiency will be defined as a triene:tetraene ratio ≥ 0.5.

Serial proteomic, microRNA, microarray analysis and analysis of fatty acids, sterols and markers of inflammation will provide insight into what proteins and genes/pathways are altered with FO treatment. Microbiome analysis will provide insight into how the intestinal microbiome is altered with PNAC and by FO. By comparing baseline analyses to analyses during specific time points during FO treatment, we will be able to detect up-regulation or down-regulation of specific proteins/genes. Fatty acid analysis of RBC membranes will provide insight in how FO alters the RBC membrane composition. Alterations in the fatty acid composition of the RBC membrane affects cell function and membrane fluidity.

Once the subject enters the study, FO will be administered for unlimited treatment period until the subject's PN is discontinued, the patient expires, or he/she receives a transplant. Should PN be terminated, FO will be stopped. If the patient receives a liver transplant, FO will be discontinued. For subjects who previously received FO treatment and re-develop biochemical evidence of PNAC off FO (direct bilirubin ≥ 2 mg/dL on 2 consecutive occasions separated by seven days), FO can be re-started if inclusion/exclusion criteria are satisfied. When FO is restarted, the protocol for laboratory and clinical monitoring will be followed. Patients who have received a liver transplant are NOT excluded from the study.

A patient can be withdrawn at any time should he suffer any side effect or adverse event that could affect his safety or jeopardize his health. The primary medical team can refer the patient for transplant evaluation at any time. This study will be conducted over 12-15 years. Prior to the beginning of the study, FDA and IRB approval will be obtained. All necessary staff education will be completed prior to enrolling patients. The study's goal is to enroll 80 subjects in 10-12 years. Final data analysis will be performed after the last subject completes the study, and will be performed in the last 4-6 months of the study. A Data Safety Monitoring Board (DSMB) will meet every 6 months to review any preliminary data, and assess for any side effects or adverse events. If after 4 or more DSMBs have been held and there are no concerns with safety, DSMBs can be held less frequently, but at a 1 year minimum. A DSMB can held at anytime should a subject, the study team, FDA or IRB have any concerns regarding the study in general. As of August 2017, a DSMB can be meet on as needed basis.

The primary endpoint will be time to resolution of PNAC (direct bilirubin < 2 mg/dL on 2 consecutive measurements). Secondary endpoints will be death, transplant, number of times FO is restarted, and time to full EN.

Statistical significance will assigned at a p value ≤ 0.05. Baseline difference and differences after intervention (liver function tests, INR, platelets, and triene:tetraene ratio, weight, length, and HC) between the two groups (FO vs SO) will be assessed by t tests for means and $\chi 2$ tests for proportions. Statistical analysis will also include, but not limited to, Kaplan-Meir survival curves, crude and hazard ratios, and logistic regression analysis for co-founding variables (ie gestational age, birthweight, number of surgeries, EN, episodes of line sepsis). The analysis will include an intention to treat analysis.

One formal interim analysis will be performed when 7 subjects have completed the study.

We will also retrospectively collect information on subjects who received FO at UCLA under compassionate use (EINDs 104,951, 104,766).

Specific Drug Information

Please see attached information regarding specific drug information provided by the supplier, Freseniurs Kabi, Hamburg, Germany.

FO, commercially available as Omegaven™ (Freseniurs Kabi), is a highly refined fish oil composed of essential fatty acids, α-tocopherol, glycerol, and purified egg phosphatide.

Therapy with FO will be initiated at starting dose of 0.5 gm/kg/d infused over 8 – 24 hours on the first and second day of therapy. On day three of the study, FO will be increased to 1 gm/kg/d and will not exceed 1 gm/kg/d (See Table 1). It will be infused intravenously through either a central or peripheral catheter in conjunction with PN. FO will always be administered with a dextrose containing solution. FO is isotonic, and will be kept at room temperature. It is compatible with PN solutions and may be co-infused via a y-site. FO may be infused through a 1.2 micron inline filter.

If subjects are hospitalized at the time of enrollment, they must be observed for 48 hours in the inpatient setting. If subjects are outpatients at the time of enrollment, the subject must be in observed in an outpatient clinical setting for the first two days that FO is administered. The outpatient subject must in observed in the clinical setting for the entire infusion and for 1 hour after the infusion has been terminated. This observational period will occur in the UCLA General Research Clinical Center (GCRC). After 48 hours of inpatient observation or 2 days of outpatient observation, and if the patient is considered medically stable and safe, FO can be administered at home by the subject's caregivers.

If FO is restarted because the subject continues to demonstrate evidence of PNAC or redevelops PNAC while off FO, and the subject tolerated FO originally without any adverse events/side effects, FO can be restarted at home.

	Dosage FO per kg body weight/day	Volume of FO per kg body weight/day
Day 1 and Day 2	0.5 g	5 mL
Day 3 +	1 g	10 mL

Table 1. Dose of FO.

FO is available as a 10% emulsion in 50 mL and 100 mL bottles. Containers will be shaken before use. When patients are hospitalized, depending upon the dose prescribed, rates less than or equal to 2 ml/hr will be provided in 30 ml syringes. Rates less than or equal to 4ml/hr will be provided in 60 ml syringes. Rates greater than 4ml/hr will be provided in glass bottles.

FO in the hospital can be split equally in 2 syringes/bottles. Because patients often only have one site for infusions, and require multiple intravenous medications, dividing the dose in 2 syringes/bottles will ensure that the subject receives the entire FO dose in a 24 hour period. The first syringe/bottle will be administered immediately when it arrives at the patient's bedside. The second syringe/bottle will be placed in fridge temporarily until the first infusion is completed. 30 minutes prior to the projected start time of the second infusion, the second syringe/bottle of FO will be allowed to come to room temperature prior to infusing.

Once the FO is placed in a syringe/bottle, it has an expiration of 24 hours. Once it is placed on a pump, it expires in 12 hours. Pharmacists will dispense the FO, and experienced health professionals or trained caregivers will administer the FO.

If additional fat calories are needed, they will be provided via the enteral route. If additional non- protein calories are needed, they will be provided as carbohydrates. No other parenteral form of fat emulsion will be used during FO therapy. The same standards of care provided to all patients receiving PN solution will be followed. The primary team will be responsible for the patient's medical care.

At home, the vial of FO will punctured with intravenous tubing, and administered with the appropriate home supplies. Parents or legal guardians will receive sufficient training on how to administer PN and FO.

If a subject is admitted to the hospital, FO will be held on the day of admission, and restarted on the second hospital day.

If a subject is scheduled for an elective operation, FO will be held the three days prior to surgery, and restarted four days after surgery. If there are any concerns for an increase risk of bleeding from baseline status, FO will be held until the concern has been addressed by the primary medical team. IF there is an emergent surgery, FO will be held prior to going to the operating room for the surgery. If at the time of consent it is known that elective operation is scheduled or required, FO will start 4-14 days after the operation is performed, depending on the patient's risk for bleeding (age, degree of IFALD, other comorbid conditions). The study team in consultation with the primary team will decided when to start FO.

To our knowledge, the drug has not been withdrawn from a study or marketing in any country for any reason related to safety or effectiveness.

Please attached copy of the drug's brochure which includes a description of the drug substance and formulation, pharmacological and toxicological effects, pharmacokinetics and biological disposition.

Please attached publications by Gura, et al, and Diamond, et al, for information relating to safety and efficacy.

Summary of Previous Human Experience with FO

PNAC

Neonates who are unable to absorb EN secondary to insufficient intestinal length and/or gastrointestinal disorders are dependent on PN. The goal of PN is to provide sufficient calories and prevent nutritional deficiencies during protracted periods of fasting in order to sustain adequate growth and development. PN, however, is associated with the development of PNAC, which carries significant morbidity and mortality in the neonatal population.

Approximately 20,000 patients with intestinal failure in the United States are dependent on PN for survival.[3] Approximately 30-60% of pediatric patients receiving PN develop PNAC.[4] PNAC is characterized by hepatic biochemical and histological alterations. Patients exhibit elevated liver function tests and direct bilirubins, and liver biopsies demonstrate fibrosis, cirrhosis, lipidosis, and steatosis.[5] The development and progression of PNAC is unpredictable in the pediatric population. Risk factors for PNAC include SBS, frequent surgical procedures, necrotizing enterocolitis (NEC), prematurity, low birth weight, small for gestational age, sepsis, and lack of EN. [6]

SBS results from a congenital disorder or surgical resection secondary to NEC or other gastrointestinal pathology that causes significant malabsorption.[7] Unable to maintain sufficient protein, fluid, electrolyte, and micronutrient intake, these patients become malnourished, and as a result, are dependent on PN for survival. SBS carries significant morbidity and mortality. The most common complication of SBS is PNAC; approximately 67% of patients with SBS develop PNAC, and 25% progress to liver failure.[8] **SBS remains the most common cause of intestinal failure in children.[9] It is the most common pre-transplant diagnosis in this population.[9] In North America, the highest death rates on the intestine transplant waiting list occur in young children who need combined intestine/liver transplants.[10]**

The ability to be weaned off of PN, or potential for bowel adaptation, depends on the primary diagnosis for intestinal failure, the amount of bowel remaining, the presence of an ileocecal valve (ICV), patient age, history of septic episodes, and presence of bacterial overgrowth.[35,36] Patients with more ileum than jejunum are more likely to achieve successful adaptation. Patients with greater than 40 cm of small bowel and an ICV are highly likely to attain indepenence from PN.[36] In fact, it has been reported that as little as 11 cm of jejunoileum with an ICV and as little as 25 cm without an ICV can survive and sustain normal growth without the assistance of PN. [37]

In addition to SBS, prematurity also places infants at high risk for PNAC.[11] Risks factors in this population include an immature biliary system, absence of EN, bacterial

overgrowth, and sepsis[12,13,8]. Moreover, 5-10% of infants who weigh less than 1,500 grams develop NEC, which may necessitate bowel resection.[14] Like SBS patients, low birth weight neonates can remain dependent for PN for long periods of time. In fact, infants with a birth weight less than 750 grams have a 39% likelihood of developing PNAC.[15]

Because small bowel length increases rapidly during gestation, it is questioned whether the amount of small bowel present in a premature infant after resection is comparable to full-term infant after resection. Rather than relying by the absolute of bowel remaining at the time of intestinal resection, the percentage of expected normal length may be more accuate in predicting ability to achieve complete bowel adaptation. Based off of existing data for the normal small bowel length in a neonate according gestational age, a percentage of their expected normal length can be calculated. Infants with less than 10% of expected normal small bowel length have a 10% chance of weaning from PN, while those with greater than or equal to 10% have an 80% chance of weaning.[38]

Prevention and treatment of PNAC remains critical, but is limited and lacking. EN can slow the progression and reverse PNAC.[16] However, for patients who are unable to wean sufficiently or are dependent on PN for extended periods of times, PNAC can progress to liver failure and in the absence of a transplant, can lead to death. **Studies report a mortality rate approaching 100% for patients unable to be weaned off PN within a year of diagnosis unless they are transplanted.[1]** Survival rates for combined small bowel and liver and liver transplants alone remain dismal. At five years post-graft, approximately 43-75% and 57-75% of patients transplanted for combined small bowel and liver and liver alone transplants are alive.[9]

The pathogenesis of PNAC is multi-factorial, and still poorly understood. The etiology likely stems from a combination of an immature liver, bacterial overgrowth, decreased secretion of bile acids and enteral hormones due to fasting, and atrophic intestinal mucosa.[17,18,19] Research has also focused on specific components or lack of components in PN that contribute to PNAC. Previous work has implicated excessive caloric administration, concentration, composition of amino acid formulations, and specific micronutritients.[20,21,22] **There is now cumulating evidence that intravenous lipids may play a crucial role development and progression of PNAC**.

Role of Intravenous Fat Emulsion on PNAC

Intralipid™ and Liposyn™ are standard lipid emulsions used to supplement PN to provide additional calories and prevent essential fatty acid deficiency. Intralipid™ is soybean based and Liposyn™is both soybean and safflower based. Both emulsions are composed of phytosterols, which are thought to impair biliary secretion. Moreover, the accumulation of lipids in Kupffer cells may further impair liver function.

There exists an abundance of literature that suggests the type of long chain polyunsaturated fatty acid (LCPUFA) present in the lipid emulsion is the primary culprit in the development of PNAC. LCPUFAs, known as omega-6 and omega-3 fatty acids, share a common metabolic pathway and rate limiting enzymes. Important omega-3 fatty acids are α-linolenic acid (ALA), eicosapentaenic acid (EPA), and docosahexaenoic acid (DHA). The human body cannot synthesize omega-3 fatty acids de novo, however, it can utilize ALA to generate other omega-3 fatty acids. These conversions compete with omega-6 fatty acids. In other words, in the presence of high concentrations of omega-6 fatty acids, the production of omega-3 fatty acids is slowed.

The omega-6 fatty acid, arachidonic acid, produces pro-inflammatory and pro-thrombotic leukotrienes, prostaglandins, and eicosanoids. As a result, the ratio omega-6 to omega-3 fatty acids (n6:n3) creates a balance between a pro-inflammatory and anti-inflammatory state. Western diets are estimated to have n6:n3 ratio of approximately 16:1, and this ratio has been implicated in many disease processes such as cardiovascular disease.[23]

Predominately composed of omega-6 fatty acids, Interlipid™ has an n6:n3 ratio of 5.5:1.[24] Omegaven™ (FO), on the other hand, is a rich in omega-3 fatty acids. FO down-regulates the production of inflammatory, thrombotic mediators, and promotes hepatic protection by inducing an anti-inflammatory state.

In addition to the ratio n6:n3 present in lipid emulsions, the mechanism of metabolism and route of administration of the fatty acid may contribute to PNAC. Enteral lipids are packaged into chylomicrons that acquire apolipoproteins, and are eventually metabolized by the liver. In contrast, the particles in Intralipid™ are similar to endogenous chylomicrons, but differ in their content. These artificial particles contain essential fatty acids and omega-6 triglycerides, and lack cholesterol and protein. Recent studies suggest that lipid emulsions rich in omega-6 fatty acids, like Intralipid™ are metabolized with less lipolysis, and may be cleared as whole particles by tissues other than the liver.[25]

The metabolism and clearance of omega-3 fatty acids, on the other hand, is unknown, but appears to independent of the pathways mentioned above.[26] Lipid emulsions rich in omega-3 fatty acids have been shown to decrease de novo lipogenesis[27], stimulate β-oxidation of fatty acids and suppress superoxidase dismutase and glutathione peroxidase, two enzymes responsible for oxidative injury.[24] All of these mechanisms are implicated in the reduction of hepatic steatosis.

Human Studies

Epidemiological and experimental studies have demonstrated that a diet rich in omega-3 fatty acids prevents certain adult-onset diseases such as the metabolic syndrome, cancer, and neurological disorders.[39] In fact, the FDA has approved a prescription form of omega-3 fatty acids as a treatment for hypertriglyceridemia.[40]

With regards to the neonatal population, **there are now published reports of the use FO for treatment of PNAC.** In 2006, Gura et al published 2 case reports of infants with PNAC treated with Omegaven™ at Boston Childrens' Hospital. In both cases, PNAC was reversed.[30] In 2007, Gura et al published their results of 18 infants who developed PNAC while receiving Intralipid™ and were treated with FO, available as Omegaven™ (Fresenius Kabi). These infants were compared to a historical cohort of 21 infants treated with Intralipid™. Patients were eligible for treatment with FO if they had a bilirubin greater than 2 mg/dL and a predicted PN duration of 30 days or more because of an acquired or congenital gastrointestinal disease. Baseline characteristics between the two populations did not differ. However, although not statistically significant, the FO cohot had a higher direct bilirubin at the entry of the study in comparison to the historical patients (5.4 vs 3.5 mg/dL). Patients in the FO cohort had their SO discontinued, and their Omegaven™ was advanced to 1 gm/kg/day by day three of therapy.

The median time to reversal of PNAC in neonates receiving FO was 9.4 weeks in comparison to 44.1 weeks in historical controls. Reversal of cholestasis was defined as a direct bilirubin less than 2 mg/dL. 38% (7 subjects total) of the FO cohort never reversed their cholestasis, while 67% (14 subjects) in the historical cohort did not reverse their cholestasis. Those receving FO were 4.8 times more likely to reverse their PNAC, and 6.8 times more likely to reverse their PNAC when the analysis was adjusted for initial bilirubin concentration, gestational age and NEC.

Two of the 7 subjects in the FO cohort who had persistant PNAC died, in comparison to 7 of the 14 subjects in the historical cohort who had persistant PNAC. 2 patients in the historical cohort received a liver transplant and were amoung the deaths. There were no transplants in the interventional group. The 2 patients that died in the FO group died of non-liver related causes. In comparison, 6 of the 7 patients who died in the historical cohort died of liver related causes.

The median time to PN cessation was statistically significant between to the two groups. In the FO cohort, the average time to be successfully weaned off PN was 13.8 weeks, in comparison to 22.9 weeks in the historical cohort. Although not statistically significant, **the risk of death between the FO and historical cohort was 11.1% and 33.3% respectively.[31]**

More recently, Diamond, et al published a retrospective cohort describing 12 children with SBS who received FO. Patients, received both Intralipids™ and FO at 1 gm/kg/d. However, if the patients did not demonstrate significant progress on this regimen, the Intralipid™ dose was reduced or discontinued completely. The median starting direct bilirubin was 8.06 mg/dL. Of the 12 patients who received FO therapy, 9 patients demonstrated complete resolution of hyperbilirubinemia. Complete resolution was defined as a direct bilirubin of zero. The median time to reverse PNAC was 24 weeks. 5 patients who received FO and Intralipids achieved complete resolution only after Intralipids™ were discontinued. All patients who demonstrated reversal of cholestasis

were delisted for transplantation. 2 of the 3 patients whose PNAC did not resolve received transplants.[32]

Safety markers have also been evaluated for patients on FO, specifically risks for essential fatty acid deficiency and bleeding. FO contains 0.1-0.7% linoleic acids, which has been demonstrated to prevent essential fatty deficiency.[34] There are no reports of essential fatty acid deficiency with the use FO in the pediatric patient population with PNAC.

Because omega-3 fatty acids inhibit cycloxygenase and other factors leading to decreased platelet aggregation, there is a potential concern that FO could increase the risk for bleeding. Both Gura, et al and Diamond, et al reported no change in coagulation studies with use of FO.[31,32] Clinical trial evidence does not support an increased risk of bleeding in adult patients with cardiovascular disease treated with fish oil even when used in combination with agents such aspirin and warafin.[33,41] Boston Childrens' Hospital has now treated over 100 patients with FO and has not reported any adverse events or side effects with this therapy.

At UCLA, FO has been provided to two patients with SBS and PNAC (IND 104766, 104951). Both patients have not experienced any side effects or adverse events. Patient 1 (IND 104766) has been on FO for approximately 16 weeks, and patient 2 (IND 104951) has been on FO for approximately 12 weeks. Both patients have demonstrated remarkable improvement in their liver function improvement. In fact, patient 2's baseline total and direct bilirubin prior to FO was 19.7 and 10.5 mg/dL; her liver enzymes, AST and ALT, were 466 and 296 respectively. In addition, she had hypertriglyceridemia with a triglyceride trough of 244. Her most recent total and direct bilirubin are 1 and 0.4 mg/dL with an AST and ALT of 71 and 98. Her triglyceride level has also improved; it was most recently measured at 78.

Animal studies and limited human studies have provided insight into how FO may possibly reverse or protect against the development of PNAC. It is postulated that FO alters glucose and lipid metabolism (specifically de novo lipogenesis), decreases inflammation and thrombosis, and alters oxidant/antioxidant activity. Further studies are needed to elucidate the exact mechanism in the SBS/PNAC population.

Lastly, despite promising FO case reports, there is not a randomized controlled trial comparing FO to standard SO at the same dose.

The Overall Plan

1. Rationale and Indication

Alternative nutritional and pharmacological strategies are imperative to improve the clinical outcome and quality of life of SBS, IF, and PNAC patients. Recent data has revealed that when standard lipid emulsions are replaced with FO, which lacks phytosterols and is rich in omega-3 fatty acids, PNAC is ameliorated or reversed. **The purpose of this pilot study is to investigate the use of FO (Omegaven™), administered at 1 gm/kg/d IV, in children with PNAC.** ***We hypothesize that FO will be a safe therapy to reduce PNAC in children*****.** Study subjects will be compared to historical controls with SBS and PNAC receiving standard SO. The following clinical outcomes will be measured:

1) Reversal of PNAC in infants receiving Omegaven™
2) Growth of infants receiving Omegaven™
3) Safety of Omegaven™ in infants with PNAC
4) Mechanism of Omegaven™

To address the above specific outcomes the following will be performed:
1) Serial laboratory tests to evaluate cholestasis and hepatic dysfunction (total and direct biliribin, ALT, AST, alkaline phosphatase, and GGT)
2) Serial anthropometric measurements (weight, length, and head circumference)
3) Serial laboratory tests to evaluate safety (coagulation parameters, platelets and essential fatty acid profile)
4) Serial serum microarray, proteomic, inflammatory markers, bile acids phytosterols and microRNA analysis and fatty acid analysis of red blood cell (RBC) membranes.
5) Serial fecal specimens will be collected to evaluate the intestinal microbiome in children at risk for PNAC.

Considering the high morbidity and mortality associated with PNAC in specific populations, this pilot study has the potential to provide valuable information about a potential treatment. Moreover, this pilot study could cautapult additional studies such as a randomized, controlled trial comparing FO to SO, and assessing FO's impact on metabolism and neurodevelopment.

2. General Approach

This study targets a high risk population for IF and PNAC; infants with SBS and/or congenital gastrointestinal disorders, and premature infants with a history of nectrotizing entrocolitis.

INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA:

Clinical evidence of PNAC
Direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements
Expected PN course greater than 30 days
Acquired or congenital GI disease
>2 weeks of age and <18 years
>60% of calories from TPN
Failed standard therapies to prevent progression of liver disease (Actigal, cyclic PN, avoidance of overfeeding, reduction/removal of copper from PN if elevated by laboratory analysis, advancement of EN)

EXCLUSION CRITERIA:

Inborn errors of metabolism
Extracorporeal Membrane Oxygenation (ECMO)
Seafood, egg, or Omegaven™ allergy
Documented cause of liver disease other than PNAC
Hemorrhagic disorder
Anticoagulant therapy
Hemodynamically unstable or in shock
Comatose state
Stroke, pulmonary embolism, recent myocardial infarction
Diabetes
Fatal chromosomal disorder
Enrollment in any other clinical trial involving an investigational agent
Patient, parents, or legal guardians unable or unwilling to give informed consent
Patient expected to weaned from PN in 30 days
Unable to tolerate necessary laboratory monitoring
Patient requiring aspirin or toradel or motrin
Patient requiring dialysis

All inclusion and exclusion criteria must be satisfied in order for the subject to be enrolled in the pilot study. If the subject meets inclusion and exclusion criteria, and the

informed consent is signed, the patient will enter the study. Baby aspirin is not considered a contraindication.

DATA COLLECTION

Demographic and clinical information will be collected when the study is initiated and throughout the study. All labs will be drawn per the primary medical team.

While it is standard that labs are drawn on MOST SBS, IF and PNAC patients on a weekly basis, the frequency and type of lab varies depends on the primary medical team and patient's condition (extent of disease, age, gestational age, presence of anemia, change in baseline status, etc). It is considered standard of care that patients who have been receiving PN for > 14 days, have electrolytes, renal function, glucose, total and direct bilirubin, and AST/ALT drawn on a weekly basis. It is also considered standard of care by many physicians to draw a weekly triglyceride trough, alkaline phosphatase, GGT, albumin/pre-albumin, total protein and complete blood count.

For patients on long-term PN (PN for couple-several months) who have demonstrated stability with regards to growth, electrolyte balance, and liver function, many physicians consider it standard of care to draw labs every 2 – 4 weeks.

For patients with advanced PNAC, it is considered standard to intermittently draw coagulation studies. Essential fatty acid profiles are collected for patients who are suspected to have an essential fatty acid deficiency (based off of clinical signs and symptoms) and who are not receiving sufficient essential fatty acids via the enteral and/or intravenous route.

All of the above labs that are performed as part of standard of care by the primary medical team will be collected and recorded in the appropriate case report form. Subjects will have labs drawn at least monthly by the primary medical team. However, labs can be drawn more frequently per the primary medical team.

Growth will be assessed monthly by measuring weight, length, and head circumference. Bedside nurses or the nurse in Intestinal Failure Clinic will perform these measurements.

Serum microarray, proteomic, inflammatory markers, phytosterols, bile acids, RBC membrane fatty acid composition and microRNA analysis will be performed at baseline, 2 weeks, 3 months, and 6 months. Thereafter, serum will be collected every six- month until FO has been stopped and for subjects who continue to remain on PN (regardless of the lipid emulsion).

Fecal specimens will be performed at baseline, 2 weeks, 3 months, and 6 months, and, thereafter every six- month until FO discontinued and/or should the subject remain PN dependent.

Data on the patient's status, specifically, liver function and growth/nutrition/development will be collected**.** Data will be collected during the study, post-FO if the patient remains PN dependent (regardless of the type of lipid emulsion) for a total of five years.

We will also retrospectively collect information on subjects who received FO at UCLA under compassionate use (EINDs 104,951, 104,766).

MONITORING

Hospitalized Subjects:

If the subject is hospitalized, he will be monitored in the inpatient setting. If hospitalized at the time of enrollment, the patient must remain hospitalized for at least 48 hours to ensure subject's tolerance to FO, observe for any side effects/adverse events, and training/counseling of the subject's parents/legal guardians. The patient will be monitored per standard clinical protocol for pediatric inpatients.

Baseline and weekly/bimonthly/monthly labs will be ordered by the primary team per standard of care. These laboratory results will be collected from the subjects' chart and will be used to monitor for any side effects/adverse events.

The PI or any co-investigator will evaluate the patient daily for the first 48 hours of therapy. Thereafter, the PI or one of the co-investigators will evaluate the inpatient subject at least 4 times per week. The PI or any co-investigator will review the subjects' medical chart, and question the bedside nurses, medical team, and parents/legal guardians regarding any side effects and/or adverse events. Please see section entitled, "Anticipated Side Effects" for specific details on anticipated side effects, monitoring, and recording of any side effect or adverse event. Physical exams will be performed daily, and bedside monitoring per inpatient protocol (ie vital signs, etc) will be performed daily.

Prior to discharge from the hospital, the Intestinal Failure Team and medical team will train the parents/legal guardians on how to administer PN and FO. Subjects will only be discharged home once caregivers demonstrate proficiency on how to administer PN and FO.

Outpatient Subjects:

If the subject is enrolled as an outpatient, the subject can receive the first two doses of FO in a clinical setting (UCLA General Research Clinical Center (GCRC)). The subject will be observed for the entire infusion period and 1 hour after the infusion. Vitals will be performed prior, every two hours, and 1 hour after the termination of FO. Trained nurses are available at the GCRC to perform vitals and observe for any side effects/adverse events.

Once the subject is discharged from the inpatient setting or has received the first two doses of FO in the GCRC, he will be monitored in the Intestinal Failure Clinic or GCRC by the PI, Dr. Kara Calkins, or any of the co-investigators. The Intestinal Failure Clinic provides a multi-disciplinary approach to children with SBS, IF, and PNAC. Trained nurses, dieticians, and social workers are available at all clinic visits. The GCRC provides outpatient rooms and support personnel (clinical nurses). Outpatient subjects will be seen on a weekly, bimonthly, or monthly basis in the Intestinal Failure Clinic or GCRC. It is standard of care for patients with SBS/IF and advanced PNAC to be seen as often as bi-weekly and as infrequently as monthly as an outpatient. Outpatient subjects can be seen by the study team as frequently as once a week. At minimum, outpatient subjects will be seen by the study team every month. The primary medical team per standard of care will determine the frequency of visits to the IF clinic. Should a patient not be scheduled for the IF clinic 4 weeks after the last clinic visit, the patient will be scheduled to be seen by the study team in the GCRC.

At each follow-up visit, parents will be questioned regarding any adverse events or side effects. A full of physical exam and review of symptoms will be performed at each clinic visit. Please see section entitled, "Anticipated Side Effects" for specific details on anticipated side effects, and monitoring and recording of any side effect or adverse event.

Should any adverse events or side effects be noted, they will be promptly reported to the IRB and FDA. All guidelines for reporting side effects and adverse events will be followed.

The primary medical team, inpatient pharmacy, and parents/legal guardians will be able to contact the PI or one of the co-investigators at anytime regarding any questions or concerns regarding the study or FO via the UCLA operator.

All subjects will be followed until one month after the termination of FO. During this 1 month follow-up period, patients will be seen in the Intestinal Failure Clinic or GCRC at least once, and laboratory monitoring will be continued per the primary medical team. Should the patient continue to demonstrate evidence of PNAC or redevelop PNAC off of FO (if FO was previously discontinued) as indicated by a direct biliribuin ≥ 2 mg/dL on 2 consecutive occasions, FO may be restarted if inclusion and exclusion criteria are still satisfied. Each time, FO is restarted, the subjects will be followed according the protocol. After the termination of each Omegaven treatment, subjects will be followed for 1 month.

Data Safety and Monitoring Board (DSMB) composed of a neonatologist (Meena Garg, MD), pharmacist (Christina Shin , Pharm D),and statistician (Kate Crespi, PhD) will met every 6-12 months to review any adverse events or side effects and assess any preliminary data. This committee will be involved in performing the early evaluations of the data at annual intervals to determine if significant results are obtained to warrant discontinuation of the trial. All complications, adverse effects, or results that warrant discontinuation of the trial will be promptly reported to the UCLA Institutional Review Board and FDA. As of date 08/03/17, the DSMB will only meet on as needed basis as

determined by the investigator, DSMB member, or another regulatory body. Annual updates will be provided to all DSMB members via email. Confirmation that these annual updates were reviewed by the respective DSMB member will be documented by a confirmatory email from the DSMB member. Should a DSMB member voice any concerns, a formal DSMB can be held to address these concerns. This documentation will be provided to the IRB.

WITHDRAWAL CRITERIA

A patient can be withdrawn at any time should he suffer any side effect or adverse event that could affect his safety or jeopardize his health.

WITHDRAWAL CRITERIA:

Patient, parent, or legal guardian request to discontinue treatment and/or observation for any reason
Uncontrolled bleeding attributed to FO
Anaphylaxis due to FO
Decision by PI that study withdrawal is in the subject's best medical interest
Patient is lost to follow-up
Serious adverse event
Physician decision based on safety of the drug in this particular patient

Possible Anticipated Risks

For contraindications to Omegaven™ see Table 2.

Impaired lipid metabolism	Severe hemorrhagic disorders
Unstable diabetes mellitus	Collapse and shock
Stroke	Embolism
Recent cardiac infarction	Undefined coma status

Table 2. Contraindications to Omegaven™.

Omegaven™ will not be administered to anyone who is hemodynamically unstable or in shock, in a comatose state, and anyone who has been diagnosised with stroke, pulmonary embolism, or recent myocardial infarction

Side Effects Specific to Omegaven™	**Side Effects Common to Fat Emulsions**
Allergy	Fat Overload Syndrome
Increased Risk of Bleeding	Metabolic Overload Syndrome
Essential Fatty Acid Deficiency	Hyperglycemia
Hyperglycemia	Hypertriglyceridemia

Hyperinsulinemia/hypoglycemia	Change in Temperature/Chills/Flushing
	Changes in Blood Pressure
	Cyanosis/Shortness of Breath
	Naseau/Vomitting
	Pain

Table 3. Possible Side Effects.

See Table 3 for possible side effects to FO and fat emulsions in general.

Omegaven™ will not be administered to anyone allergic to seafood or egg, who has a hemorrhagic disorder, or is on anticoagulant therapy. Patients will continue to receive heparin in their PN.

Possible Side effects specific to Omegaven™:

Allergic Reaction:
Definition: Allergy is defined as a disorder of the immune system; it is characterized by excessive activation of mast cells and basophils by IgE resulting in an inflammatory response. The presentation of allergy varies. It can present with a rash (eczema or hives), itching or red eyes, airway symptoms (wheezing, dyspnea, or angioedema), gastrointestinal symptoms (abdominal distention, vomiting, or diarrhea), or anaphylaxis.
How to Identify: The patient will be assessed for any signs or symptoms by physical exam and medical and patient report. Parents will be questioned at each clinic visit for any signs or symptoms of allergy.
Treatment/Withdrawal: In the case that the patient develops an allergy in response to the FO, the patient will be given Benadryl, Albuterol for respiratory symptoms, or other treatments per the primary medical team. If the symptoms continue to coincide with additional uses of FO, the patient will be withdrawn from the study or premedicated with Benadryl. The decision to withdraw or continue the patient in the study will be made after a discussion with the parents/legal guardians of the subject. If the patient develops an anaphylactic reaction or angioedema to FO, the FO will be discontinued, and the appropriate steps, such as Benadryl, epinephrine, steroids, and volume and airway resuscitation will be given per the primary medical team. The patient will be withdrawn from the study immediately.
Note: Patients allergic to fish or egg are excluded from receiving FO.

Increased Risk of Bleeding
Definition: increased bleeding above baseline
How to Identify: Subjects will be monitored according to protocol and questioned regarding an increase in bleeding above baseline
Treatment/Withdrawal: Treatment for bleeding will be according routine practice and the medical team. If bleeding is primarily attributed to the intervention, the patient can be withdrawn from the study. All subjects prior to an elective operation will have Omegaven held for 3 days prior and 4 days after the elective operation. If at the time of consent it is known that an elective operation will be performed or schedule, FO will

start 4-14 days after this surgery, depending on the patient's risk for bleeding (age, degree of IFALD, other comorbid condition). The study team in consultation with the primary team will decided when to start FO. Any subject with an active bleed, that is considered greater than baseline, will have their Omegaven held until the bleeding has decreased or resolved. All decisions to hold Omegaven will be discussed with the primary medical team and legal guardians/parents. In order to reduce the risk for bleeding, subjects who require Motrin, Toradel or Aspirin, along with dialysis will be excluded from the study. All subjects on the study should be maintained on an acid suppressor to reduce the risk for a gastrointestinal ulcer. During the study, Motrin, Toradel and Aspirin is contraindicated; if these drug are medically necessary, the patient will be withdrawn from the study.
Note: All subjects enrolled this study have an increased baseline risk for bleeding because they have cholestasis. Subjects with advanced cholestasis and portal hypertension, have an even greater risk for bleeding. While Omegaven may carry the risk of increased bleeding, it is unclear if the benefit outweighs the risk.

Prolonged Bleeding Time
Definition: INR > 1.4
How to Identify: Coagulation studies will be collected and reviewed. The patient will be assessed for any clinical bleeding by physical exam and medical and patient report. Parents will be questioned at each clinic visit and asked to notify the PI or any co-investigator of any signs of clinical bleeding (petechiae, nosebleeds, blood per rectum, gum bleeding, hematemesis, etc)
Treatment/Withdrawal: Product replacement (FFP, platelets, etc) will be given per the primary medical team.
Notes: FO will not be administered to patients on anti-coagulant therapy or who have a hemorrhagic disorder. Should the patient require anticoagulant therapy during the study period, the subject will be withdrawn. The patient will continue to receive heparin in their PN. Although there is a theoretical risk that FO could prolong a patient's bleeding time, published literature demonstrates that there is no difference between the INRs of PNAC patients on FO versus those on SO[31].

Essential Fatty Acid Deficiency
Definition: Essential fatty acid definition will be defined as triene:tetraene ratio ≥ 0.5.
How to Identify: The ratio will be determined by an essential fatty acid profile, which will be monitored per the primary medical team. The patient will assessed for any clinical signs of essential fatty acid deficiency by physical exam and medical and patient report. Parents will be questioned at each clinic visit and asked to notify the PI or any co-investigator of any signs of an essential fatty acid deficiency (alopecia or dermatitis). Moreover, since thrombocytopenia can occur with essential fatty acid deficiency, platelets will be monitored. Should the patient exhibit possible signs or symptoms of this deficiency, an essential fatty acid profile will be performed by laboratory analysis.
Treatment/Withdrawal: If the patient demonstrates an essential fatty acid deficiency (triene:tetraene ratio ≥ 0.5), the patient will be withdrawn from the study.

Hyperinsulinemia/Hypoglycemia

Definition: insulin concentration > 25 uU/mL and persistent hypoglycemia (glucose concentration < 40 mg/dL after PN has been discontinued), history of significant exposure to a high glucose delivery rate (glucose delivery rate > 16 mg/kg/min with continuous PN), and Endocrinology consult verifying hypoglycemia is not secondary to advaced liver disease.

How to Identify: Should the subject demonstrate persistent hypoglycemia when PN is weaned or discontinued, have been exposed to a high glucose delivery rate for a prolonged period of time, and the primary team have a high suspicion of hyperinsulinemia, a serum insulin concentration can be checked when the subject is hypoglycemic. Should the insulin level be elevated, while the glucose concentration is depressed, the patient MAY have hyperinsulinemia. Further investigation may be warranted, and a consult with Endocrinology should be sought to determine the exact etiology.

Treatment/Withdrawal: The family will be informed, and given the option to withdraw. The primary team will also be encouraged to slowly decrease the glucose delivery rate. Recommendations from Endocrinology should be followed.

Side effects common to FO and other fat emulsions such as Intralipids™ include:

Fat Overload Syndrome

Definition: Overdose of FO or SO can result in Fat Overload Syndrome. Fat Overload Syndrome occurs when a fat emulsion is infused too rapidly, or when the patient experiences a change in clinical status such as sepsis or renal impairment, and the patient's triglyceride trough is abnormally high (> 300 mg/dL).

How to Identify: Bedside nursing and parents or legal guardians will be counseled to infuse the FO at the prescribed rate. The PI or any co-investigator will review the medical charts to ensure the FO was administered at the proper rate. Moreover, bedside nursing and parents or legal guardians will be questioned at what rate the FO is being administered. Should the FO be infused too quickly, nurses and caregivers will be counseled to promptly inform the PI or any co-investigator.

Treatment/Withdrawal: If the patient is given a rapid infusion, the FO will be infused at the proper rate, and triglyceride troughs will be followed serially until they normalize.

Metabolic Overload Syndrome

Definition: Metabolic Overload Syndrome is characterized by a constellation of symptoms that include hepatomegaly with or without icterus, a change in coagulation parameters leading to an increased risk to bleed, splenomegaly, anemia, leucopenia, thrombocytopenia, altered liver function test, hyperlipidemia, hyperglycemia, headache, abdominal pain, and fatigue.

How to Identify: Patients will have physical exams on a regular basis in the inpatient setting and on a weekly or bimonthly basis on the outpatient setting to assess for skin color, signs of bleeding, or splenomegaly. Labs will be drawn on a weekly-monthly basis to assess for thrombocytopenia, anemia, leukopenia, liver function, hyperlipidemia, and hyperglycemia. Parents or legal guardians and medical staff will be counseled to report any fatigue or abdominal pain to the PI or any co-investigator. Parents will questioned at

all clinic visits regarding any signs or symptoms concerning for Metabolic Overload Syndrome.
Treatment/Withdrawal: If the subject is diagnosed with Metabolic Overload Syndrome, the patient will be discontinued from the study.
Notes: Metabolic Overload Syndrome usually occurs with the concomitant administration of cottonseed oil. Cottonseed oil will not be administered with FO.

Hyperglycemia
Definition: Hyperglycemia is defined as a glucose ≥ 150 mg/dL
How to identify: Plasma glucoses will be monitored at least every 12 hours in the inpatient setting, and every week - month in the outpatient setting.
Treatment/Withdrawal: If hyperglycemia occurs (glucose ≥ 150 mg/dL), the following steps will be taken:

1.) The study team will ensure the blood drawn to measure the glucose level was not drawn from a port infusing a dextrose containing solution.
2.) If mild hyperglycemia (glucose level between 150-200 mg/dL) occurs, the glucose will be monitored for twenty-four hours. Should the glucose level remain elevated (150-200 mg/dL), the glucose delivery rate (GDR) will be decreased by 1-2 milligrams/kilogram/minute (mg/kg/min) and then on a daily basis until the patient becomes normoglycemic (<150 mg/dL).
3.) If the glucose level is ≥ 200 mg/dL, but < 300 mg/dL, serial glucose levels will be followed over six hours. If after serial glucose levels, the glucose remains elevated (150-300 mg/dL), the GDR will be decreased by 1-2 mg/kg/min. Should the hyperglycemia persist, the GDR will be slowly decreased to achieve normoglycemia.
4.) If the glucose is ≥300 mg/dL at anytime, a repeat glucose will be drawn. If the glucose is in fact ≥ 300 mg/dL, the GDR will be decreased by 1-2 mg/kg/dL. Serial glucose levels will be followed, and the GDR will be slowly decreased over twenty-four hours to achieve normoglycemia.
5.) Insulin use will be at the discretion of the primary medical team.
6.) Other causes for hyperglycemia will be ruled out by the primary medical team.
7.) In addition to reducing the GDR, the PN may be given over an extended period of time.

Hypertriglyceridemia
Definition: Hypertriglyceridemia is defined as a triglyceride (TG) trough > 300 mg/dL
Identification: TG troughs will be drawn weekly – monthly
Treatment/Withdrawal: If hypertriglyceridemia occurs, the following steps will be taken:

1.) The study team will ensure that TG trough was measured when the patient was off FO for at least 4-6 hours.
2.) If the TG was drawn at the appropriate time, it will be repeated in 1-7 days.

Note: Although there is a theorectical risk that FO could cause hypertriglyceridemia, it should be noted that FO is now commonly prescribed for hypertriglyceridemia. In fact,

Gura et al described a case report of 9 year old patient with severe hypertriglyceridemia secondary to fat-free PN that was successfully treated with FO[42].

Temperature
Definition: A fever is defined as a rectal temperature ≥ 100.4° F.
Identification: As an inpatient, the subject's temperature will be monitored per hospital protocol (every 2-6 hours). Should the subject feel febrile, the medical caregiver or parent or legal guardian will instructed to measure the subject's temperature. Should the patient have a fever, the PI or any co-investigator will be notified. The parents or legal guardian will be questioned regarding any recent fevers at all outpatient visits. Vital signs will be performed at each outpatient visit.
Treatment/Withdrawal: If the patient experiences a fever, the appropriate work-up will be completed by the primary medical team to ensure the patient does not have an infection. If the fever is not attributed to an infection or other causes, and the source is thought to be FO, Tylenol or Motrin will be administered at the appropriate dose. If a fever reoccurs and is attributed to FO, the patient will be withdrawn from the study.

Chills/Cyanosis/Shortness of Breath/Nausea/Vomiting
Definition: self-explanatory
Identification: The medical caregivers and parent or legal guardian will be counseled to report any of these symptoms. Should the patient have a any of these symptoms, the PI or any co-investigator will be notified. The parents or legal guardian will be questioned regarding these symptoms at all outpatient visits.
Treatment/Withdrawal: If the patient experiences cyanosis, shortness of breath, nausea, or vomiting, the appropriate work-up will be completed by the primary team to rule out other causes, specifically infection. Should the symptom or symptoms be attributed to FO, the patient will be withdrawn from the study.

Pain
Definition: self-explanatory
Identification: The medical caregivers and parent or legal guardian will be counseled to report any signs or symptoms of pain. Should the subject have a any of these symptoms, the PI or any co-investigator will be notified. The parents or legal guardian will be questioned regarding these symptoms at all outpatient visits. Physical exams will be performed on a regular basis in the inpatient setting, and weekly-bimonthly on an outpatient setting to assess for pain.
Treatment/Withdrawal: If the patient experiences pain, the appropriate work-up will be completed by the primary team to rule out causes other than the FO. Should the symptom or symptoms be attributed to FO, the patient will be administered Tylenol or Motrin at the appropriate dose for pain relief. If the pain is not relieved or reoccurs, the patient will be withdrawn from the study.

The above side effects/adverse events will be monitored by the following specific means (See Table 4 and Figure 3):

- **Allergy**—clinical observation, patient report, vital signs
- **Prolonged Bleeding Time**—PT/PTT/INR, clinical observation, patient report
- **Essential Fatty Acid Deficiency**—essential fatty acid profiles, clinical observation, and patient report
- **Hyperglycemia**—glucose levels
- **Fat Overload Syndrome**—triglyceride level, patient report
- **Metabolic Overload Syndrome**—TG level, complete blood count, liver function tests, PTT/PT/INR, glucose levels, physical exam, patient report, clinical observation
- **Hypertriglyceridemia**—TG levels
- **Changes in Blood Pressure/Chills/Cyanosis/Shortness of Breath/Nasuea/Vomitting**—clinical observation, patient report, vital signs
- **Pain**—clinical observation and patient report
- **Hyperinsulinemia/hypoglycemia**—serum glucose and insulin concentrations
- **Increased risk for bleeding**—clinical observation, patient report

Should a side effect be noted, the following specific responses may occur:

Side Effect	**Response**
Allergy	Assess and treatment by Medical Team, Benadryl, Albuterol, possible withdrawal
Anaphylaxis	Assess and treatment by Medical Team, withdrawal
Bleeding	Treatment by Medical Team, consideration of withdrawal
Essential Fatty Acid Deficiency	Treatment by Medical Team, withdrawal
Hyperglycemia	Changes in PN therapy, insulin
Fat Overload Syndrome	Monitoring of triglycerides
Metabolic Overload Syndrome	Withdrawal

Hypertriglyceridemia	Monitoring of triglycerides
Changes in Temperature/Chills/Flushing	Assess and treatment by medical team, Tylenol, Motrin, withdrawal
Changes in Blood Pressure/Shortness of Breath/Cyanosis	Assess and treatment by medical team, withdrawal
Pain	Assess and treatment by Medical Team, Tylenol, Motrin, possible withdrawal
Hyperinsulinemia/hypoglycemia	Endocrinology consult

Table 4. Responses to Side Effects.

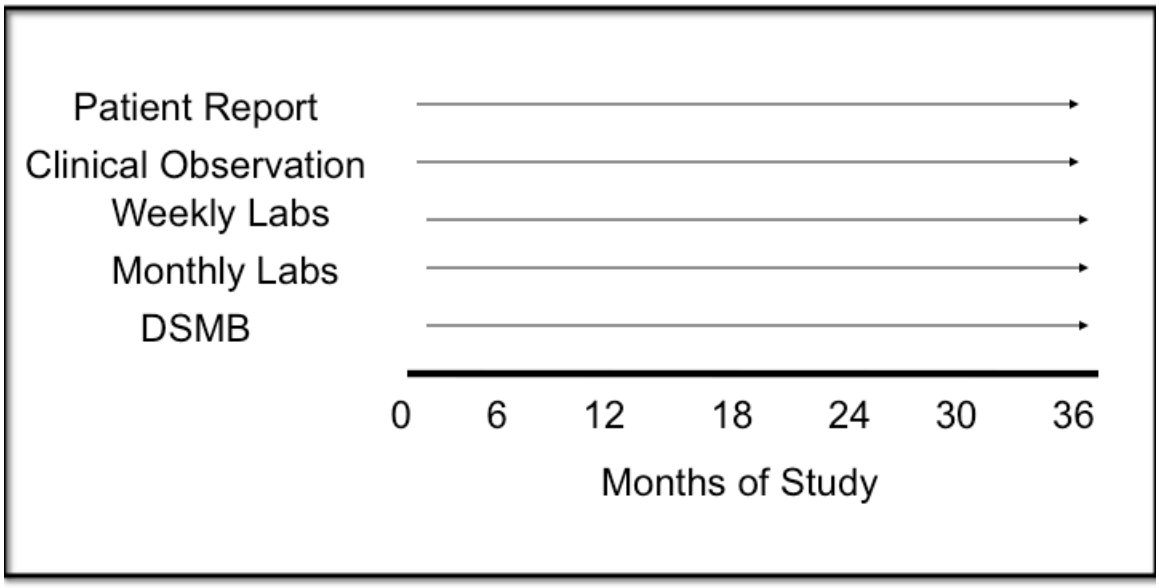

Figure 3. Monitoring of Side Effects or Adverse Events.

See attached CRF 4 for recording of side effects, adverse events, or serious adverse events.

Protocol

1. Purpose of the study

The specific aim of this pilot study is to investigate if providing FO, at 1 gm/kg/d IV, to children with PNAC will safely reverse or ameliorate their PNAC. Study subjects will be compared to 40-80 historical controls with SBS and PNAC, who received standard SO, 1gm/kg/d IV, available as Intralipid™ for > 60 days. The following clinical outcomes will be measured:

1) Reversal of PNAC in infants receiving Omegaven™
2) Growth of infants receiving Omegaven™
3) Safety of Omegaven™ in infants with PNAC
4) Mechanism of Omegaven™

To address the above specific aims the following will be performed:
1.) Serial laboratory tests to evaluate cholestasis and hepatic dysfunction (total bilirubin, direct bilirubin, ALT, AST, alkaline phosphatase, and GGT)
2.) Serial anthropometric measurements (weight, length, and head circumference)
3.) Serial laboratory test to evaluate safety (coagulation parameters, platelets and essential fatty profile)
4.) Serial serum proteomic, inflammatory markers, bile acids, phytosterols, microRNA and microarray analysis and fatty acid analysis of red blood cell (RBC) membranes.
5) Serial fecal specimens will be collected to evaluate the intestinal microbiome in children at risk for PNAC.

PNAC will be defined as a direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements. Reversal of PNAC will be defined as direct bilirubin < 2 mg/dL. Essential fatty acid deficiency will be defined as a triene:tetraene ratio ≥ 0.5.

2. Names and Addresses of Investigators, Facilities, and Institutional Review Board

See Form 1572.

3. Criteria for Patient Selection/Estimate Number of Patients to be Studies

The study will target to enroll 80 subjects.

INCLUSION CRITERIA:

-Clinical evidence of PNAC
-Direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements
-Expected parenteral nutrition course greater than 30 days
-Acquired or congenital GI disease
->2 weeks of age and <18 years of age
->60% of calories from TPN
-Failed available, standard therapies for PNAC (ie advancement of feeds, cycle PN, Actigal, avoidance of overfeeding, removal or reduction of copper in PN if elevated by laboratory analysis)

EXCLUSION CRITERIA:

-Inborn errors of metabolism
-Extracorporeal Membrane Oxygenation (ECMO)
-Seafood, egg, or Omegaven™ allergy
-Documented cause of liver disease other than PNAC
-Hemorrhagic disorder
-Anticoagulant therapy
-Hemodynamically unstable or in shock
-Comatose state
-Stroke, pulmonary embolism, recent myocardial infarction
-Diabetes
-Fatal chromosomal disorder
-Enrollment in any other clinical trial involving an investigational agent
-Patient, parents, or legal guardians unable or unwilling to give Informed consent
-Patient expected to weaned from PN in 30 days
-Unable to tolerate necessary laboratory monitoring
-Patient requiring aspirin or toradel or motrin
-Patient requiring dialysis

The gender and ethnic make-up of the subjects will reflect the gender and ethnic make-up of the patients admitted and seen at Mattel Children's Hospital and Pediatric outpatient clinics.

4. Description of the Study Design

Experimental Group (FO):

If the patient meets the inclusion and exclusion criteria, and the parents/legal guardians sign the consent form, the subject will be enrolled. Demographic, clinical, laboratory, and safety monitoring data will be collected. Patients will receive FO at 0.5 gm/kg/d IV

for 2 days, then 1 gm/kg/d IV for the remainder of the study. Subjects will be followed for 1 month after the termination of FO. Data will be collected for up to 5 years after the termination of FO intervention.

Once the subject enters the study, FO will be administered for the duration of PN. Should PN be terminated, FO will be stopped. If the subject re-develops biochemical evidence of PNAC (direct bilirubin ≥ 2 mg/dL on 2 consecutive occasions) at later date and is PN-dependent, but not receiving FO, FO can be re-started as long as inclusion and exclusion criteria are satisfied. FO will be continued for the duration of PN. Should FO be restarted at a later date, subjects will be followed for 1 month after the termination of FO.

Historical Controls (SO):
Subjects will be compared to 40-80 historical controls with SBS and PNAC who have received SO. PNAC will be defined as a direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements.

Methods:
The following clinical outcomes will be measured:

1) Reversal of PNAC in infants receiving Omegaven™
2) Growth of infants receiving Omegaven™
3) Safety of Omegaven™ in infants with PNAC
4) Mechanism of FO

To address the above specific outcomes the following will be performed:

1) Serial laboratory tests to evaluate cholestasis and hepatic dysfunction.
The total and direct bilirubin, ALT, AST, alkaline phosphatase, and GGT will be collected. These labs are performed as part of standard care for patients with SBS/IF and PNAC. Reversal of cholestasis will be defined a direct bilirbuin < 2 mg/dL.

2) Serial anthropometric measurements of weight, length, and head circumference
The above measurements will be performed on a monthly basis. The information will be collected from the subject's chart. The bedside nurse or the Intestinal Failure nurse will perform these measurements.

3) Serial laboratory test including coagulation parameters, platelets and essential fatty profiles.
Coagulation parameters (PTT/PT/INR), and platelets will be collected. These labs are considered standard of care of patients with SBS/IF and PNAC. Essential fatty acid profiles will be performed per the primary team, and results will be collected if the profile is performed.

4) Serial serum proteomic, inflammatory markers, bile acids, phytosterols, microRNA and microarray analysis and fatty acid analysis of the RBC membrane
Serum will be collected at baseline, 2 weeks, 3 months, and 6 months, and every six months while the subject is PN dependent. Samples will be coded and collaborators will not be provided the key code. Proteomic analysis will be performed in conjunction with the laboratories of James LeBlanc, PhD (UCLA). Microarray analysis will be conducted in conjunction with the Microarray Core (UCLA). Fatty acid analysis of the RBC membrane in conjunction with Susan Henning, PhD (UCLA). MicroRNA analysis will be performed in conjunction with Sherin Devaskar, MD (UCLA). Phytosterol analysis will be performed in conjunction with Robert Steiner, MD (University of Wisconsin) and Jogchum Plat (Maastricht University, Netherlands). Bile acid analysis will be performed in conjunction with Robert Steiner, MD (University of Wisconsin). These analyses will only be performed before, during, and after the first 6 months of FO treatment. In other words, if a subject redevelops or continues to have PNAC and FO is restarted, these analyses will not be performed.

For microarray analysis, 1 cc of blood will be collected at room temperature, and placed into a PAXgene tube. RNA will then be isolated using the QiAMP RNA blood mini kit (Qiagen, California), and then frozen and stored at -80°C. Microarray analysis will be performed using an Affymetrix microarray (Affymetrix, California).

For proteomic analysis, phytosterols, bile acids, microRNA and RBC membrane fatty acid composition, 2.5 cc of blood will be collected into an EDTA tube. The blood will be separated into RBCs and plasma. The plasma will be stored at -80 °C for proteomic analysis via mass spectroscopy. The RBCs will also be stored at -80 °C for RBC fatty acid membrane composition analysis via thin layer chromatography.

If there is concern that a subject is septic at the time of blood collection, the collection will be delayed until there is indication that the sepsis has resolved. Resolution of sepsis is determined by a negative blood culture. If blood is unable to be obtained at the scheduled collection time if the subject is an outpatient due to problems with the subjects' line, blood can be collected at the next clinic visit scheduled for routine purposes.

5) Serial fecal specimens will be performed to evaluate of the intestinal microbiome in children at risk for PNAC. Fecal specimens will be collected at baseline, 2 weeks, 3 months, and 6 months of FO therapy, and every 6 months thereafter for subjects who remain PN dependent. The OMR-200 device (OMNIgene-gut, OMR-200, DNA Genotek) will be used for fecal specimens. This collection kit is intended for the non-invasive self-collection and stabilization of microbial DNA in human stool. The device is designed to enable safe and effective collections by naive users and medically trained personnel, alike. It is to be used for research purposes only. Detailed pictorial instructions are included with each collection device. Users may also view an online video which demonstrates proper sample collection with OMR-200. Per the provided instructions, bulk stool (approximately 400 g) is collected from the diaper, stoma bag or toliet into an

appropriate receptacle. The user then takes a small amount of stool using the provided 'spatula', fills the top of the collection device, and caps the tube. The action of capping seals the device and automatically extrudes the stool sample from the measuring chamber into the collection tube. Inside the tube is a non-toxic stabilizing reagent and mixing apparatus. After the sample is collected, and the tube is capped, the user then vigorously shakes the tube for 30 seconds to homogenize it. At that point, the sample is liquefied, and the relative abundance of DNA contributed by the gut microbiota is stabilized, such that a 'snapshot' of the in vivo state is preserved for 14 days at ambient temperatures. If stool cannot be collected at the time of research visit and the subject is not in the inpatient setting, the family will be provided with OMR-200 kit for stool collection. The specimen will be returned to the study team within 14 days of the next research/clinic visit. A stool sample collected in this device is compliant with IATA regulations, and may be shipped at ambient temperatures internationally.

DNA Genotek designs and manufactures products in accordance with the ISO 13485 Quality System (for medical devices), Good Manufacturing Practices, and FDA QSR 820.DNA extraction and 16S rRNA microbiome profiling will be performed by DNA Genotek. After no longer than 14 days, samples will be frozen for future analysis.

All plasma and RBC samples will be stored in the Neonatal Research Center.

A DSMB will meet every 6 months to evaluate any side effects or adverse events, assess the subject's progress, and analyze any preliminary data. After several DSMBs, the frequency of DSMB meetings can be decreased to at minimum once a year. As of 08/03/17, DSMB will only meet on as needed basis.

All deviations to the protocol will be recorded and described. All deviations will be reported to the appropriate personnel (ie UCLA IRB) according to IRB guidelines/regulations. Moreover, the reason for the deviation, and response to the deviation will be recorded. See CRF 5 for specifics.

5. Method for Determining Dose, Maximum Dose, and Duration

The dose of Omegaven™ was previously published by Gura, et al[31,32]. Therapy will begin at 0.5 gm/kg/d IV. After 2 days, therapy will be advanced to 1 gm/kg/d IV. The dose will remain at 1 gm/kg/d. FO can be restarted if the subject continues to demonstrate evidence of PNAC or redevelops PNAC off FO.

6. Description of the Observations to be made to fulfill the Study Objectives

PNAC

Improvement in PNAC will be assessed by labs evaluating cholestasis and liver function (total and direct bilirubin, ALT, AST, alkaline phosphatase, and GGT). These labs will be ordered per the primary medical team,

Safety

Safety of Omegaven™ will be assessed by laboratory studies and monitoring for any side effects or adverse events. These labs will be ordered per the primary medical team.

PN labs, coagulation parameters (PT/PTT/INR), and platelets will be drawn per the primary team... Subjects will be monitored either in the inpatient or outpatient setting for adverse events or side effects. All adverse events and side effects will be described, tracked, and reported to appropriate persons/institutions.

Growth

Growth (weight, length, and head circumference) will be measured monthly by the bedside nurse or nurse in the Intestinal Failure Clinic.

Mechanism

Currently, there is a paucity of information regarding how FO reverses IFALD. Since SO contains phytosterols and FO lacks phytosterol. As a result, the liver's exposure to phytosterols is decreased with FO. Sterols are known to interfere with bile acid transport resulting in cholestasis—the hallmark of pediatric IFALD. Major sterols include stimgasterol, sitosterol, and campesterol. SO is made up of 57% phytosterols. FO is made up of cholesterol only.[43] Approximately 5% of orally ingested phytosterols are absorbed by the intestine, while 95% are excreted in the feces. However, intravenous fatty acid emulsions bypass the intestine, and rely up on the canicular ABCG5/G8 transporter to secrete phytosterols into the bile. Sitosterol inhibits the cholesterol 7α-hydroxlase, the rate-limiting step that converts cholesterol into bile acids, which act as an important lipid emulsifier and stabilizer and plays an important role in cholesterol homeostasis (Boberg, 1989). Moreover, stigmasterol antagonizes the farnesoid X receptor (FXR), a nuclear receptor that protects the liver from hepatoxic bile acids by: 1. reducing bile acid import via suppressing a bile acid co-transporter (NTCP, SLC10A1), 2. reducing bile acid synthesis by suppressing CYP7A1, and 3. increasing bile acid efflux by up-regulating the bile salt export pump (BSEP) ABC11 and organic solute transporter (OST). In animal models, FXR knockouts develop liver injury, while treatment with FXR agonists protect against cholestasis.[44] In cell lines, stigmasterol antagonizes BSEP (Carter 2007). In fact, mice infused with PN and FO exhibited less liver injury compared to those infused with PN and SO. When stigmasterol was added to FO, these animals developed cholestasis.[45] In humans, phytosterol concentrations in control children have been reported at 40 μmol/L, while concentrations in children with IFALD were as high as 1500 μmol/L.[46] A linear correlation between PN duration with serum phytosterols has been documented.[47,48]

Bile acid transport is not only regulated by phytosterols, but also inflammation. Children with IF at are high risk for inflammation secondary to gut injury and bloodstream

injections. Sepsis is considered be a major culprit in the pathogenesis of IFALD.[8,49] Lipopolysaccharides and cytokines are known to alter BSEP and NTCP expression.[50,51] When liver macrophages were stimulated with either stigmasterol, lipopolysaccride, or placebo, cells subjected to stigmasterol or lipopolysaccride exhibit increased transcription of IL-6 and TNF-α.[45]

The dose and polyunsaturated fatty acid composition of the IFE product may further exacerbate inflammation. SO is composed mainly of pro-inflammatory omega-6 fatty acids. FO, on the other hand, is composed of mainly anti-inflammatory omega-3 fatty acids. As a result, FO alters downstream production of pro- and anti-inflammatory compounds.[52] In fact, prior to transitioning to FO, animals and children receiving SO exhibited a shift from high concentrations inflammatory eicosanoids to anti-inflammatory eicosanoids and pro-resolving lipid mediators.[52,53]

The dose and type of fatty acid emulsion may also effect lipid metabolism and hepatic architecture. In animal experiments, high omega-6:omega-3 fatty acid ratios correlate with hepatic lipid accumulation and steatosis by regulating peroxisome proliferator-activated receptor Y (PPAR-Y) and sterol regulatory element binding proteins (SREBPs).[52,54,55] PPAR-Y regulates fatty acid storage, while SREBPs are involved in cholesterol synthesis and fatty acid uptake. As fat accumulates in hepatic cells, phagocytic dysfunction and impaired endotoxin clearance occurs, resulting in hepatic injury.

In summary, we believe that phytosterolemia and inflammation, which are influenced by sepsis and polyunsaturated fatty acids, act synergistically and simultaneously to: 1. inhibit the liver's ability to promote bile acid secretion and 2. promote hepatic remodeling—subsequently, resulting in the biochemical and histological features of IFALD. While there is evidence to suggest these links, there is a paucity of human studies investigating this hypothesis.

Proteomic, microarray, and microRNA analysis will provide insight into what proteins and genes are altered (up-regulated or down-regulated) by FO intervention in this specific population. Specific microRNAs are small RNAs that contain seed sequences that complement the 3'-untranslated region of mRNAs, post-transcriptionally regulating gene expression. MicroRNAs are seen in non-cell based serum or plasma, these levels correlate with cellular injury or inflammation. In the case of IFALD, where there is established cholestatic liver injury, certain liver-specific miRNAs are seen in the serum as biomarkers of liver injury. In this study, we plan to examine subjects who have incurred liver injury as seen with elevation of serum direct bilirubin and transaminases, to determine if liver-specific microRNAs increase. With intervention with FO, when the liver injury abates, liver specific microRNAs will be evaluated in serum to see if their concentrations diminish. This study is designed to see if the microRNAs are specific and sensitive in predicting liver injury and resolution of the same. With regards to the proteomic and microarray studies, areas of interest include: inflammation, thrombosis, lipid and glucose metabolism, insulin resistance, and oxidation/antioxidation.
Phytosterol and bile acid analysis will provide insight into how FO alters sterols over time.

Serial fecal specimens will provide insight into how the gut microbiome is altered by intravenous fat and cholestasis. Animal models of PNAC have demonstrated that: *1.)* phytosterols in soybean oil cause dysbiosis and *2.)* dysbiosis causes hepatic injury via the activation of the toll-like receptor-4 by bacteria. In rodents receiving PN and soybean oil who have PNAC, FXR/LXR expression is reduced and colonic *Erysipelotrichacease* (a gram negative bacteria) is increased by 20-30% in comparison to controls. In contrast, in rodents who received PN and fish oil, biliary flow is preserved and their fecal microbiota is distinctly different when compared to rodents who received soybean oil. In addition, lack of enteral nutrition results in intestinal atrophy and inflammation. As the gut's permeability increases, bacteria secondary to bacterial overgrowth translocate into the bloodstream resulting in infections and hepatic inflammation. Like phytosterols, the cytokine interleukin-6 (IL-6) inhibits FXR/LXR, causing cholestasis. High omega-6:omega-3 ratios further increase hepatic inflammation and accelerate liver injury. Studies have demonstrated that the type of dietary fat (oral or IV) alters the microbiome. In fact, fish oil positively influences the microbiome resulting in improved metabolic and liver health. However, it is unknown how the microbiome is altered in children receiving PN and whether the type of IV fatty acid alters intestinal bacteria.

These time-points were selected for specific reasons. The literature supports that the biochemical effect of FO can be detected as early as 6 weeks, but as late as 3-6 months[30,31]. Collecting blood 4 months after FO has terminated, will provide information on whether the effect of the drug is sustained.

By assessing the RBC membrane over time, we will be able to detect changes in the fatty acid composition. Changes in the lipid content of the cell membrane effects cell function and fluidity which may impact transcription and translation.

7. Clinical Procedures, Laboratory Tests, and other Measures to be taken to Monitor for Side Effects

Clinical Procedures

Monitoring for any possible side effects or adverse events will include, but not limited to:

INPATIENT SETTING:

-bedside monitoring per inpatient protocol (vital signs, etc)
-daily physical exams
-primary care and observation by primary medical team
-labs per primary team
-patient report
-clinical observation
-assessment by PI or any co-investigator at least 4 times/week
-medical staff and parents/legal guardians will be questioned for any of the anticipated side effects of FO at least 4 times/week
-medical chart review

OUTPATIENT SETTING:

-weekly/bimonthly/monthly clinic visits to the Intestinal Failure Clinic per the primary medical team or GCRC
-labs per protocol
-patient report
-clinical observation
-full physical exams
-full review of systems
-parents/legal guardians will be questioned for any of the anticipated side effects
-medical chart review

Laboratory Tests

All blood will be taken from existing catheters using a sterile technique. In the rare case that the subject does not have an intravenous tube to draw blood, blood will be taken from a vein or small artery. All labs will be per the primary medical team and part standard of care.

At baseline and at the 2 weeks and 3 and 6 months, and every 6 months thereafter (for subjects who remain PN dependent), blood (3.5 cc) will be collected every six- month until FO or PN has been stopped. The microarray requires 1/5 (0.2) teaspoon (1 cc) of blood. The mircoRNA analysis requires 1/10 (0.1) teaspoon (0.5 cc) of blood. The proteomic, phytosterols, and bile acid analysis and/or RBC membrane fatty acid analysis will require 2/5 (0.4) teaspoon (2 cc) of blood.

Study	Amount of Blood Required
Microarray	1 cc
MicroRNA	0.5 cc
Proteomic, Inflammatory markers, Phytosterols, Bile acids, RBC fatty acids	2 cc
Fecal specimens collection	400 g at least

Due their small size and limitations in blood sampling, neonates weighing less 1.5 kg at enrollment, microarray, microRNA, inflammatory markers, phytosterols, bile acids, proteomic, and RBC membrane composition analyses will not be performed during the study period.

The microarray, proteomic, inflammatory markers, phytosterols, bile acids, microRNA and RBC fatty acid membrane composition analysis are not considered standard labs for routine care. The study will pay for these labs.

At baseline and at the 2 week and 3 and 6 month, thereafter every six-months (if the subject continues to receive PN), 400 g of fecal specimens will be collected to evaluate the intestinal microbiome.

Other Measures

Collection of Safety Data:

If an adverse event or serious adverse event occurs, it will be described accordingly per 312.32 and 312.33:

1) *"Associated with the use of the dru*g: There is a reasonable possibility that the experience may have been caused by the drug.
2) *Disability.* A substantial disruption of a person's ability to conduct normal life functions.
3) *Life-threatening adverse drug experience*: Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more se vere form, might have caused death.
4) *Serious adverse drug experience*: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
5) *Unexpected adverse drug experience* : Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not

being anticipated from the pharmacological properties of the pharmaceutical product."

In addition to the above classification, further details that will be recorded:

6) *Timing:* when the event occurred and any temporal associations.

7) *Resolution*: If and when the event resolves, details of the resolution will be recorded.

8) *Response to Event*: Response to event includes any treatment, withdrawal from the study, and communication with the IRB and FDA

9) *Other Event*: Not a serious adverse drug experience, life-threatening, or associated with a disability

8. Chemistry, Manufacturing, and Control Information

(a-c) Please see attached information from the manufacturer of the drug, Fresenius Kabi.

(d) Labeling—n/a

(e) A claim for categorical exclusion under 25.30 or 25.31 is being made.

Pharmacology and Toxicology Information

Pharmacology and drug disposition

Studies in animal are limited with respect to Omegaven™.

However, there are studies comparing FO to standard soybean oil and their effect on PNAC. Studies have demonstrated that animals receiving standard lipid emulsions develop histological evidence of hepatic steatosis, reduced bile flow, and alterations in liver enzymes. These changes, however, can be ameliorated or prevented when standard lipids are replaced with FO. Van Aerde et al. compared bile flow rates in piglets administered FO or SO. While the piglets who received soybean fat emulsion had elevated bilirubin concentrations and reduced bile flow, the piglets who received FO had similar bilirubins and bile flow rates when compared to control.[28]

A murine model was employed by Alwayn et al. to collaborate the above findings. Mice received a fat-free, high carbohydrate diet in combination with FO, either enteral or intravenous, or standard intravenous Intralipids™. Animals that received the high-carbohydrate, fat free diet alone or in combination with Intralipids™ demonstrated severe liver disease, characterized by elevations in hepatic enzymes, abnormal liver biopsies, and increased hepatic fat content. Histological staining revealed diffuse steatosis, and MR-spectroscopy demonstrated marked increase in hepatic fat content. Liver biopsies from the group that received enteral or intravenous FO, however, were similar to control, and had significantly decreased hepatic fat content compared to the other experimental groups.[29]

There is theoretical concern that the use of FO as the sole source of fat in PN could lead to essential fatty acid deficiency. However, when mice were fed various percentages of fish oil for 9 weeks, 1 and 5% fish oil groups demonstrated evidence of essential fatty acid deficiency, while the 10% fish oil group did not display any evidence of essential fatty acid deficiency as measured by a triene-tetrane ratio.[34]

Please see information supplied by the manufacturer of FO for additional information. Please see section entitle, “Previous Human Experience with FO” for information regarding human experience with this drug.

Additional Information

This study will be performed in children who are considered a vulnerable population. All regulations will be adhered to as dictated by the governing IRB.

Human Subjects Research:

PROTECTION OF HUMAN SUBJECTS

1. Risks to Subjects:

Human subjects involvement and characteristics:
Subject identification and recruitment will be carried out in the at Mattel Children's Hospital Neonatal Intensive Care Unit, Pediatric Floor, Pediatric Intestinal Failure Clinic at UCLA Medical Center, and Santa Monica UCLA Medical Center and Orthopedic Hospital. UCLA Medical Center is one of the largest medical centers in Southern California. The NICU, Pediatric Floor, and clinics are state-of-the-art clinical facilities with a well respected, a group of pediatricians and subspecialists with diverse expertise,. The subjects involved in this must have clinical evidence of PNAC, a direct bilirubin ≥ 2 mg/dL on 2 consecutive measurements, expected PN course > 30 d, and be > 2 weeks of age and < 18 years of age. The gender and ethnic backgrounds of the eligible neonates will undoubtedly reflect the background of children admitted to the Pediatric service. Patients with inborn errors of metabolism, on Extracorporeal Membrane Oxygenation (ECMO), a seafood, egg or Omegaven™ allergy, documented case of liver disease other than PNAC, hemorrhagic disorder, on anticoagulant therapy, hemodynamically unstable or in shock, in a comatose state, have a history of stroke, pulmonary embolism, or recent myocardial infarction, diabetes, or a fatal chromosomal disorder are excluded from the study. All subjects enrolled will be vulnerable patients as they are children.

Sources of Materials:
Type of Specimens, Data, or Records:
The specimens and data collected from patients enrolled in this study will used specifically for this study alone. The only specimens collected are blood samples. The data collected concerning the subject will be obtained from the subject's medical records. The study team will review the entire medical record, including the following: diagnosises, birthweight, gestational age, hospital where the subject was born, PN data (PN calories/day, EN calories/day, PN dextrose dose/day, PN protein dose/day, PN lipid dose/day), feeds with breastmilk, candidate for transplant, liver biopsy data, abdominal ultrasound data, types of gastrointestinal surgeries, episodes of sepsis, date and cause of death.

The following growth measurements and laboratory information will be collected: weight, length, head circumference, fluid balance, vital signs, urine sugar/ketones, laboratory tests (sodium, potassium, chloride, carbon dioxide, bicarbonate, glucose,

BUN, creatinine, triglycerides, calcium, magnesium, phosphorus, prealbumin, albumin, total protein, alkaline phosphatase, bilirubin (total and direct), GGT, ALT, AST, essential fatty acid profile, hemoglobin, hematocrit, red blood cell, white blood cell, platelets, PT, PTT, selenium, copper, zinc, iron, carnitine, Vitamins A, D, and E, and aluminum).

Blood will also be used for microarray, proteomic, micro RNA, inflammatory markers, phytosterol, bile acids, and RBC membrane fatty acid analysis.

No identifying information will be included in any of the manuscripts or reports of the data.

Data Collection, Storage, and Confidentiality:
Data will collected on Case Report Forms (CRFs) (See attached CRFs). The subject will be assigned a code to protect his or her privacy. The coded CRFs, along with consents, will be stored in a locked file/protected computer database accessible only by study personnel. Only the study staff will be able to link the subject ID numbers to personal identifiers. No data with subject identifiers will be released to anyone as required by law. All data will be stored by the principal investigator in a locked cabinet in locked room or protected computer database and kept in the manner and length of time required by the federal and state authorities. All data collected under this protocol will be confidential. When the results of the research are published or discussed in conferences, no information will be included that would reveal the patients identity. If photographs, videos, or audiotape recordings will be used for educational purposes, then the patient's identity will be protected or disguised.

All blood (plasma and RBCs) will be stored in the Neonatal Research Center. Tubes will be labeled with the subject's assigned code.

Potential Risks:
Risks of Fish Oil (Omegaven™):
Omegaven™ is not approved for use in the United States and the clinical experience with it is limited. Due to lack of experience in certain life threatening situations, the manufacturer does not recommend the use of this product in certain patients. The manufacturer specifically does not recommend Omegaven™ in patients with severe liver or renal insufficiency, children, or premature infants. The manufacturer also states that patients should not receive Omegaven™ for more than four weeks. Therefore, considering this study is targeting children with liver disease, there may be risks that are currently unforeseeable with the use of this unapproved drug.

In general, Omegaven™ is not recommended in patients who are in a coma, or who have unstable diabetes, or had a heart attack, stroke, or clot. Patients who are in shock should not receive Omegaven™. The manufacturer has indicated that there may be increased risk of an allergic reaction to this product in patients with egg or shellfish allergies. Omegaven™ can cause problems with bleeding. It is therefore not recommended for

anyone who has severe hemorrhagic disorders. There is a concern that patients receiving Omegaven™ could develop an essential fatty acid deficiency.

In order avoid potential risks, patients must met inclusion and exclusion criteria. In an effort to minimize risks, patients with a hemorrhagic disorder, on anticoagulant therapy, hemodynamically unstable or in shock, in a comatose state, who have a history of stroke, pulmonary embolism, recent myocardial infarction, or diabetes are excluded from the study. Moreover, coagulation parameters and liver function tests will be monitored on a weekly basis, and an essential fatty acid profile will be monitored at baseline, and 3 and 6 months of therapy..

Risks of Intravenous Fats:
Preliminary studies in animals and limited studies in humans have indicated that the risks of Omegaven™ are similar to the risks other intravenous fat. Omegaven™, like other intravenous fats, can cause hyperglycemia, hypertriglyceridemia, and a metabolic acidosis. If a patient receives an overdose of an intravenous lipid, or if it is given too quickly, "fat overload syndrome" can occur. It is also possible that an intravenous lipid can cause "metabolic overload."

In order to assess for these conditions or complications, patients will be monitored by report, physical exam, and scheduled laboratory tests. While patients are hospitalized, glucoses will monitored on a daily basis, and with any change in TPN. As an outpatient, glucoses will monitored, and with any change in TPN. A triglyceride trough, along with electrolytes, and arterial, venous or capillary gas will be performed on a weekly basis.

In the advent of hyperglycemia occurs (glucose ≥ 150 mg/dL), the following steps will be taken:

1.) We will ensure the blood drawn to measure the glucose level was not drawn from a port infusing a dextrose containing solution.

2.) If mild hyperglycemia (glucose level between 150-250 mg/dL) occurs, the glucose will be monitored for twenty-four hours. Should the glucose level remain elevated (150-250 mg/dL), the glucose delivery rate (GDR) will be decreased by 1-2 milligrams/kilogram/minute (mg/kg/min). If the patient remains hyperglycemic, the GDR will slowly be decreased until the patient becomes normoglycemic (<150 mg/dL).

3.) If the glucose level is ≥ 250 mg/dL, but < 300 mg/dL, serial glucose levels will be followed over twelve hours. If after serial glucose levels, the glucose remains elevated (150-300 mg/dL), the GDR will be decreased by 1-2 mg/kg/min. Should the hyperglycemia persist, the GDR will be slowly decreased to achieve normoglycemia.

4.) If the glucose is ≥300 mg/dL at anytime, a repeat glucose will be drawn. If the glucose is in fact ≥ 300 mg/dL, the GDR will be decreased by 1-2 mg/kg/dL. Serial glucose levels will be followed, and the GDR will be slowly decreased over twenty-four hours to achieve normoglycemia.

5.) Insulin use will be at the discretion of the primary medical team.

6.) Other causes for hyperglycemia will be ruled out by the primary medical team.
7.) In addition to decreasing the GDR, the daily dose of PN may be given over a longer period of time to prevent hyperglycemia.

Hypertriglyceridemia is defined as a TG> 300 mg/dL. In the advent of hypertriglyceridemia, the following steps will be taken:

1.) The study team will ensure that the triglyceride (TG) level was not drawn when the patient was receiving FO or SO. All TG trough levels will be obtained ~6 hours after lipid infusion.
2.) If the TG level was drawn at the appropriate time, the TG level will be repeated in 1-7 days.

All other changes to the PN will be made at the discretion of the treating physician.

Hyperinsulinemia and hypoglycemia are defined as an insulin concentration > 25 uU/mL and persistant hypoglycemia (glucose < 40 mg/dL) in the face of a prolonged high glucose delivery rate (> 16 mg/kg/min) along with an Endocrinology consult confirming that the hyperinsulinemia and hypoglycemia are NOT secondary to advanced liver disease.

Also like other intravenous fats, Omegaven™ can be associated with changes in body temperature, chills, flushing, cyanosis, changes in blood pressure, decreased appetite, nausea or vomiting, shortness of breath, headache, and chest, back, or bone pain. In very rare cases, intravenous fats can cause an erection.

Risks of blood drawing:
All blood will be obtained from existing catheters. In the event that an intravenous catheter is not present, the blood draw may be associated with pain from a heel stick or blood draw from a small vein or artery. Mild pain and bruising may be associated with these types of blood draws but every effort will be made to take the blood test at the same time that other tests are obtained.

Parents or legal guardians will counseled regarding all potential risks associated with this study.

Risk Classification:
Because Omegaven™ is not an FDA approved drug, the overall risk is greater than minimal.

Therapeutic Alternatives:
Children not participating in this study will receive standard PN supplemented with a standard intravenous fat emulsion.

Risk/Benefit Ratio:

There is greater than minimal risk because Omegaven™ is an unapproved by the FDA. Potential benefits of this study include a reduction in morbidity and mortality children with PNAC. The knowledge gained from this study will contribute to the general knowledge of how alternative nutritional strategies can not only reduce morbidity and mortality associated with PNAC, but also other inflammatory diseases is enormous.

Information Withheld from Subjects:
No information about the research purpose, design, and procedures will be withheld.

2. Adequacy of Protection Against Risks:

Requirement and Informed Consent:
Capacity to Consent:
Informed consent will be obtained from the parents or guardians of subjects designated to give consent for their children.

Personnel Inviting Participants:
Potential subjects will be known to the investigators by virtue of their involvement in their standard medical care. In addition, faculty in the Departments of Pediatrics and Pediatric Surgery will be made aware of this study and may refer potentially eligible subjects to the investigators.

Process of Consent:
Parents/legal guardians of potential subjects and potential subjects who meet inclusion and exclusion criteria will be identified by the principal and co-investigators. Potential subjects will be approached by either Kara Calkins, MD, Robert Venick, MD. A consent form, and when appropriate, an assent form, will be provided to the subject and/or their family/caretakers in their primary language for their consideration . Upon agreement, the patient may be approached by the principal investigator or co-investigators who will further explain the study, procedures involved, and potential risks and benefits. Potential participants will be invited to read the informed consent. Potential participants will be encouraged to ask questions, and consult other health professionals for a second opinion. They will be encouraged to take ample time discuss the study with other physicians, family, and friends. All verbal and written information will be provided in the subject's and/or parents'/legal guardians' primary language. The person inviting participants will be a legally licensed physician or surgeon (California licensure) experienced in delivering healthcare to the pediatric population. All members of this research team have completed UCLA PHRS and HIPAA clinical research certifications. All subjects will continue to receive standard of care irrespective of their decision to participate in the study.

This study targets children 2 weeks - < 18 years of age. However, it is possible, that a subject may reach 18 years of age, during the study period.

Consent for Children < 7 years of age:

For children < 7 years of age, one parent or legal guardian will be required to sign the consent form ("Parent Permission for a Minor to Participate in Research").

Consent/Assent Process for Children 7 – 12 years of age:
For children 7 -12 years, the child will be required to sign to the assent ("Assent to Participate in Research"), and ONE parent/legal guardian will be required to sign the consent form. ("Parent Permission to a Minor to Participate in Research").

Consent/Assent Process for Children 13 – 17 years of age:
For children 13 – 17 years age, the subject must sign the assent form ("Youth Assent/Adult Consent"), and ONE parent/legal guardian must sign the consent form ("Parent Permission for a Minor to Participate in Research").

Consent Process for Adults ≥ 18 years of age:
Should a subject reach the age of 18 years of age during the study, the adult subject must provide a consent ("Youth Assent/Adult Consent").

Comprehension of the Information Provided:
The parent(s) or legal guardian(s) of the subject and subject when appropriate, will be given time to read the consent form and ask questions regarding the study prior to deciding to allow their child to participate in the study. The parents will be questioned by the investigator to ensure their understanding of the information in the consent form including the risks, the potential benefits, and the alternatives to participation in the study. The investigator will also explain that the decision to participate or not to participate in this research study does not affect the care of their child and that they have the right to withdraw consent at any time without any consequences.

Consent/Assent Forms:
This study targets children 2 weeks - < 18 years of age. However, it is possible, that a subject may reach 18 years of age, during the study period.

Consent for Children < 7 years of age:
For children < 7 years of age, one parent or legal guardian will be required to sign the consent form ("Parent Permission for a Minor to Participate in Research").

Consent/Assent Process for Children 7 – 12 years of age:
For children 7 -12 years, the child will be required to sign to the assent ("Assent to Participate in Research"), and ONE parent/legal guardian will be required to sign the consent form. ("Parent Permission to a Minor to Participate in Research").

Consent/Assent Process for Children 13 – 17 years of age:
For children 13 – 17 years age, the subject must sign the assent form ("Youth Assent/Adult Consent"), and ONE parent/legal guardian must sign the consent form ("Parent Permission for a Minor to Participate in Research").

Consent Process for Adults ≥ 18 years of age:
Should a subject reach the age of 18 years of age during the study, the adult subject must provide a consent ("Youth Assent/Adult Consent").

A consent/assent forms will be used to obtain consent/assent.. Consents/assents will also be available in Spanish for Spanish speaking families.

Protection Against Risk:
The procedural risks from the study are greater than minimal since Omegaven™ in not approved by the FDA. In order to minimize risk, patients will be screened to ensure they met inclusion and exclusion criteria. Side effects will assessed by physical exam, patient report, and scheduled laboratory tests. All attempts will be made to obtain blood from pre-existing sampling catheters whenever available and/or obtain the blood sample at the same time as other scheduled blood tests. To date, there are no published side effects of Omegaven™ in infants with PNAC.

Financial Obligations of the Subjects:
The hospital will attempt to obtain reimbursement for Omegaven™ provided to the subject while in the hospital. The home health agency providing the subject's IV nutrition and Omegaven™ at home will attempt to obtain reimbursement from the insurance company to pay for the cost of Omegaven™ and home health supplies.

It is possible that the medical insurer will not pay for all the treatments and tests the subject receives in this research study. That is because many insurance companies, HMOs, and health benefit plans do not cover for experimental treatments. If that happens, the charges the subject will have to pay are as follows:

1.) home health supplies (IV infusion pumps, IV tubing, and outpatient pharmacy dispensing charges).
2.) Omegaven™ at home

It is estimated that home health supplies may cost approximately $500.00-$900.00 per month. It is estimated that Omegaven™ may cost $1500.00-$2100.00 per month

If the insurance company, HMO, or health benefit plan refuses to pay for some costs, the study will cover the cost of the following:

1.) Omegaven™ in the hospital

The study will not cover the cost of labs consider standard of care.

Microarray, proteomic, inflammatory markers, microRNA, phytosterol, bile acids, and RBC membrane composition analysis will be paid for the study.

Emergency Care and Compensation for Research-Related Injury:

In the unlikely event of an injury or illness resulting from this study, emergency treatment will be provided.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Potential Benefits:
Participation in this study may not directly benefit the subject. However, knowledge gained from this study may used to support the use of parenteral FO in infants with PNAC.

Payment for Participation:
There is no payment for participation in this study.

DATA AND SAFETY MONITORING PLAN

A committee comprised of a pediatrician (Dr. Meena Garg), research pharmacist (Christina Shin, Pharm D), and statistician (Kate Crespi, PhD) will review potential complications or adverse effects that arise from the study. This committee together will also be involved in performing the early evaluations of the data at annual intervals to determine if significant results are obtained to warrant discontinuation of the trial. All complications, adverse effects, or results that warrant discontinuation of the trial will be promptly reported to the UCLA Institutional Review Board and FDA.

As of August 2017, annual updates will be provided to all DSMB members via email, and annual meetings will not be required. Confirmation that these annual updates were reviewed by the respective DSMB member will be documented by a confirmatory email from the DSMB member. Should a DSMB member voice any concerns, a formal DSMB can be held to address these concerns. This documentation will be provided to the IRB.

INCLUSION OF CHILDREN

The subjects involved in this study must have clinical evidence of PNAC, a direct bilirubin $\geq$ 2 mg/dL on 2 consecutive measurements, expected TPN course > 30 d, and be > 2 weeks of age and < 18 years of age. The purpose of this study is focused on this patient population as they are at a discernable propensity for specific diseases that may be alleviated by omega-3 fatty acids. The principal investigator and other contributors involved in the study demonstrate expertise in the care of this patient population.

Table 6
Monitoring Schedule for Patients with SBS/IF and PNAC

Parameter	Weekly-Monthly	Periodic	Comments
Weight			
Fluid Balance			
Vital Signs			
Urine Sugar/Ketones			
Cathetar site/function			
Labs			
Sodium	X		2 yellows (0.5 cc each)
Potassium	X		
Chloride	X		
Glucose	X		
BUN	X		
Creatinine	X		
Magnesium	X		
Phosphorous	X		
ALT	X		
AST	X		
Total Bilirubin	X		
Direct Bilirubin	X		
Total Protein		X	
Albumin		X	
Pre-albumin		X	
WBC	X		1 lavendar (0.5 cc)
RBC	X		
Hemoglobin	X		
Hematocrit	X		
Platelets	X		
Triglyceride	X		2 reds (0.5 cc each)
GGT		X	
PT/PTT/INR		X	1 blue (1.8 cc)
Essential Fatty Acids		X	1 red (0.8 cc)
Vitamins A, D, E		X	2 reds (0.5 cc each)
Selenium		X	
Copper		X	
Zinc		X	
Aluminum		X	

Iron		X	
Carnitine		X	
Microarray Analysis		X	Paxgene (1 cc), research lab
Proteomic, Inflammatory Marker, Phytosterol, Bile Acid, RBC Fatty Acid Analysis		X	EDTA (2 cc), research lab
microRNA Analysis		X	EDTA (0.5 cc) research lab
Fecal specimens		X	400 g in MOR-200 device, research lab

All of the above labs except for the microarray, microRNA, proteomic, inflammatory makers, phytosterols, and bile acids and RBC membrane analysis are considered standard of care for SBS/IF subjects with PNAC and will be ordered per the primary medical team.

Case Report Form 4: Side Effects, Adverse Events, Serious Adverse Events

Subject:
Date:
Time:
Side Effect or Adverse Event Description:

Intervention:

Resolution:

Is this Event:
Unexpected? Y or N If yes, desribe.

Associated with a Diability? Y or N If yes, describe.

Life Threatening? Y or N If yes, describe.

Serious Drug Expereince? Y or N If yes, describe.

Withdrawal from Study Warranted: Y or N

Date Reported to IRB and FDA:

Case Report Form 5: Deviations from Protocol

Subject:
Date:
Deviation

Why deviation occurred:

Response to deviation:

References

1. Wales, PW. Neonatal short bowel syndrome; a cohort study. J Pediatr Surg;2005;40:755-62.
2. Jugie, M. Study of the impact of liver transplantation on the outcome of intestinal liver transplantation on the outcome of intestinal grafts in children. Transplantation;2006;81:992-97.
3. Digestive Diseases Interagency Coordinating Committee (DDICC). Short Gut and Small Bowel Transplantation. Meeting Minutes. June 25, 2002. Available at: www2.niddk.nih.gov/NR/rdonlyres/9CCC34A3-3CFE-4D89-93DF-1ADEE3AA3E32/0DDICC_June-25-2002_Minutes.pdf.
4. Buchman, A. Total parenteral nutrition-assocated intrahepatic liver disease. JPNEN J Parenter Enteral Nutr. 2002;26:S43-8.
5. Freund, H. Abnormalities of liver functin and hepatic damage associated with out total parenteral nutrition. Nutrition;1991;7:1-5; discussion 6.
6. Beath, S. Parenteral nutrition-related cholestasis in postsurgical neonates:multivariate analysis of risk factos. J Pediatr Surg;1996;31;604-6.
7. O'Keefe, S. Short bowel syndrome and intestinal failure: consensus definaitions
    a. and overview. Clin Gastroenterol Hepatol;2006:6-10.
8. Sondheimer, J. Infection and cholestatsis in neonates with intestinal resection and long-term parenteral nutrition. J Pediatr Gastroenterol Nutr. 1998;27:131-7.
9. Grant, D. 2003 Report of the Intestin Transplant Registry: a new era has dawned. Ann of Surg. 2005;241:607-13.
10. Fryer, J. Mortality in candidates waiting for combined liver-intestin transplants exceeds that for other candidates waiting for liver transplants. Liver Transpl. 20039:748-53.
11. Shin, J. Could lipid infusion be a risk for parenteral nutrition-associated cholestasis in low birth weight infants? Eur J Pediatr. 2008;167:197-202.
12. Btaiche, I. Parenteral nutrition-associated liver complications in children. Pharmacotherapy.2002;22:188-211.
13. Owings, E. Management of cholestasis in infants with very low birth weight. Semin Pediatr Surg. 2000;9:96-102.
14. Thompson, A. Necrotizing enterocolitis in newborns, pathogenesis, prevention, and management. Drugs. 2008;68:1227-38.
15. Christensen, R. Identifiying patients, on the first day of life, at high-riskof developing parenteral nutrition-associated liver disease. Journ of Perinatology. 2007;5:284-90.
16. Javid, P. The role of enteral nutrition in the reversal of parenteral nutrition-associated liver dysfunction in infants. Journ of Ped Surg. 2005;40:1015-18.
17. Greenberg, G. Effect of total parenteral nutrition on gut hormone release in humans. Gastroenterology. 1981;80:988-93.
18. Yeh, S. Effects of L-glutamine oninduced hepatosteatosis in rats receiving total parenteral nutrition. J Formos Med Assoc. 1995;94:593-9.

19. Kubota, A. Total parenteral nutrition-associated intrahepatic cholestasis in infants: 25 years experience. J Pediatr Surg. 2000;35:1049-51.
20. Moss R. Hepatobiliary dysfunction during total parenteral nutrition is caused by infusate, not the route of administration. J Pediatr Surg 1993;28:391-6; discussion 396-7.
21. Helms RC. Comparison of a pediatric versus standard amino acid formulation in preterm neonates requiring parenteral nutrition. J Pediatr 1987;110:466-70.
22. Moss R. Methionine infusion reproduces liver injury of parenteral nutrition cholestasis. Pediatr Res 1999;45:664-8.
23. Simopoulos, A. Evolutionar aspects of diet and essential fatty acids. World Rev Nutr Diet. 2001;88:18-27.
24. Diamond, I. The rationale for the use of parenteral omega-3 lipids in children with short bowel syndrome. Pediatr Surg Int. 2008;24:773-78.
25. Hultin M. Intravenous lipid emulsions: removal mechanisms as compared to chylomicrons. J Lipid Res 1995;36:2174-84.
26. Qi K. Effects of particle size on blood clearance and tissue uptake of lipid emulsions with different triglyceride compositions. JPEN J Parenter Enteral Nutr 2003;27:58-64.
27. Nestel P. Effects of N-3 fatty acids on lipid metabolism. Annu Rev Nutr 1990;10:149-67.
28. Alwayn, I. Omega-3 fatty acid supplementation prevents hepatiti steatosis in a murine model of nonalcoholic fatty liver disease. Pediat Res 2005;57:445-452.
29. VanAerde, J. Intravenous fish oil emulsion attenuates total parenteral nutrition induced cholestasis sin new born piglets. Pediatr Res;1999;45:202-8.
30. Gura, K. Reversal of parenteral nutrition-associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. Pediatr. 2006;118(1).
31. Gura, K. Safety and efficacy of a fish oil based fat emulsion in the treatment of parenteral nutrition associated liver disease. Pediatr. 2008:121:678-86.
32. Diamond, I. Changing the paradigm: Omegaven for the treatment of liver failure in pediatric short bowel. Journ of Ped Gastroent and Nutr. 2009:48:209-15.
33. Bays, H. Safety considerations with omega-3 fatty acid therapy. Am Journ of Card. 2007:99:35-43.
34. Strijbosch, R. fish oil prevents essential fatty acid deficiency and enhances growth: clinical and biochemical implications. Metabol. 2008:57:698-707.
35. Duro, D. Overview of pediatric short bowel sydnrome. JPGN. 2008:47:S33-6.
36. Diamanti, A. Irreversible intestinal failure: prevalence and prognositic factors. JPGN. 2008:47:450-7.
37. Spencer, A. Pediatric short bowel syndrome: redefining predictors of success. Ann of Surg. 2005:242:403-12.
38. Dorney, S. Improved survival in very short small bowel of infancy with use of long-term parenteral nutrition. J Pediatr. 1985:107:521-5.
39. Assissi, A. Fish oil and mental health: the role of n-3 long chain polyunsaturated fatty acids in cognitive development and neurological disorders. International Clin Psychopharm. 2006:21:319-36.

40. McKenney, J. Role of prescription omega-3 fatty acids in the treatment of hypertriglyceridemia. Pharmacotherapy. 2007:27:715-28.
41. Harris, W. Expert opinion: omega-3 fatty acids and bleeding—cause for concern? Amer Journ of Cardio. 2007:99.
42. Gura, K. The role of an intravenous fat emulsion composed of fish oil in parenteral nutrition dependent patient with hypertriglyceridemia. Nutr Clin Prac. 2007;22;664-72.
43. Forchielli ML, Bersani G, Tala S, Grossi G, Puggioli C, Masi M. The spectrum of plant and animal sterols in different oil-derived intravenous emulsions. *Lipids.* 2010; 45(1):63-71.
44. Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. *J Clin Invest.* 2003;112(11):1678-1687.
45. Kasmi KC, Anderson AL, Devereaux MW, et al. Phytosterols promote liver injury and kupffer cell activation in parenteral nutrition-associated liver disease. *Sci Transl Med.* 2013;5(206):206ra137.
46. Clayton PT, Bowron A, Mills KA, Massoud A, Casteels M, Milla PJ. Phytosterolemia in children with parenteral nutrition-associated cholestatic liver disease. *Gastroenterology.* 1993;105(6):1806-1813.
47. Bindl L, Lutjohann D, Buderus S, Lentze MJ, Bergmann KV. High plasma levels of phytosterols in patients on parenteral nutrition: a marker of liver dysfunction. *J Pediatr Gastroenterol Nutr.* 2000;31(3):313-316.
48. Kurvinen A, Nissinen MJ, Gylling H, Miettinen TA, Lampela H, Koivusalo AI, et al. Effects of long-term parenteral nutrition on serum lipids, plant sterols, cholesterol metabolism, and liver histology in pediatric intestinal failure. *J Pediatr Gastroenterol Nutr.* 2011;53(4):440-446.
49. Squires RH, Duggan C, Teitelbaum DH, Wales PW, Balint J, Venick R, et al. Natural history of pediatric intestinal failure: initial report from the pediatric intestinal failure consortium. *J Pediatr.* 2012.
50. Moseley RH, Wang W, Takeda H, et al. Effect of endotoxin on bile acid transport in rat liver: a potential model for sepsis-associated cholestasis. *Am J Physiol.* 1996;271(1 Pt 1):G137-146.
51. Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular relocalization of hepatic RXRalpha: a novel mechanism for reduced hepatic gene expression in inflammation. *Nucl Recept.* 2004;2(1):4.
52. Kalish BT, Le HD, Gura KM, Bistrian BR, Puder M. A metabolic analysis of two intravenous lipid emulsions in a murine model. *PloS One.* 2013;8(4):e59653.
53. Kalish BT, Le HD, Fitzgerald JM, et al. Intravenous fish oil emulsion promotes a shift towards anti-inflammatory-pro-resolving lipid mediators. *Am J Physiol Gastrointest Liver Physiol.* doi: 10.1152/ajpgi.00106.2013.
54. Perez-Echarri N, Perez-Matute P, Marcos-Gomez B, Marti A, Martinez JA, Moreno-Aliaga MJ. Down-regulation in muscle and liver lipogenic genes: EPA ethyl ester treatment in lean and overweight (high-fat-fed) rats. *J Nutr Biochem.* 2009;20(9):705-714.

55. Takeuchi H, Kojima K, Sekine S, Murano Y, Aoyama T. Effect of dietary n-6/n-3 ratio on liver n-6/n-3 ratio and peroxisomal beta-oxidation activity in rats. *J Oelo Sci.* 2008;57(12):649-657.
56. Harris JK, El Kasmi KC, et al. Specific microbiome changes in a mouse model of parenteral nutrition associated liver injury and intestinal inflammation. *PLoS One*. 2014 Oct 20;9(10):e110396.

ATTACHMENTS

Manufacturer's Drug Information
Published Studies
Curriculum Vitae for Physicians