Clinical Protocol

Study Title: Standard IV lipid dosing compared to IV lipid minimization for the prevention of PNAC/PNALD (HUM00075458)

Sponsor Investigator: Dr. Meghan Arnold

IND: 121287

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Study Significance/Background

Parenteral nutrition (PN) is the intravenous administration of complete and balanced nutrition; it has transformed nutritional support for patients who cannot maintain adequate nutrition via the gastrointestinal tract.[1] PN is associated with a number of significant infectious and metabolic complications including parenteral nutrition-associated cholestasis (PNAC) which can lead to parenteral nutrition-associated liver disease (PNALD). Both are considered among the most challenging complications associated with prolonged PN administration. PNAC is generally accepted to occur when the direct bilirubin rises above 2 mg/dL after other potential etiologies of direct hyperbilirubinemia have been eliminated.[2-5] The reported incidence of liver dysfunction in children on long term PN in the literature ranges from 7.4% to 84%; this variety is attributed to study heterogeneity – the patient population studied, the definition of liver dysfunction used and different risk factors associated with the development of liver disease itself.[6,7]

Well-characterized risk factors for the development of PNAC include prematurity, low birth weight, repeated infectious/septic episodes, a lack of enteral feeds, delayed initiation of enteral feeds and the length of time on PN.[8-10] Progressive PNAC may lead to PNALD as demonstrated by severe liver injury and fibrosis which can ultimately lead to death from liver failure if transplantation is not achieved. The development of PNALD itself is associated with increased morbidity and mortality related to recurrent line infections, sepsis, the need for repeated hospitalizations and operative procedures, biliary sludge and gallstones, bacterial overgrowth and translocation.[6,9,11] In a study of surgical neonates, almost one-third died with long-standing PNALD.[12] In a more recent study of children with short bowel syndrome maintained on long term PN, PNALD was the greatest risk factor for mortality.[13]

Parenteral nutrition is made up of amino acids, dextrose, intravenous fat emulsion (IVFE), micronutrients, fluids, and electrolytes. In addition to the patient-related factors listed above which are known to contribute to the development of PNAC, the IVFE component of PN, and specifically soybean-based emulsions, have been shown to be an independent causative factor in the development of PNAC.[14,15] Soybean-based fat emulsions contain high levels of phytosterols which have been shown to impair bile drainage and contribute to hepatobiliary dysfunction.[16] Given these findings, several strategies have been undertaken to minimize the effects of IVFE on the liver; these strategies include soybean-based IVFE reduction or the use of alternative IVFE such as marine oil-based emulsions or combination fat emulsions. Data supporting these approaches, however, have been based on retrospective or uncontrolled studies thus limiting the strength of the supporting evidence. Further, soybean based fat emulsions remain the only approved IVFE products for use in pediatric patients in the United States.

The standard dosing for IVFE in neonates is 3 g/kg/day. The development of PNAC while on this dosing regimen often leads practitioners to reduce the IVFE dose in an attempt to reverse or keep the liver dysfunction and fibrosis from progressing. The data supporting IVFE reduction (IFER) focus on the efficacy of this modification after the first signs of PNAC have developed rather than as a preventative approach. The benefit of IFER was first described in the adult literature in 1982 but it was not until 2000 that a similar approach was reported in a pediatric population with severe cholestasis.[17,18] In this report by Colomb et al the IVFE component of PN was acutely terminated once cholestasis developed (defined as a plasma bilirubin ≥30 μmol/L). Patients were subsequently noted to have a marked decline in bilirubin levels.[18] A prospective study by Cober and Teitelbaum supported this concept of IFER for the prevention of PNAC progression.[19,20] In this single-center study, patients who developed PNAC (defined as a direct bilirubin >2.5 mg/dL) on standard soybean-based IVFE at a dose of 3 g/kg/day were switched to a restricted regimen of IVFE at a dose of 1 g/kg/day twice a week. These infants were then compared to a well-matched historical controls who were maintained on standard IVFE dosing regardless of bilirubin level. Thirty-one patients were included in the study group and were noted to have a significant decline in their serum direct bilirubin levels over time when compared to the control group. Specifically, the IFER group was found to have a downward trend in bilirubin whereas the standard cohort was found to have increased bilirubin levels across study weeks (estimated slope -0.73 mg/dL/week compared to 0.29 mg/dL/week; p=0.0017). In addition, the number of patients achieving resolution of PNAC was significantly greater in the IFER group compared to the control group (n=13 vs. n=3; p=0.013). It is important to note that eight patients in the IFER group developed mild, reversible essential fatty acid deficiency (EFAD) which was

defined as a triene:tetraene ratio ≥0.05. No physical manifestations of EFAD were observed in this group, however, and those who developed EFAD had their IVFE dose increased to 1 g/kg/day three times a week. If EFAD persisted the IVFE dose was increased to 2 g/kg/day. Increased IVFE dosing led to resolution of EFAD in all patients. No significant differences in growth parameters were noted between groups.

A reduction in the dose of soybean-based emulsion has also been studied as a preventative approach. In a study by Nehra and colleagues, neonates requiring long-term parenteral nutrition support, defined as ≥21 days of therapy, were divided into two groups: those receiving IVFE at a dose of 1 g/kg/day (n = 29) and those receiving IVFE at doses between 2 and 3 g/kg/day (n=32).[21] Groups were then evaluated for the primary outcome of the development of cholestasis, defined as a direct bilirubin >2 mg/dL for ≥2 consecutive weeks. The incidence of cholestasis was not significantly different between the groups with 15/29 (52%) developing cholestasis in the lower IVFE group compared to 14/32 (44%; p=0.61) in the standard IVFE group. The time to cholestasis was also similar between groups. In this study, once cholestasis developed patients were expeditiously transitioned to full enteral feeds or to a marine oil-based fat emulsion. Limitations of this study include its retrospective nature, slight differences between the study groups and unclear indications for the different IVFE doses between groups.

A retrospective study was recently published which compared the development of cholestasis in surgical infants treated with 1 g/kg/day of IVFE relative to a historical cohort who received standard dosing.[22] Starting in 2009 all surgical neonates at a single center who were expected to require PN for at least 2 weeks were administered a soybean-based fat emulsion at a dose of 1 g/kg/day. A total of 82 infants were treated in this manner and were compared to the 132 infants treated with standard dose soybean-based IVFE (2 to 3 g/kg/day) at the same institution from 2005 through 2008. Infants treated with IFER were found to have a significantly lower incidence of PNAC (defined as a serum bilirubin ≥2 mg/dL for a minimum of 2 weeks and for at least 2 measurements): 22% compared to 43% (p=0.003). IFER was also associated with a reduction in the peak direct bilirubin levels observed (p<0.0001). Overall, infants treated with standard dosing were 1.77 times more likely to develop PNAC than those treated with IFER (CI 1.17 – 2.68; p=0.007). Growth parameters were not different between groups. Limitations of this study include its retrospective nature and the lack of data regarding the development of EFAD in the IFER group.

In the first known study of its kind, Rollins et al recently published pilot data of a prospective, randomized trial comparing IFER to standard IVFE dosing in surgical neonates.[23] In this study 28 surgical patients ≥26 weeks gestation anticipated to require PN for at least 2 weeks were randomized to reduced (1 g/kg/day) or standard (3 g/kg/day) IVFE. Because this was a feasibility study it was not powered to detect significant differences between the groups. Despite this limitation, infants who received the reduced dose were observed to have lower overall direct bilirubin levels (p=0.04). Subjects randomized to standard dosing had a 13-fold higher median increase in total bile acids compared to a 2-fold increase in the reduced group (p=0.02). While weight z-scores increased more in the standard group (p=0.006), average weight gain was similar between the groups (23.7 g/day in the standard group and 20.8 g/day in the reduced group). No patient in the reduced IVFE group developed clinical or laboratory-proven EFAD which was assessed using triene:tetraene ratios.

These studies of IFER reveal important but occasionally conflicting findings relevant to the administration of a reduced dose of IVFE and its effect on the prevention and treatment of PNAC. Each study has limitations and because of their occasionally differing conclusions, questions remain regarding the optimal dose of IVFEs to promote growth and optimize brain development while preventing the development of PNAC and its progression to irreversible liver disease. These questions include the optimal dose of IVFE, the timing of the initiation of IFER, short- and long-term outcomes related to IFER as well as the safety associated with this treatment approach. These questions will be best answered in a well-designed prospective study that randomizes patients to IFER and standard dosing in order to evaluate the effectiveness of this strategy in maintaining adequate growth while reducing the development of PNAC and without leading to EFAD.

Essential fatty acids are known to be important for neurodevelopment. Specifically, docosahexaenoic acid (DHA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and long-chain polyunsaturated fatty acids are important for neural development and visual development within the first year of life.[24] The essential fatty acids

linoleic and alpha-linolenic acids are converted to AA and DHA and are typically provided through enteral nutrition (e.g. breast milk or enteral formulas). However, for PN-dependent patients, essential fatty acids must be provided through IVFEs. The optimal dose and type of IVFE is a topic of current investigation, and the effects of exogenous supplementation via the intravenous or enteral route on the developing brain have not been determined.[25] The balance between providing these essential nutrients during infancy and preventing the development of PNAC has led to concerns regarding the possible effects on neurodevelopment. In a recent pilot study completed at The University of Michigan, the association between IFER treatment and poor neurodevelopmental outcomes was evaluated.[26] Of the IFER-related variables analyzed (mean IVFE dose, IFER duration, and development of EFAD), none were found to be predictive of poor neurodevelopmental outcomes. However, this pilot study was retrospective in nature, relied on parent-reported outcomes, and was limited by small sample size. Further data is needed to confirm the effects of early IFER on neurodevelopmental outcomes.

Primary Hypothesis

IVFE reduction (IFER) during the administration of neonatal parenteral nutrition will lead to a significant decrease in the rate of rise of direct bilirubin (mg/dL/week) when compared to standard therapy.

Secondary Hypotheses

1. The time to development of PNAC, when present, will be longer with IFER when compared to standard IVFE therapy.
2. IFER will not be associated with a significant increase in failure to thrive (as assessed by growth parameters) or the development of essential fatty acid deficiency (EFAD) when compared to standard IVFE therapy.
3. Neurodevelopmental outcomes (NDOs), when assessed at 12 and 24 months of corrected gestational age, will be similar for those treated with IFER and standard IVFE therapy.

Specific Aims

1. To determine whether neonates and infants receiving PN with IFER (1 g/kg/day) will have lower rates of rise of direct bilirubin compared to those receiving standard IVFE therapy (3 g/kg/day)
2. To compare the time to development of PNAC in neonates and infants receiving PN with IFER (1 g/kg/day) compared to those receiving PN with standard IVFE administration (3 g/kg/day)
3. To determine if IFER results in normal growth and essential fatty acid (EFA) parameters by measuring weight, length, head circumference (weekly) and EFA profiles (every 2 weeks in the IFER group) during the treatment period.
4. To compare NDOs at 12 and 24 months of corrected gestational (±2 weeks) age of those treated with IFER with those treated with standard IVFE doses using a combination of neurodevelopmental screening tools (e.g. Bayley Scales of Infant Development, MacArthur Communicative Development Inventory, etc.) aimed at capturing several developmental domains such as motor, cognition, speech and language, sensory, and behavior.

Study Definitions

Subjects will be considered “on treatment” from the time of randomization until 7 days after PN has been discontinued, but not to exceed a total of 84 days. Subjects will be considered “on study” until they complete their neurodevelopmental testing at 24 months corrected gestational age (±2 weeks).

Study Design

This is a multi-institution, prospective, randomized study evaluating IVFE dose reduction on the development of PNAC as a proxy measure of PNALD in neonates requiring prolonged PN. The definition of PNAC will be that used predominantly in the literature – a direct bilirubin level of ≥2 mg/dL, documented on two occasions 1 week apart, in patients who have required parenteral nutrition for more than 2 weeks. **Eligible neonates will be randomized into two treatment arms: standard IVFE administration (3 g/kg/day) or IVFE reduction (IFER) (1 g/kg/day).** A total of 60 subjects (30 subjects per arm) will be included in the study. The study will evaluate

short- and long-term outcomes of this strategy. In the short-term, infants will be followed for up to 12 weeks for the development of PNAC. If a subject's PN is discontinued before 12 weeks, and if they remain in the hospital, follow-up data will be collected for an additional 7 days but not beyond a total of 84 days. Only standard-of-care data will be collected during these 7 days. No procedures will be performed for the purpose of this study during these 7 days. In the long-term, subjects' neurodevelopmental outcomes will be assessed at 12 and 24 months corrected gestational age (±2 weeks).

Primary outcome measure:

1. The rate of rise of direct bilirubin as a function of time (mg/dL/week) between the two groups over 12 weeks or until the subject has been off of PN for >7 days, whichever comes first.

Secondary outcome measures

1. Initial 12 week period:
    a. The incidence of PNAC (direct bilirubin ≥2 mg/dL) and severe PNAC (direct bilirubin ≥4 mg/dL in subjects on parenteral nutrition for at least 2 weeks)
    b. The time to development of PNAC and severe PNAC
    c. The peak total and direct bilirubin levels
    d. The incidence of EFAD
    e. Adequacy of growth as evaluated by z-scores for weight, height, and head circumference
    f. Other safety measures assessed during the initial 12 week period will include adverse events (e.g., episodes of sepsis and catheter-related blood stream infections).
2. Long-term follow-up:
    Neurodevelopmental assessments will include:
    a. At 12 months corrected gestational age (±2 weeks):
        i. Bayley Scales for Infant and Toddler Development (BSID-III)
    b. At 24 months corrected gestational age (±2 weeks):
        i. Bayley Scales for Infant and Toddler Development (BSID-III)
        ii. MacArthur-Bates Communicative Development Inventories (CDI)
        iii. Brief Infant Toddler Social Emotional Assessment (BITSEA)
        iv. Gross Motor Function Classification System (GMFCS)
        v. Behavioral Assessment System for Children-Second Edition (BASC2)

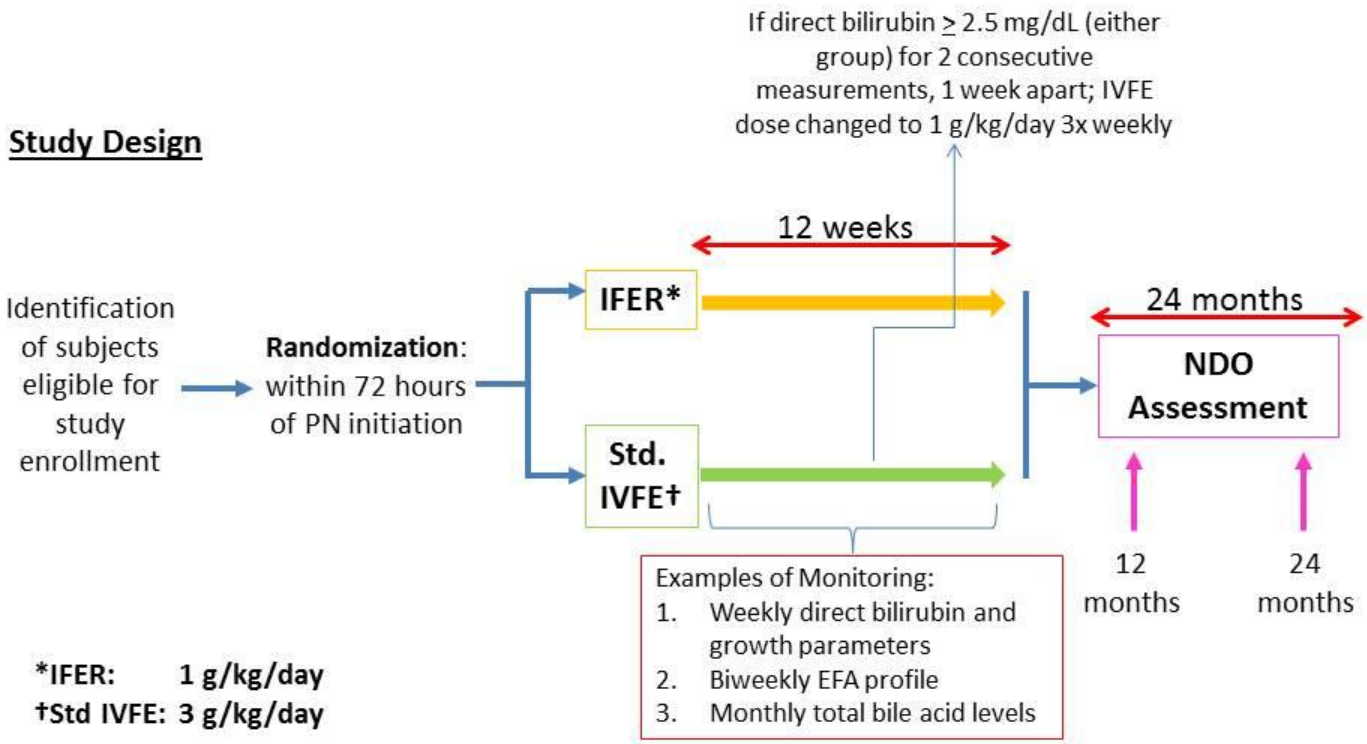
Study Arms:

Study Arm 1 (Standard IVFE dosing): Subjects in the standard IVFE dosing arm (3 g/kg/day) will be on treatment for a total of 12 weeks or until the subject has been off of parenteral nutrition for >7 days, or if they are discharged from the hospital, whichever comes first.

Study Arm 2 (IFER dosing): Subjects in the IFER dosing arm (1 g/kg/day) will be on treatment for a total of 12 weeks or until the subject has been off parenteral nutrition for >7 days, or if they are discharged from the hospital, whichever comes first.

All subjects will be maintained at their assigned dosing regimen for the duration of the treatment period (12 weeks) or until they are transitioned off of PN for >7 days, are discharged or until the direct bilirubin is ≥2.5 mg/dL for 2 consecutive weeks, whichever occurs first. Data will be collected for the duration of the study for those subjects who remain on parenteral nutrition for the full 12 weeks. For those who transition off of PN prior to the end of the 12 week period, data will be collected for 7 days after termination of PN if the subject remains in the hospital. The clinical course of these subjects who wean off PN prior to 12 weeks will continue to be monitored according to standards of practice. If serum direct bilirubin reaches a level of ≥2.5 mg/dL for 2 consecutive weeks during the course of the treatment, subjects will be transitioned to an IVFE dose of 1 g/kg/day three days per week. Those who remain cholestatic at the termination of their treatment period will be evaluated on a monthly basis for the persistence or resolution of cholestasis. This will be done by obtaining monthly liver function tests and bilirubin fractions for 6 months.

Overview of Study Design:



Inclusion Criteria

Study subjects will be neonates and infants who are ≥28 weeks corrected gestational age at the time of enrollment who are parenteral nutrition naïve (<72 hours of PN exposure prior to randomization) and whose direct bilirubin has never been >2 mg/dL with at least one of the following diagnoses:

1. Meconium ileus and peritonitis
2. Gastroschisis
3. Omphalocele >4 cm in diameter or with liver herniated outside of the abdominal cavity
4. Necrotizing enterocolitis requiring surgical intervention (drain placement, laparotomy, bowel resection)
5. Volvulus
6. Intestinal atresia with >50% bowel loss as defined by bowel length nomogram (**Figure 1**)

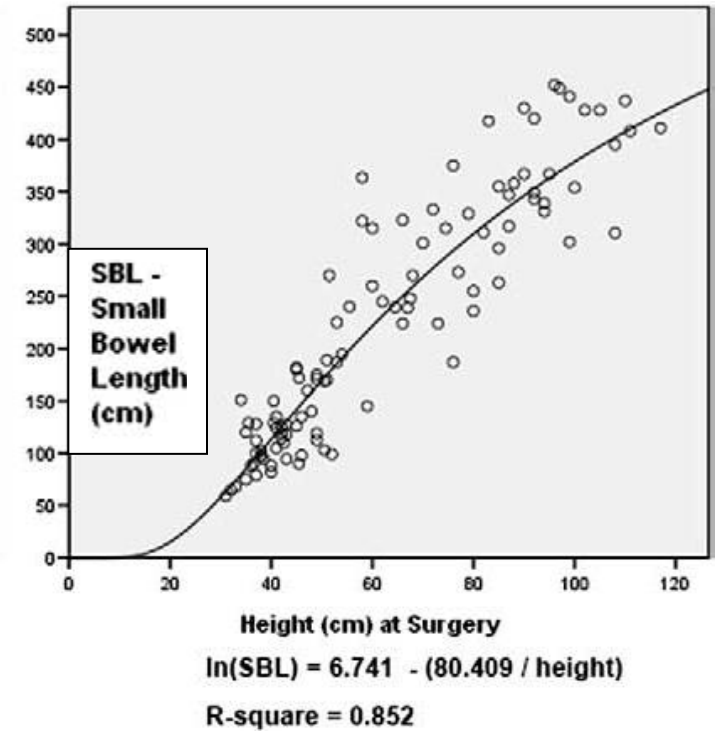


Figure 1. Nomogram estimating normal small bowel length based on height at the time of surgery.[26]

This population was selected because of their high risk for developing PNAC due to their underlying disease process and limited ability to tolerate enteral feeds. Using this group of subjects will allow for sufficient data to be obtained with the fewest number of subjects over the shortest period of time.

No preference will be given to sex, race or ethnic background during the enrollment of subjects into the study.

Exclusion Criteria

1. Birthweight <1kg
2. A metabolic pathway defect which is associated with liver dysfunction in the neonatal period including: hereditary fructose intolerance, galactosemia due to transferase deficiency and neonatal tyrosinemia, and/or disorders of lipid metabolism
3. A history of hepatic insufficiency as defined by either a biopsy with cirrhosis and/or a marked aberration in synthetic function (defined by elevated prothrombin time (INR ≥2.0) with no evidence of a systemic coagulopathy and no administration of an anticoagulant)
4. Primary or secondary liver disease, including hepatitis, as defined by either an elevated prothrombin time (INR ≥2.0)or elevation of liver function tests (AST and ALT) greater than twice the upper limit of the age-adjusted norm
5. Congenital obstruction of the hepatobiliary tree (e.g. biliary atresia, choledochal cyst)
6. History of PNAC or a direct bilirubin >2 mg/dL at any time prior to enrollment/randomization
7. Renal failure (as defined by a creatinine >1.5 mg/dL)
8. Use of extracorporeal membrane oxygenation (ECMO), as hemolysis from the ECMO circuit may cause a mixed hyperbilirubinemia unrelated to the development of PNAC
9. Documented active infection which may be communicable, including infectious hepatitis or human immunodeficiency virus
10. Previous receipt of choleretic agents (e.g. ursodiol, phenobarbital, cholecystokinin). This is done in order to avoid the use of an agent which may reduce cholestasis and the development of PNAC.
11. Current administration of phenobarbital or other barbiturates
12. No infant with a congenital or acquired anomaly which will require major cardiovascular surgery
13. Major congenital or chromosomal anomaly
14. Hypoxic ischemic encephalopathy

15. Congenital defect of the brain
16. Major seizure disorder

Removal from Study Protocol After Initiation of Study
The following criteria may require study removal:

1. Parent or guardian request for removal
2. Transfer to a non-study hospital
3. Development of multiple nutritional problems which are unable to be managed (e.g. more than mild undernutrition, irreversible undernutrition, hyperglycemia, or hypertriglyceridemia refractory to intervention)
4. At the discretion of the PI

Informed Consent
Informed consent will be obtained from the parents or legal guardian of the child. Consent will be obtained in person whenever possible. In the rare circumstance that a parent or legal guardian cannot be present in person, consent will be obtained via telephone. We expect this to be an unusual occurrence as subjects can be randomized up to 72 hours after PN initiation.

Randomization
Subjects will be randomized within 72 hours of PN initiation. Stratified, equal within-center randomization will be performed after consent is obtained. Group assignment will be determined by a random permutation of blocks of randomly determined sizes of 2 or 4. Randomization will be stratified based on gestational age (<32 and ≥32 weeks gestation). Enrollment and randomization will occur as early as feasible after identification of subjects who meet eligibility criteria. Randomization MUST occur within 72 hours of PN initiation to minimize the number of days of PN prior to treatment group assignment. All subjects will be treated according to standard management until consent is obtained and randomization occurs. Once randomization occurs the PN dose the subject is currently on may change based on group assignment.

Blinding
Subject's family members, the statistical team and those administering the neurodevelopmental tests will be blinded to treatment assignment. Blinding of families and those evaluating neurodevelopmental outcomes is particularly important as these are subjective assessments which we do not want to be biased by the treatment assignment. Adequacy of blinding will be assessed at the end of the initial 12 week treatment period by asking parents which treatment arm they believe the subject has been assigned to. Assessment of the adequacy of blinding will be performed and changes made if blinding of parents is deemed to be suboptimal. It is not possible to blind the inpatient care team and the investigators as the remainder of the PN formulation (dextrose, protein) must be adjusted to deliver adequate caloric needs to the subjects based on their IVFE treatment assignment. The lack of blinding during this phase of the study will not influence the short-term outcomes as they are discrete measurements that cannot be biased by subjectivity.

Standardization of Parenteral Nutrition Across Study Centers
The following guidelines for parenteral nutrition administration have been established to provide uniformity among study sites. Each site will follow these guidelines.

Energy
Total caloric delivery will range between 100-120 Kcal/kg/day when PN is the only source of calories
Total non-protein caloric delivery will not exceed 100 Kcal/kg/day when PN is the only source of calories

Carbohydrate
Dextrose infusion rate (DIR) will be increased in the following manner, provided there is no aberration in the serum glucose levels:

Day 1	DIR 4-8 mg/kg/min
Day 2 and beyond	Advancement of DIR by 1-2 mg/kg/min per day to a goal of 11-18 mg/kg/min

	For study subjects 0-30 days old the maximum DIR will be 16 mg/kg/min For study subjects >30 days old the maximum DIR will be18 mg/kg/min

If hyperglycemia develops (defined as serum glucose >150 mg/dL), subjects will be managed in accordance with standard guidelines[28]

1. Confirmatory point of care glucose test performed
2. If confirmatory glucose >150 mg/dL, the DIR will be reduced daily in increments of 1-2 mg/kg/min as tolerated until the serum glucose normalizes
3. Insulin will only be administered if hyperglycemia has not been controlled by adjusting the DIR

Protein

Protein, in the form of neonatal IV amino acids solutions, will be initiated on the first day of PN at the highest delivery possible based on the following ranges:

Body weight:	<1.5 kg	1.5 – 2.5 kg	>2.5 kg
Protein Needs (g/kg):	3.4 – 4	3.2 – 3.8	2 – 3

If protein is not able to be initiated at the goals indicated above, protein will be advanced over the first 2-3 days of PN administration to reach specified goals.

In order to maintain uniformity in the PN administered between study centers, formulations will use neonatal-specific amino acid solutions that contain supplementation with taurine, glutamic acid and aspartic acid, all of which are not routinely contained in conventional adult crystalline amino acid infusions. Uniformity of amino acid solutions is particularly important because a lack of taurine may have a role in the development of PNAC.[29,30] Ensuring that all infants receive this amino acid formulation will ensure uniformity between centers.

IVFE

Standard Arm: IVFE to begin at 1 g/kg/day on the first day of PN and advance by 0.5-1 g/kg/day to a maximum of 3 g/kg/day.

IFER arm: IVFE administration to begin and remain at 1 g/kg/day for the duration of the study unless inadequate weight gain/growth occurs and/or the subject develops EFAD.

Hypertriglyceridemia

If hypertriglyceridemia develops (serum triglycerides (TG)>250 mg/dL), subjects will be managed as follows:

1. Repeat laboratory measure to confirm diagnosis
2. Screen for carnitine deficiency (if present, supplement as appropriate)
3. If confirmatory TG >250 mg/dL, then:

TG 250-400 mg/dL AND glucose >150 mg/dL	**TG 250-400 mg/dL AND glucose ≤150 mg/dL**	**TG ≥400 mg/dL AND glucose >150 mg/dL**	**TG >400 mg/dL AND glucose ≤150 mg/dL**
1. Decrease DIR by 1-2 mg/kg/min as tolerated until serum glucose normalizes 2. Decrease current IVFE infusion by 50% for the remainder of the current infusion	1. Decrease current IVFE infusion by 50% for the remainder of the current infusion 2. Hold IVFE x 48 hours and repeat TG level*	1. Decrease DIR by 1-2 mg/kg/min as tolerated until serum glucose normalizes 2. Discontinue current IVFE infusion 3. Hold IVFE x 48 hours and repeat TG level*	1. Discontinue current IVFE infusion 2. Hold IVFE x 48 hours and repeat TG level*

3. Hold IVFE x 48 hours and repeat TG level*			

* Upon repeat TG level:
1. If TG are <250 mg/dL: restart IVFE at 50% of previous dose
2. If TG are ≥250 mg/dL: continue to hold IVFE infusion and repeat TG level every 48 hours until <250 mg/dL. If IVFE is held for ≥7 days with continued TG levels ≥250 mg/dL, restart IVFE at 0.5 g/kg/day

Essential Fatty Acid Deficiency (EFAD)
The detection of EFAD will be assisted by a biochemist and expert in the area of lipid biology.

The IVFE dose will be adjusted if subjects develop EFAD. EFAD will be defined by the development of any of the following:
1. Clinical evidence of EFAD deficiency (i.e. scaly skin)[31,32]
2. Triene:tetraene ratio of >0.20
3. Arachidonic acid (ARA), (EPA) and/or docosahexaenoic acid (DHA) levels below normal as defined by Mayo Laboratory reference ranges

In the presence of EFAD, the IVFE dose will be increased in 0.5 g/kg/day increments based on biweekly essential fatty acid profiles until resolved.

Cycled Parenteral Nutrition
Study subjects will not receive cycled parenteral nutrition (i.e. parenteral nutrition administered over less than 24 hours per day) during the study period, as cycled PN has been shown to decrease the development of PNAC and may represent a confounder.[33,34]

Adjustment of Parenteral Nutrition if Inadequate Weight Gain Occurs
(Lipid Restricted Group Only)

Subjects with inadequate weight gain that cannot be attributed to other physiologic causes (e.g. low urine sodium, low hematocrit, insufficient respiratory support) will be managed according to the protocol described below.

Indicators of acute and chronic malnutrition will be assessed using z-scores.[35] For the purposes of the initial 12 weeks, subjects will only be assessed for **acute** malnutrition as chronic malnutrition occurs after 3 months. Z-scores will be obtained using the Peditools.org calculator (https://peditools.org/). Fenton scores[36] will be used for infants born <37 weeks gestation, until 50 weeks gestation, at which time the WHO scores will be used, using corrected gestational age. WHO scores[37] will be used for infants born at ≥37 weeks gestation. Z-scores will be documented for birth anthropometrics. Z-scores will be assessed at 1 month of age and weekly thereafter (allowing 1 month before reassessment to account for natural weight loss at birth in addition to weight loss from acute illness at birth). Z-scores for all anthropometrics will be followed, however, the z-score for weight-for-age will be used as the primary assessment for acute malnutrition and will be used to prompt intervention. If intervention is required, PN will be adjusted as described in **Table 1**.

Table 1. PN Adjustments Based on Change in Z-score (after 1 month of age)

Decrease in z-score by 0.5 standard deviation (SD) (17 percentiles decrease) from birth	Increase DIR by 2 mg/kg/min (as clinically tolerated) Maximum DIR for study subjects 0 – 30 days old: 16 mg/kg/min Maximum DIR for study subjects >30 days old: 18 mg/kg/min
Decrease in z-score by 1 SD (34 percentiles decrease) from birth	1. Increase DIR by 2 mg/kg/min (as clinically tolerated)

	a. Maximum DIR for study subjects 0 – 30 days old: 16 mg/kg/min b. Maximum DIR for study subjects >30 days old: 18 mg/kg/min 2. If Z-score is not improving in response to increase in DIR after 1 week, or if DIR is already at max of 18 mg/kg/min, increase IVFE by 0.5 g/kg/day to a maximum of 1.5 g/kg/day 3. If poor weight gain continues and consideration is being made to increase the lipid dose to 2 g/kg/day the coordinating center must be contacted to determine if the subject should be withdrawn.
Decrease in z-score by 2 SD from birth x2 consecutive measurements separated by 7 days or Lipids need to be increased to >1.5 g/kg/d d/t poor growth	Subject deemed to have growth failure, prompting exit from study in order to allow for liberalization of the IVFE dose to promote adequate growth

Data Collection

1. Demographic information
2. Birth weight, gestational age
3. Weight, gestational age at the time of enrollment/randomization, and weekly thereafter
4. Inclusion diagnosis
5. Number of septic episodes according to standard definitions[38]
6. Use of choleretic medications
    a. Ursodiol
    b. Cholecystokinin
    c. Phenobarbital
7. Nutritional data
    a. Parenteral nutrition data
        i. IVFE dose
        ii. Dextrose infusion rate
        iii. Total calories and % calories obtained via PN
    b. Enteral nutrition data
        i. Total calories and % calories being obtained via enteral route
        ii. % calories obtained via breast milk
8. Growth parameters (whenever possible, obtain all growth measurements on the same day of the week)
        i. Weight (with z-score) weekly (every 7 days ± 2 days)
        ii. Height (with z-score) weekly (every 7 days ± 2 days)
        iii. Head circumference (with z-score) weekly (every 7 days ± 2 days)
9. Additional safety data to be obtained from human subjects is summarized below, indicating any key differences in frequency of monitoring between the two groups:

Laboratory Parameter	Frequency (+24 hours)*
Fractionated bilirubin panel (includes total, indirect, and direct bilirubin)	One time weekly (Monday)
Serum bile acids (total)	One time monthly (every 4th Monday on treatment)

Point of care or serum glucose measures	- Daily for the first 5 days of PN, then twice weekly (Monday, Thursday) - Confirmatory test if serum glucose is >150 mg/dL on electrolyte panel
Serum Triglycerides	One time weekly (Monday)
Essential Fatty Acid Profile	**IFER Group:** Every 2 weeks (Thursday) **Standard Group:** At the time of restriction of IVFE to 1 g/kg/day three days per week, then every 2 weeks (Thursday)
Comprehensive metabolic panel	Twice weekly (Monday, Thursday)
Magnesium level	Twice weekly (Monday, Thursday)
Phosphorus level	Twice weekly (Monday, Thursday)
Complete blood count	One time weekly (Monday)

* Lab draw volumes will be below each institution's daily limits

Definition of Sepsis

Sepsis will be defined in a standard manner using the following criteria (**Table 2**):[38]

The presence of at least two of the following four criteria ***in the presence of or as a result of suspected or proven infection***:

1. Core temperature of >38.5C or <36C
2. Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over 0.5 to 4 hour time period OR for children <1 year old: bradycardia, defined as a mean heart rate <10th percentile for age in the absence of external vagal stimulus, beta-blocker drugs, or congenital heart disease, or otherwise unexplained persistent depression over 0.5 hour time period
3. Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia
4. Leukocyte count elevated or depressed for age or >10% immature neutrophils

Table 2. Age specific criteria for the criteria listed above; adapted.[38]

Age	Tachycardia (bpm)	Bradycardia (bpm)	Respiratory Rate (breaths/min)	Leukocyte Count Leukocytes x $10^3/mm^3$	Systolic Blood Pressure (mmHg)
0 days – 1 week	>180	<100	>50	>34	<65
1 week – 1 month	>180	<100	>40	>19.5 or <5	<75
1 month – 1 year	>180	<90	>34	>17.5 or <5	<100

Monitoring and Follow Up of Subjects

Monitoring during the 12 week treatment period includes the amount and type of parenteral and enteral nutrition (total calories, expressed as kcal/kg/day, and amounts of parenteral protein and IVFE, expressed as g/kg/day, type of enteral formula (e.g. breast milk, formula). Clinical diagnoses, clinical occurrences other than adverse events, clinical complications, and drugs listed on the medication form will be recorded. If a patient goes home on PN prior to the end of 12 weeks from randomization, data will be collected according to standards of that institution.

Infants completing the initial 12 week period will be monitored according to the institution's standard of care during the remainder of their hospitalization. Subjects who are cholestatic at the termination of the initial 12 week period will be followed with monthly LFTs and bilirubin fractions to assess for resolution or progression of PNAC. These monthly labs draws will continue for 6 months. If patients remain cholestatic, a referral for further gastrointestinal workup will be made.

Among subjects not followed for cholestasis, a final morbidity and mortality assessment will be made, by phone, 6 months after completion of the initial phase of the study. To ensure that follow-up is achieved after the infant is discharged and for attaining the neurodevelopmental follow-up at 12 and 24 months of age, a contact sheet will be held by the study coordinator which will contain the names, addresses and phone numbers of all participants.

Neurodevelopmental Outcome Measures

After the initial 12 week treatment period, study subjects from both treatment arms will be evaluated at 12 months and 24 months (corrected age) in order to assess the influence of early IFER treatment on neurodevelopmental outcomes. Early outcome assessments are typically conducted at a corrected age of approximately 24 months.[39] Recommended outcome domains have included motor, cognition, speech and language, sensory and other disabilities. Developmental assessments typically involve a medical examination, direct assessment with an instrument such as the Bayley Scales of Infant Development (Bayley-III) and developmental survey instruments. While there is evidence that a number of common survey screens lack validity when conducted below 15 months of age,[40] by 24 months of age specific survey instruments have been shown to be reliable and valid measures of behavioral risks.[40-44] For the purposes of this study the 12 month assessment is included to examine the possibility with our target population of very early identification of developmental lags and predictors of risk. These assessments are crucial in order to investigate whether an association exists between reducing the dose of IVFE during a critical period of growth and brain development (i.e. the neonatal and infant period) with long-term neurodevelopmental outcomes.

Bayley Scales of Infant and Toddler Development (BSID-III):[45] The BSID-III is designed to assess developmental functioning of infants and toddlers, ages 1 month to 42 months. The instrument includes five distinct scales, of which three scales and associated subscales are utilized for the purposes of this study: cognitive, language (receptive and expressive communication) and motor (fine motor and gross motor).

The MacArthur-Bates Communicative Development Inventories (CDI):[46] The MacArthur-Bates CDI is the most widely used parent report measure of language development. The CDI includes two versions: Words and Gestures for ages 8-16 months and Words and Sentences for children ages 16-30 months. Scores are reported as percentiles compared to age-standardized norms.

Brief Infant Toddler Social Emotional Assessment (BITSEA):[43,44,47] The 42-item BITSEA is a parent report measure of social-emotional and behavior problems in children ages 1-3. The Problem Index includes internalizing, externalizing and dysregulation problems. The internalizing scale has items that assess depression, anxiety and negative emotionality.

Gross Motor Function Classification System (GMFCS):[48,49] The GMFCS is a five level classification system that assess the gross motor function of children with and at risk for cerebral palsy, from infancy through age 12. It differentiates children into one of five levels of functioning, based on the child's current gross motor ability, limitations in function, and need for assistive technology.

Behavior Assessment System for Children – Second Edition Preschool, Parent (BASC-2 Preschool Parent's version):[50] The BASC-2 PRS-P is a 134 item questionnaire that assesses psychological status and adaptive behavior. The parent/caregiver rates the occurrence of behaviors using a 4-point rating scale. The profile yields eight clinical scales (hyperactivity, aggression, anxiety, depression, somatization, atypicality, withdrawal and attention problems) and four adaptive scales (adaptability, social skills, activities of daily living and functional communication) as well as four composite scores (externalizing problems, internalizing problems, adaptive skills and the Behavioral Symptoms Index).

These neurodevelopmental tools will be evaluated on the following schedule:

12 month follow-up	24 month follow-up

BSID-III	BSID-III, CDI, BITSEA, GMFS, BASC-2

We recognize that neurodevelopmental outcomes may be influenced by other factors that extend beyond early interventions during the neonatal and infant periods. Such factors may include duration of parenteral nutrition, duration of IFER, mean IVFE dose, percent nutrition obtained via the enteral route compared to the parenteral route, type of enteral intake (i.e. breast milk), the development of essential fatty acid deficiency, as well as early intervention programs, attendance at daycare/preschool, and socioeconomic status. As such, we will evaluate study subjects every 6 months (± 30 days) and collect the following data, which will be used in final statistical analyses to evaluate predictive variables on neurodevelopmental outcomes:

1. Current parenteral and enteral nutrition parameters
2. Current IVFE dose/mean IVFE dose over course of previous 6 months
3. Essential fatty acid profiles (if available, according to each institution's standard of care), evaluating for development of essential fatty acid deficiency
4. Attendance at early intervention programs and/or daycare/preschool

Monitoring for Adverse Events

The study coordinator and investigators at each site will monitor for adverse reactions. Although generally very well-tolerated, the administration of PN with Intralipid® 20% is associated with a number of adverse events.

Determination of Expectedness:

Adverse events will be classified as expected or unexpected. Expected adverse events include those listed in the clinical protocol, the package insert for PN with Intralipid® 20%, and those events listed in the informed consent document.

These may include, but are not limited to:

1. Line complications including:
    a. Catheter-related blood stream infection (CRBSI)
        i. Defined as: A laboratory-confirmed bloodstream infection (LCBI) where central line (CL) or umbilical catheter (UC) was in place for >2 calendar days on the date of event, with day of device placement being Day 1, *and* a CL or UC was in place on the date of event or the day before. If a CL or UC was in place for >2 calendar days and then removed, the LCBI criteria must be fully met on the day of discontinuation or the next day[51,52]
        ii. This is in distinction to a catheter-related blood stream infection (CRBSI) whose definition requires removal of the catheter[51]
    b. Need for catheter replacement
2. Hyperglycemia > 300 mg/dL
3. Hypertriglyceridemia >400 mg/dL
4. Development of essential fatty acid deficiency
5. Moderate undernutrition (decreased in z-score by ≥2 standard deviations) to severe undernutrition (decreased in z-score by ≥3 standard deviations)
6. Thrombophlebitis
7. Hypercoagulopathy
8. Thrombocytopenia (platelet count <50 k/mm^3)

Determination of Relatedness:

Generally, the definition of an adverse event could include worsening of a pre-existing condition and investigators could be asked to make the distinction between exacerbation of a pre-existing condition and an event related to the study protocol when recording and reporting adverse event. However, due to the nature of the population being studied, the typical effects of the underlying condition may not yet be present at Baseline.

Based on clinical information, an adverse event will be determined by the site PI to be definitely not related, unlikely to be related, possibly related, definitely related or insufficient information to Intralipid® 20% administration or other study procedures. Usual effects from the subject's condition that are considered by the site PI to be not related to Intralipid® 20% administration or other study procedures will be noted in the subjects' medical records but will not be recorded or reported as an adverse event for this study. Examples include emesis, reflux and pain due to an expected surgery/procedure.

Adverse Event Reporting

All adverse events that occur from the time of consent until the patient completes 12 weeks of TPN or has been off TPN for 7 days, whichever comes first will be assessed for expectedness and relatedness and recorded in the subject's study binder.

Only adverse events considered to be possibly related to Intralipid® 20% administration or other study procedures will be reported to the sponsor, the IRB, the FDA, and the DSMB.

Severe Adverse Event Reporting

Although no serious adverse reactions are anticipated, study participants will be monitored, and any such events will be recorded and reported to the appropriate regulatory committees. These include:

- Death while on the study
- Any study-related event that prolongs hospitalization
- Any study-related event that requires an operative procedure

Mortality will be defined as a death occurring at any time during the child's admission to the hospital. The cause of death, autopsy findings and whether the subject was on study drug at the time of death will also be recorded.

All adverse events will be graded as follows in their relation to IFER: definitely not related, unlikely to be related, possibly related, definitely related or insufficient information. All cases rated as possibly or definitely related will be defined as an SAE. These SAEs will be reported to the University of Michigan Sponsor-Investigator within 48 hours. The site PI will be contacted immediately and the case will be evaluated for reporting to IRB, FDA and study termination, if deemed necessary. Each clinical site will report SAEs to their local IRB as per their standard reporting policy. As per 21 CFR 312.32(c), the sponsors will also notify FDA and all participating investigators in an IND safety report of any adverse experience associated with the use of the drug that is both serious and unexpected.

Data Safety Monitoring Board

An independent data safety monitoring committee will be established to monitor the study for safety and to determine if a statistical difference has been achieved between the two groups for the primary outcome measure at interim data analyses. The data safety monitoring committee will review the data at the halfway point of the study to assess the safety and efficacy of the study to that point. If safety issues are revealed the study investigators and each site's IRB will be notified immediately. The data safety monitoring committee will also ensure that adverse events are promptly reported to the FDA, and all participating investigators will be notified as well of any adverse experience associated with the use of the drug that is both serious and unexpected.

Sample Size Determination

The sample size was calculated for the primary outcome (rate of rise of direct bilirubin) using previously published data as well as our clinical experience.[22,23] We have assumed that the mean bilirubin levels at baseline, 4 weeks, 8 weeks and 12 weeks in the standard dosing group (2-3 g/kg/day) will be 0.55 mg/dL, 1 mg/dL, 1.5 mg/dL and 2 mg/dL corresponding to a slope of 0.125 mg/dL/week over 12 weeks. The standard deviation of the bilirubin measures at each of these four time points is 1. Likewise, we have estimated that the mean bilirubin levels at baseline, 4 weeks, 8 weeks and 12 weeks in the minimization group (1 g/kg/day) will be 0.45 mg/dL, 0.5 mg/dL, 0.45 mg/dL and 0.5 mg/dL (a slope of roughly zero). The standard deviation of the bilirubin measurements at

each of these four time points is 1. The correlation of any two adjacent measures on the same individual is 0.5. This correlation drops to 0.354 between baseline and 8 weeks and to 0.25 between baseline and 12 weeks.

Using the above assumptions, a sample size of 15 subjects per group using a repeated measures ANOVA will have 85% power to detect the change in slopes as being significant at the 0.05 level, 79% power to detect a difference in means overall between groups and 88% power to detect the general change in means over time across the two groups. When the sample size is increased to 20 subjects per group, the above power numbers increase to 95%, 90% and 96%, respectively. If 30 subjects are recruited per group this design will allow for a 33% attrition rate by twelve weeks as we expect that not all patients will require PN for the full 12 weeks of the initial study period. Thus, we will recruit a minimum of 30 subjects per group to ensure adequate power to detect our primary outcome measure.

Of the secondary outcomes, sample size was also calculated for the incidence of cholestasis. Assuming an incidence of 50% in the standard group and an incidence of 10% in the minimization group, with alpha = 0.05 and power of 80%, a sample size of 23 is required for each group. Regarding the other secondary outcomes, our sample size is large enough to allow for establishing trends which may then highlight avenues for future research.

Planned Statistical Analysis

All data analysis will be directed and performed by a statistician through the Center for Statistical Consultation and Research at the University of Michigan (http://cscar.research.umich.edu/about/). Data will be analyzed using SAS software release 9.3 for Windows (Copyright, SAS Institute Inc., SAS and all other SAS Institute Inc. product of service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA). All study subjects will be included in the statistical analysis in an intent-to-treat manner.

Descriptive statistics using standard statistical tests (e.g. Student's t-test, paired sample t-test, Wilcoxon rank sums tests, Fisher's exact tests, Chi-square test, McNemar's test, etc.) will be used throughout the statistical analysis of data. Logistic regression will be used to compare differences between groups. Because septicemia can exacerbate PNAC, we will include the incidence of septicemia as a covariate in our analysis. Septic episodes will be defined as positive cultures or clinical presentation consistent with sepsis during the 7 day period corresponding to the subject's weekly bilirubin value. The number of septic episodes will be accounted for using a Poisson regression analysis (or possibly a negative binomial regression if there is more variability than expected for a Poisson distribution), with the total time on PN as an offset, allowing us to compare the rate of occurrence of septic episodes in the two groups.

Primary Outcome Measure

The primary outcome measure – change in direct bilirubin as a function of time (mg/dL/week) between the two groups – will be evaluated using a linear mixed model analysis to compare the slope of total bilirubin levels between the standard and reduced groups. This model will allow us to examine the degree of variability between subjects in the rate of change over time, and will take this variability into account in the model by including a random effect for the slope of time for each subject as well as a random intercept for each subject.

Secondary Outcome Measures

1. **Time to PNAC (direct bilirubin ≥2 mg/dL) and time to severe PNAC (direct bilirubin ≥4 mg/dL)**
   The time to development of a first occurrence of PNAC and of severe PNAC will be evaluated using survival analysis. Initially, we will compare the two groups using Kaplan-Meier curves, but the eventual analysis will take into account important covariates, such as development of sepsis, average IVFE dose and percentage of calories from enteral feeds using a Cox proportional hazards survival analysis. We will test whether the proportional hazards assumption is met for the analysis by including an interaction term between treatment group and time to see if any differences between the groups are consistent or increase/decrease over time. The effect of important time-varying covariates (e.g., septic episodes) can also be taken into account using a Cox model.

2. **Level of peak total and direct bilirubin**

These measures will be compared between the two treatment groups using a linear mixed model.

3. **Incidence of PNAC, severe PNAC, and EFAD**
   The number of occurrences of each of these outcomes will be compared using either a Poisson regression model or a negative binomial model if the variance in these counts is greater than expected for a Poisson distribution. We will also include the time on TPN as an offset, so that we can compare the rates of occurrence of each of these outcomes for the two treatment groups. These regression models will be adjusted for the covariates such as development of sepsis, average IVFE dose, and percentage of enteral feeds.

4. **Adequacy of growth as evaluated by z-scores for weight, height and head circumference**
   The z-scores for each of these measures will be compared between the two treatment groups using a linear mixed model. For normally growing infants, we would expect that there would not be a trend in z-scores because we would expect them to maintain their curve. However, for the subjects in this study, there may be a loss in z-score over time due to inadequate growth. The slopes of the changes in these z-scores over time will be compared between the two treatment arms. A random intercept will be included for each subject to allow for individual variability in average values as well as a random slope to allow for variability in the rate of change over time across subjects. Important covariates, such as gestational age (<32 weeks gestation vs. ≥32 weeks gestation) and birth weight will also be used as covariates in this analysis.

5. **Neurodevelopmental Outcomes**
   Bayley Scales of Infant and Toddler Development (BSID-III):[45] Raw scores are converted to composite and subscale scores using age-standardized norms. Scale composite average internal consistency reliability coefficients range from .91 to .93 with subtest reliability ranging from .86 to .91. Validity-related studies indicate relatively high correlations (.72-.79) with the Wechsler Preschool and Primary Scale of Intelligence – Third Edition (WPPSI-III)[53] and with the Preschool Language Scale – Fourth Edition (PLS-4)(.71-.83).[54]

   The MacArthur-Bates Communicative Development Inventories (CDI):[46] The MacArthur-Bates CDI is the most widely used parent report measure of language development. The CDI includes two versions: Words and Gestures (ages 8-16 months) and Words and Sentences (ages 16-30 months). Scores are reported as percentiles compared to age-standardized norms.

   Brief Infant Toddler Social Emotional Assessment (BITSEA):[44,47,55] Dichotomous scores are generated based on cut-off scores, which identify subjects to be at risk. Internal consistency coefficients range from.80 to.82. Test-retest reliability is acceptable at .80-.85. Recent evidence identifies the BITSEA as the best short tool for early detection of psychosocial problems in two-year olds.[40]

   Gross Motor Function Classification System (GMFCS):[48,56] Interrater reliability is 0.75 and both content and predictive validity are well-demonstrated in child and adult populations. [56-58]

   Behavior Assessment System for Children – Second Edition Preschool, Parent Preschool Parent's version (BASC-2 PRS-P):[50] Limited construct validation data indicate strong correlations with similar scale scores. Primary measurements from the BASC-2 including the Adaptive Behavior Composite and the Behavior Symptoms Index will be reported as T-scores and as percentiles. Secondary analyses will be conducted on the subscales.

For the comparison of the two treatment arms across time for neurological measures that are approximately continuous, a linear mixed model will be used with a repeated measures covariance structure. The best covariance structure will be chosen based on a comparison of the Akaike Information Criterion (AIC) and Bayes Information Criterion (BIC). This will allow us to adjust for correlated measures within a subject and possibly for unequal variances across times points and across groups. Each measure will be compared between the groups at each time point, controlling for important demographic variables, such as gestational age, diagnosis, bilirubin (total and direct), as well as a time-varying covariate for development of essential fatty acid deficiency. Importantly, we will also evaluate the influence of three IFER-related variables on neurodevelopmental

outcomes: mean IVFE dose, duration of IFER and the development of essential fatty acid deficiency. Since total and direct bilirubin are expected to be highly collinear, we will use only one of these predictors and compare the models using AIC and BIC to decide which is a better predictor of the particular outcome being assessed.

A logistic regression with Generalized Estimating Equations (GEE) approach will be used to compare the binary outcomes between groups.

Intent to Treat
All statistical analyses will be performed using an intent to treat approach.

Potential Confounding Variables:
Nutritional intake: Differences in nutritional intake alter the risk of PNAC as any amount of enteral nutrition has been shown to enhance rehabilitation.[59] Infants able to tolerate even trophic amounts of enteral calories will be at decreased risk compared to those than those who are completely PN-dependent. Every attempt will be made to standardize enteral feeding advancement. This will be possible because of the relatively homogeneous nature of the subjects enrolled and their similar expected ability to tolerate enteral feeds. Statistical analyses will account for variations in enteral intake. Overfeeding may result in hepatic steatosis that can also worsen PNAC, therefore all subjects will follow a strict guideline for PN administration. Additionally, the administration of breast milk, which contains proteins necessary for optimal brain development, may be a confounding variable and will be accounted for in the final data analysis. PN duration (with or without IFER) may also affect neurodevelopmental outcomes and will be included as a covariate in the statistical analysis.

Variation in IVFE dose: The IVFE dose may vary depending on the clinical status of the subject (e.g. elevated TG, EFAD, direct bilirubin ≥2.5 mg/dL, nutritional and/or fluid status). Every attempt will be made to maintain dosing within the assigned treatment arm. Based on previous experience using IFER at the University of Michigan, hypertriglyceridemia and EFAD are exceedingly rare occurrences, therefore, the IVFE dose is not expected to be adjusted frequently based on these findings. Statistical analysis will account for differences in IVFE dose by using the mean IVFE dose as well as the duration of IVFE/IFER as a covariate.

Critical medical incidences: As differences in the degree of medical illness between groups may adversely affect the development of PNAC, a number of complications will be examined as secondary outcome measures including sepsis and mortality rates as well as days in the neonatal intensive care unit.

Occurrence of sepsis: Because sepsis may lead to increased levels of direct bilirubin and may worsen PNAC, the occurrence of sepsis is being recorded and a marker of morbidity.

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