PNALD: Study Design

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Etiologic Factors Associated with PNALD

- Prematurity
- Sepsis
- Absence of Enteral nutrients
- Endotoxin and Pro-inflammatory cytokines
- Disturbance in Bile acid transport
- TPN constituents and administration
Spectrum of PNALD

- Hepatocellular injury
- Steatosis/steatohepatitis
  - More common in adults
- Cholestasis
  - More common in neonates/infants
  - Advanced liver disease
- Fibrosis without cholestasis
Consequences of PNALD-Associated Cholestasis

• Known
  – Poor bile flow
  – Jaundice
  – Malabsorption
  – Fibrosis
  – Cirrhosis/portal hypertension
  – Bleeding from gastric/intestinal stoma
  – Associated with poor outcome (death/liver transplantation)

• Possible
  – Impact on intestinal adaptation
  – Increased risk for sepsis
Study Design Strategies

- Intervention after development of cholestasis
  - Fewer patients than in the past
  - Investigator reluctance to “wait” for cholestasis
  - Best for referred patients

- Prevention
  - Identify high risk populations likely to require long-term PM
  - Small numbers remain problematic

- Both will require a consortium to complete
Recent Management Changes in U.S. Patients to Treat or Prevent Cholestasis

- Early lipid reduction or intervention strategies
  - Intralipid and Omegaven
  - Particularly in “high risk” patients

- PN cycling; careful attention to glucose infusion rate

- Aggressive enteral/oral feeding regimens

- Preventive strategies for catheter related blood stream infections
  - Antibiotic and Ethanol lock therapy
Recent management changes will alter study design

- Prevalence of hyperbilirubinemia in PNALD has likely decreased as a consequence of these changes at major centers. We know little of the other features of PNALD (e.g., fibrosis, bile flow).

- Entry criteria requiring hyperbilirubinemia (i.e., intervention study) may result in a futile effort to satisfy enrollment requirements.
Assuming the prevalence of PNALD-associated hyperbilirubinemia has decreased as a result of changes in local “standards of care”, what clinical questions can be addressed regarding PNALD if “cholestasis” is not required for study entry?

- Can a cohort be identified early as “high risk” for developing hyperbilirubinemia?
- Can hyperbilirubinemia be prevented in a “high risk” cohort?
- If prevention is possible, is it associated with lipid composition, amount administered, or other factors?
• Must a “prevention strategy” be permanent, or is limited intervention during a “vulnerable period” sufficient to keep the total bilirubin $\leq 2.0$ mg/dL?

• If the total bilirubin remains $\leq 2.0$ mg/dL, can liver injury still occur? Do non-invasive markers of liver cell injury or fibrosis provide evidence of liver injury despite a total bilirubin $\leq 2.0$ mg/dL?

• Do lipids with anti-inflammatory potential alter the immune/inflammatory cytokine or serum lipid profile compared to standard lipid?

• Do lipids with anti-inflammatory potential, provide benefit beyond PNALD such as reduced frequency of infection or decrease time to enteral autonomy.
Hypothesis

• Children at risk for hyperbilirubinemia as a consequence of prolonged PN who receive early introduction of an anti-inflammatory mixed lipid infusion (1 gram/kg/day) for up to 4 months are less likely to have a total bilirubin of ≥ 2.0 mg/dL at 12 mo following study entry than similar children who receive early introduction of soy lipid (1 gram/kg/day) for up to 4 months.
Entry Criteria

- On PN for no longer than 14 days
- Age: < 90 days
- Diagnosis associated with need for PN for at least 4 mo
  - Short bowel syndrome with*
    - Remaining small bowel length is less than 85% of estimated bowel length and/or less than 110 cm total length
    - Absent ileo-cecal valve
  - Congenital enterocyte defect

Exclusion Criteria

- Gastroschisis with primary closure
- PN for 15 days or more
- Hemodynamic instability (to be defined)
- Cardiopulmonary instability (ECMO, oscillating ventilator)
- Other known hepatobiliary condition (biliary atresia, Alagille syndrome)
## Potential Study Lipids

<table>
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<tr>
<th>Component</th>
<th>SMOFlipid 20%</th>
<th>Intralipid 20%</th>
<th>Omegaven 10%</th>
<th>ClinOleic 20%</th>
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<td>348 + 33</td>
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Study Design

• Prospective, randomized, double-masked
• Early introduction (within 14 days of PN initiation) of the Study Lipid for up to 4 months
  – Mixed Lipid (SMOF) 1 g/kg/d vs
  – Soy (Intralipid) 1 gm/kg/d

• After 4 months, if on PN, child is placed on Intralipid 1 gm/kg/d
  – Total bilirubin > 5 mg/dL: Omegaven (compassionate use)
Study Design

Lipid A

Lipid B

Intralipid 1 gm/kg/d

Omegaven Rescue

Qualifying Diagnosis

14 days 4 months 12 months
Study End-Points

• Primary Outcome: Total bilirubin < 2.0 mg/dL at 12 mo.
• Secondary Outcomes at 3, 6, 9, and 12 mo following study entry
  – Total bilirubin, direct bilirubin*, ALT, GGTP
  – Growth, weight gain, head circumference parameters
  – Inflammatory and lipid profiles
• Secondary outcomes at 12 months
  – Duration of PN
  – Need to increase Lipid over 1.5 gm/kg to increase calories
  – Number of catheter related blood stream infections (time to the first, rate/PN days)
  – Time to enteral autonomy (off PN for > 3 mo)
  – Need for Omegaven rescue
  – Time to 50% total calories provided enterally
  – Overall survival with or without native bowel
  – Intestinal or liver transplant: listed, received

*A Central Lab will be required for direct/conjugated bilirubin measurements for research analysis.
Management Variables

- Trophamine between 2-3 gm/kg/d
- Glucose infusion rate not greater than 16mg/kg/min
- Enteral feeding regimen with breast milk or pregestimil?
- Use of IV antibiotic / ETOH lock therapy?
- Oral antibiotics
- Pro/anti-motility agents
- Acid suppression
Clinical and Research Labs

• Clinical / Standard of Care
  – TPN labs: weekly while on PN; at least every 2 months when off PN
  – Vitamins and Micronutrients: at least every 4 months

• Research: Biomarkers for
  – Oxidative stress
  – Serum lipid profile
  – Inflammation
  – Liver fibrosis
  – Intestinal mass
  – Intestinal inflammation
Clinical / Standard of Care Labs

- TPN Labs at least weekly while on PN, every month when off PN for 3 mo then every 2 months
  - Hgb, Hct, WBC, platelets
  - AST, ALT, GGTP, Total protein, albumin, bilirubin total/direct
  - Calcium, Magnesium, Phosphorous
  - Na, K, Cl, bicarbonate (CO2), BUN, creatinine, glucose
  - Triglyceride
- Vitamins at least every 4 and 8 months if on PN, q 6 months off PN
  - Vitamin E, 25-OH Vitamin D, Retinol, Retinol binding protein
  - PT/INR, Vitamin B12
- Trace elements at 5 and 10 months on PN, every 6 months off PN
  - Copper, manganese, selenium, zinc, chromium,
Research Labs

- **Oxidative stress**: monthly on PN, every 2 mo off PN
  - 8-isoprostan, 8-hydroxydeoxyguanosine, glutathione peroxidase
  - Vitamin E, total lipids, phytosterol level
- **Lipid profile**: monthly on PN and for 3 mo off PN, then 12 mo.
- **Inflammation**: weekly on PN, monthly off PN for 3 mo, then at 12 mo
  - Luminex markers
- **Liver fibrosis/cholestasis**: monthly on PN, every 3 mo off PN
  - AST-to-platelet ratio index
  - Fibro-scan, Fibro-test
  - Total bile acids
- **Intestinal mass**: monthly on PN at time of clinical labs off PN (no more frequently than q month)
  - Plasma citrulline
- **Intestinal inflammation**: monthly on PN and for 3 mo if off PN, the at the time of a clinical visit, no more frequently than every 2 mo
  - Stool for calprotectin
Blood Volumes

- **Oxidative stress:**
  - 8-isoprostane
  - 8-hydrosydeoxyguanosine
  - glutathione peroxidase
  - Vitamin E
  - total lipids
  - phytosterol level

- **Lipid profile:** 150 µl serum or plasma

- **Inflammation:** Luminex markers—200 µl serum

- **Liver fibrosis/cholestasis:**
  - AST-to-platelet ratio index
  - Fibro-scan, Fibro-test
  - Total bile acids

- **Intestinal mass:** Plasma citrulline

- **Intestinal inflammation:** Stool for calprotectin
Research Tissue

• If liver biopsy is performed for clinical purposes or liver explant is available; obtain unstained slides for research purposes is tissue is available.
• Can we get tissue for research purposes?
  – At the time of abdominal surgery?
Extra Slides
INFLAMMATION

- Bacterial Overgrowth
- Intestinal Adaptation
- Intestinal Microbiome
- PN Components
- Catheter Related Blood Stream Infections
- Surgery
- Liver Injury
- Fasting
PNALD is more than Cholestasis

- In the absence of jaundice
  - Aminotransferase elevations may persist
  - Hepatic fibrosis may progress
- Inflammatory markers are associated with PNALD
- Oxidant stress is associated with structural and functional liver injury