**Methods**

This was a prospective, open label, compassionate use study conducted under IND 111,397 from the U.S. Food and Drug Administration. The protocol was approved by the Institutional Review Boards of the University of Texas Health Science Center at San Antonio and University Hospital, San Antonio, Texas. The study was registered with the NIH Clinicaltrials.gov service, identifier #NCT01425567 prior to initiation of study.

All neonates admitted to the Neonatal Intensive Care Unit (NICU) at University Hospital between March 2011 and December 2013 were considered for enrollment. Inclusion criteria included patients >14 days and <24 months of age; a diagnosis of anatomic short gut defined as >50% of small bowel surgically removed for any reason, or known severe dysmotility of the gut defined as inability to tolerate minimal enteral feeds unrelated to anatomy; a serum direct bilirubin ≥4mg/dL; and receiving at least 60% of their calories from intravenous parenteral nutrition and expected to require intravenous nutrition for at least an additional 28 days. Not meeting the anatomic short gut definition or severe dysmotility criterion but with serum bilirubin ≥6 mg/dL and receiving at least 60% of their calories from intravenous parenteral nutrition and expected to require intravenous nutrition for at least an additional 28 days were also included. Exclusion criteria included infants who had a congenitally lethal condition; severe bleeding despite standard management; evidence of viral hepatitis or primary liver disease as the etiology of their cholestasis; other health problems such that survival was extremely unlikely even if cholestasis improved; or had a known allergy to eggs or fish products.

Written informed consent was requested from a parent or legal guardian of every patient who met the entry criteria. After obtaining consent, the patient was switched to FishLE from Intralipid® (Baxter Healthcare Corporation, Deerfield, Illinois), a soybean-based lipid emulsion and the usual starting lipid component of parenteral nutrition at University Hospital. Patients who were transported from an outside institution with a previous diagnosis of PNALD and who met study criteria were started on FishLE upon admission.

FishLE was infused continuously over 24 hours via a peripheral or central catheter at 1 g/kg/day along with standard parenteral nutrition per hospital guidelines: dextrose (glucose infusion rate (GIR) goal 13-15 mg/kg/min. If no weight gain for 2 weeks, GIR advanced to a max of 17 mg/kg/min); amino acids (goal 3-4 g/kg/day Trophamine 10% solution, BBraun, Bethlehem, PA); electrolytes (adjusted per daily/weekly labs); vitamins (Pediatric MVI, in daily PN); and pediatric trace minerals (1mL/kg, three times per week, Fresenius Kabi, Bad Homburg, Germany) although it varied considerably due to shortages. FishLE was administered daily, barring an unforeseen reason such as transfer or requiring a procedure prohibiting it. If patients did not receive parenteral nutrition for seven consecutive days or 10 intermittent days within a 2 week period, or had a direct bilirubin <1 mg/dL for four consecutive weeks, they were dropped from the study and Intralipid® was re-instituted. Returning to FishLE later would require re-initiating study entry procedures.

In the event that hypertriglyceridemia developed (serum triglyceride >300 mg/dL), the subsequent total daily FishLE dose was administered over 20 hours and another serum triglyceride was obtained before starting the next infusion 4 hours later. If the elevated serum level persisted, the dose of FishLE was reduced by 25%. FishLE could be discontinued if serum triglyceride >400 mg/dL persisted and no other cause of hypertriglyceridemia was identified (renal disease, drug side effects).

Patients continued in the study until they were weaned from total parenteral nutrition for 7-10 days or if serum direct bilirubin was <1 mg/dL for four consecutive weeks, whichever came sooner. There were stopping rules that could apply before the study discontinuation criteria were met: severe clinically apparent bleeding that was not present before FishLE was started and required blood products or surgical intervention; severe hypertriglyceridemia as above; severe hyperglycemia (persistently >200 mg/dL despite reduced dosage) not secondary to infection, drugs or high dextrose infusion; overdose of FishLE (i.e. inadvertently given over a short period of time or at a higher dose); or metabolic acidosis if inadvertent overdose occurred.

Detailed demographic characteristics and outcomes were recorded prospectively. Monitoring of nutritional intake included parenteral nutrition (total calories and daily dose of glucose, amino acids, and lipids) and enteral nutrition (total calories, intake, and source). Weekly monitoring included anthropomorphic measurements (weight, height and head circumference), complete blood count with manual differential, serum chemistry, triglyceride and liver function testing including serum total and direct bilirubin, AST, ALT, alkaline phosphatase, total protein, albumin, and GGT. Non-esterified free fatty acid concentrations (NEFA) were measured by enzymatic methods at 4 week intervals to ensure fatty acid deficiencies did not develop. Other laboratory results obtained for clinical reasons were also recorded.

Study infants were evaluated in the NICU preterm infant follow-up (PREMIEre) program at 6 and 12 months corrected postmenstrual age (PMA). At each evaluation, patients were tested using the Bayley Scales of Infant Development III to assess development. The tool was administered by a certified developmental psychologist. Cognitive, motor, and language scores were obtained for each patient. Patients were also evaluated at the Pediatric Gastroenterology clinic to monitor liver function tests, nutrition, and management of specific morbidities or sequelae.

*Data Analysis*

Study laboratory data including serum direct bilirubin, total bilirubin, AST, ALT, GGT, triglyceride levels, and albumin were analyzed longitudinally to assess the response to treatment and the average rate to normalization of elevated serum direct bilirubin in the FishLE treated group. Clinical data, including gestational age (GA), birth weight (BW), length, head circumference, and specific diagnoses leading to anatomic or functional short gut were summarized. Clinical morbidities were summarized to assess their frequencies, including intraventricular hemorrhage, bronchopulmonary dysplasia, central line-associated blood stream infections, and death in the FishLE treated group. Total NEFA were analyzed longitudinally to evaluate for deficiencies that may have occurred while on protocol. Student T-test was utilized for continuous data, chi-square and Fisher exact test for dichotomus data, Mann-Witney U for non-normally distributed data, and logistic regression analyses were used as applicable.

Follow-up growth and neurodevelopmental outcome data were compared to cohorts of non-study infants who were evaluated in the PREMIEre program during the same study period (2011-2013) and matched by GA and gender 1:5. However, due to a high proportion of small for gestational age (SGA) infants and a prolonged length of stay (LOS) in the FishLE treated group, the matched control cohort was increased to include all infants that matched by gestational age and gender during the entire period studied. Neurodevelopmental scores were analyzed by Student T-test and dichotomized by severity for each category and analyzed by binary logistic regression.