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Neurodevelopmental Effects of Donor Human Milk vs. Preterm Formula in ELBW infants

The MILK trial

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Research Plan

A. Specific Aim: There is strong evidence that maternal breast milk feedings in infancy confer multiple health benefits in the extremely preterm population (< 29 GA). Studies suggest an IQ advantage of up to 8 points conferred by *maternal* milk feeding in this population. Rates of sepsis and necrotizing enterocolitis are also lower in human milk fed ELBW infants, and they experience shorter hospital stays and fewer re-hospitalizations in the first year of life. When mothers choose not to or are unable to provide milk, preterm formula is usually used. Recently, pasteurized *donor* human milk is available in some NICUs in the US as an alternative to preterm formula. Donor milk has not been well studied with regard to its safety and efficacy. *It is unknown if donor human milk confers the same benefits as maternal milk with regard to neurodevelopmental and health outcomes.* **The proposed study will be the first US multicenter randomized trial of the health and developmental effects of donor milk as compared to preterm formula in ELBW infants receiving little or no maternal milk.** Our long-term goal is to optimize neurodevelopmental and health outcomes for ELBW infants, maximizing their quality of life and societal functionality throughout their lives. If donor human milk has similar effects to maternal milk, the public health benefit of donor milk feedings in ELBW infants unable to receive maternal milk would be considerable.

The specific aim of this research proposal is:

1. To determine the effect on neurodevelopmental outcomes at age 22-26 months of donor human milk as compared to preterm infant formula as the in-hospital diet for ELBW infants whose mothers choose not to provide breast milk or are able to provide only a minimal amount. We will conduct a multicenter randomized controlled trial of donor milk vs. preterm formula in ELBW infants in the centers of the NICHD Neonatal Research Network (NRN).
 - Hypothesis: Among ELBW infants who receive no or minimal maternal milk, those fed donor human milk will have better neurodevelopmental outcomes at 22-26 months than those fed formula, as tested by the Bayley Scales of Infant Development, III.

B. Background and Significance:

B.1. Public Health Impact of Neurodevelopmental Outcomes of VLBW infants

12.7% of infants born in the US today are born prematurely (before 37 weeks' completed gestation) with 2% born before 30 weeks' gestation ¹. The rate of premature birth in the US has increased 18% since 1990. Although the number of VLBW infants is small, they experience a disproportionately high rate of morbidity, and have high rates of medical resource usage. In 2001, \$13.6 billion was spent in the US on hospital stays for all premature infants, and that figure is undoubtedly an underestimate of current expenditures.

In addition to the initial medical costs to society, these infants are at high risk for lifetime disability, including chronic lung disease, mental retardation, cerebral palsy, and behavior disorders. 9-26% of surviving ELBW infants have cerebral palsy, and 6-42% demonstrate cognitive disability in the toddler years. When these infants are assessed at older ages, 50% of school-age former ELBW infants have educational disabilities requiring special education. Among a group of disabled premature survivors in the UK, special education costs incurred by 8-9 years of age exceeded the medical costs for the same 8-9 year period, including initial hospital costs ². In the teen years, vocational limitations are present in 27-71% of survivors, indicating significant concern for these young adults' ability to function independently in society ³. Additional research has demonstrated that ELBW infants, when compared to their full-term siblings, had lower IQ scores by 10 points, suggesting that these children are not meeting their genetic intellectual potential ⁴.

These functional intellectual disabilities result in reduced quality of life and continued high use of medical and education resources among ELBW survivors. According to the Center for Special Education Finance, the annual cost of education for a non-special education elementary student in the US in 1999 was \$6,566. The cost for the average special education student was \$10,000, which increased dramatically to \$35,000 for the

most disabled students. Therapies or interventions leading to improvements in neurodevelopmental outcomes in this particularly high-risk population would confer significant public health benefit and quality of life improvement. Even a movement to less severe cognitive impairment in the disabled ELBW survivor population would result in significant cost savings in education.

B.2. Maternal Milk and Neurodevelopmental Outcomes in Preterm Infants

Breastfed infants have higher IQ in adulthood than do formula-fed infants⁵. A meta-analysis of 20 studies regarding breastfeeding and intelligence demonstrated a 3.16 point IQ advantage in breastfed children, with premature infants experiencing the highest benefit, 5.2 points⁶. In a trial of breastfeeding interventions conducted in Belarus, enrolling 17,046 infants, the experimental interventions led to significant increase in breastfeeding through the first year of life. At age 6.5, the full scale IQ scores of children in the intervention group were 5.9 points higher than the control group⁷.

In the UK, a cohort of 771 premature infants fed a variety of diets has been followed for neurodevelopmental outcomes. At 18 months, infants whose mothers chose to provide breast milk for their infants scored 4.3 points higher on the Bayley Mental Development Index than their formula-fed peers, adjusting for socioeconomic variables⁸. In the same population, tested at 7.5-8 years of age, breastfed children scored 8.3 points higher on the Weschler Intelligence Scale for Children, after adjustment for socioeconomic and maternal education variables. This represents more than half of a standard deviation of IQ, an extremely significant difference for an individual child, and a potential source of massive public health benefit across large populations.

More recently, the investigators of the NICHD Neonatal Research Network reported the effects of maternal milk feeding on neurodevelopmental outcomes of ELBW infants. Comparing 775 breastfed infants to 260 formula fed peers revealed clear dose-dependent advantages for the breastfed group in all the Bayley domains: mental development (MDI), psychomotor development (PDI), and behavioral rating scales. For every 10ml/kg per day of maternal milk ingested, MDI scores increased by 0.53 points, and PDI scores increased 0.63 points. Comparing the infants who received predominantly maternal milk to those receiving solely formula, a third of a standard deviation difference in IQ is predicted (11.5 point difference in MDI)⁹. These differences are remarkable when the length of hospitalization and discharge diets of the infants are considered. The infants in this study were hospitalized for an average of 98 days, and their diets were as described. However, although 75% of the infants received maternal milk in the hospital, only 30% were still receiving maternal milk when they were discharged. In addition, when the infants receiving maternal milk after discharge were excluded from the analysis, the magnitude and significance of the differences remained. **This suggests that a dietary intervention, maternal milk, delivered over a short time period, approximately three months, has measurable neurodevelopmental effects 18 months later.**

It is unknown whether donor human milk confers the same neurodevelopmental advantage as maternal milk for ELBW infants. There have been no studies directly comparing donor human milk to formula in non-breastfed infants, which would be required to assess this question. Some of the infants in the UK cohort described earlier received some donor milk as a supplement to maternal milk, but amounts are unknown¹⁰.

B.3. Other Health Benefits Associated with Maternal Milk Feeding in VLBW infants:

Maternal milk confers protection against infection, a major source of morbidity in this population. Blood-culture proven sepsis occurred in 21% of 6215 VLBW infants admitted to NICHD Neonatal Network (NRN) centers in 1998-2000. These infants were 2.5 times more likely to die before discharge than uninfected infants¹¹. VLBW survivors of late-onset neonatal sepsis followed in NRN centers between 1993 and 2001 had a 1.3-1.7 times increased risk of cerebral palsy, and impairment in mental and motor development, compared with uninfected infants¹².

Maternal milk feeding of VLBW infants is associated with lower rates of sepsis¹³⁻¹⁵ than in formula fed peers. El-Mohandes et al reported a 2.5 times increased risk of sepsis in formula fed infants admitted to the NICU when compared with those fed maternal milk¹⁶. Maternal milk feeding resulted in a 50% reduction in risk for sepsis and meningitis in another cohort of VLBW infants, adjusted for confounding factors¹⁷.

The incidence and severity of necrotizing enterocolitis (NEC), a potentially fatal illness, is reduced by maternal milk feeding^{14, 18}. Lucas and Cole reported a 6-10 times increased risk of NEC in formula fed infants as compared with those fed maternal milk, in their cohort of 926 preterm infants participating in a randomized feeding study¹⁹. In a study of feeding methods in VLBW infants, Schanler et al reported that NEC occurred in 1.6% of maternal milk fed infants, as compared with 13% of formula-fed²⁰. Intestinal perforation, a complication of NEC requiring surgical intervention, increases the risk of death from NEC and lengthens hospital stay²¹. Case fatality rates with surgical intervention are as high as 50%²². In one series, the incidence of intestinal perforation in formula fed infants with NEC was 39%, compared with 7% in breastfed infants²³. Another, smaller cohort demonstrated a perforation rate of 0% in maternal milk fed infants, and 50% in formula fed²⁰. As case fatality rates in non-surgical NEC are much lower (11%), human milk may literally save lives in this population, by decreasing the risk of both NEC and the complication of perforation²⁴.

These decreased short-term morbidities result in economic savings as well as human savings. Maternal milk feeding is associated with shorter hospital stays for ELBW infants¹⁴ than in their formula fed peers. By combining the costs of increased hospital days, episodes of sepsis and NEC the cost of not using maternal milk in ELBW infants has been estimated at \$9669 per infant²⁵.

There is also evidence that there are long-term health benefits to maternal milk feeding in preterm infants. In a cohort of preterm infants (mean GA 29 weeks) formula fed infants were 1.7 times as likely to be rehospitalized in the first year of life as those fed mother's milk²⁶. The same cohort also demonstrated a lower rate of overall serious adverse events during the first year of life in maternal milk fed infants compared to formula fed peers²⁶.

The basis of maternal milk protection is unknown, so it is not known whether the same benefits should be expected from donor milk. Currently, maternal milk is not available to all ELBW infants. Some mothers are unable to express milk for their infants due to maternal illness or medications, or lack psychosocial support or financial resources necessary to do so. The challenges of breastfeeding an ELBW infant are much greater than those in the term population. A qualitative analysis of studies of breastfeeding barriers in mothers of premature infants shows that fatigue, maternal illness, difficulty in expressing milk, and low milk volumes are significant impediments to providing milk for preterm infants²⁷. Our recent analysis of the CDC Pregnancy Risk Assessment Monitoring System survey results found that infants born at <32 weeks' gestation were breastfed at significantly lower rates than healthy term infants ($p < 0.001$)²⁸. Infants whose mothers are unable to provide milk have historically been fed infant formula, which has been associated with poorer neurodevelopmental and health outcomes.

B.4. Donor Human Milk

Pasteurized donor human milk, a promising alternative to formula in the case of unavailable maternal milk, is available, and use of donor milk in the preterm population is rapidly rising in the US. The 13 banks of the Human Milk Banking Association of North America (HMBANA), a non-profit organization, process most donor milk dispensed in the United States. In 2007, HMBANA banks dispensed 1,166,336 ounces of donor milk, up 45% from 2000. 37% of that milk was used in an inpatient hospital setting, most in NICUs. In 2004, 32% of recipients of HMBANA milk were premature infants.

Milk donors are volunteer women in the first year of lactation, most of whom delivered healthy term infants. All donors are screened for HIV, HTLV, Hepatitis C, Hepatitis B, and syphilis, according to FDA blood and tissue bank regulations. Donors undergo extensive screening regarding prescription medications, over-the-counter medications, and herbal supplements, few of which are compatible with milk donation. Medical clearance is also sought from the donor's physician and the donor's child's physician.

Milk is expressed by donors and frozen until delivery to the milk bank, where it is pasteurized by heating for 30 minutes at 62.5 C (Holder pasteurization). Milk from 3-10 donors is pooled to make a large volume, which is then separated into aliquots for pasteurization. Each donor may contribute milk to multiple pools.

Pasteurization kills bacteria and white blood cells that may be found in milk, and the combination of heating and then deep freezing inactivates viruses. Holder pasteurization has been shown to inactivate HIV, HTLV,

CMV, and many other viruses^{29, 30}. There has never been a reported case of disease transmission, including HIV, Hepatitis B, and Hepatitis C from donor milk in the 30 years of operation of HMBANA, but there is undoubtedly some small risk of viral transmission remaining.

Pasteurization decreases the levels of some infection fighting and immune stimulating factors in human milk and kills the milk leukocytes. Secretory IgA levels are decreased by up to 40% by Holder pasteurization, and lactoferrin is decreased 57%³⁰. Oligosaccharides are unaffected by pasteurization³⁰. Although these infection-fighting compounds are decreased by pasteurization, the alternative to pasteurized donor milk, i.e., preterm infant formula, contains none of these anti-infective biologic compounds.

B.5. Donor Human Milk, Including Donor Human Milk-Derived Fortifier And Outcomes In VLBW Infants

Although no large studies of a sole donor milk diet compared to a sole formula diet (such as we propose), have been performed, a trial comparing a diet composed only of maternal milk plus donor milk and donor milk-derived human milk fortifier to a diet composed of maternal milk plus preterm formula and bovine milk-derived human milk fortifier has recently been published. Schanler and colleagues randomized infants weighing less than 1250 g at birth whose mothers were providing milk to receive fortification in the form of donor human milk-derived HMF, and donor milk when mother's milk supply was inadequate, or to receive fortification as powdered bovine HMF, and supplemental feeds as preterm formula. They found no significant differences in length of stay, duration of TPN (the primary outcome of the study), growth, BPD, or a variety of other outcomes. They did note a higher incidence of NEC or death in infants in the bovine fortifier group, 20% vs. 8.5% and 6% for the donor human milk fortifier groups. All cases of surgical NEC occurred in participants who received bovine derived products at some time prior to the onset of NEC. This study did not examine neurodevelopmental outcomes. The study population received an average of 70% of total intake during the study as maternal milk, which is in contrast to the study population targeted in this proposal, those for whom little or no maternal milk is available. However this study adds evidence for improved short term outcomes with human milk diets as compared with bovine milk diets³¹.

Schanler and colleagues also studied donor milk vs formula as a supplement to maternal milk in ELBW infants, comparing NEC and sepsis rates³². Infants in this study received maternal milk as available, and were randomized to donor milk (n=78) or preterm formula (n=88) for any supplements needed. This trial was not blinded, and included a reference cohort of infants receiving all maternal milk (n=70). Infants receiving all maternal milk had the lowest rates of sepsis and NEC, with donor milk supplemented infants with intermediate rates, and formula supplemented infants with the highest rates. The difference in infection outcomes between donor milk and preterm formula groups was not significant, but all infectious outcomes were lower in the donor milk group than the formula group, a trend toward significance. Neurodevelopmental outcomes were not assessed as a part of this study. Rates of NEC and sepsis were significantly lower than predicted (55% predicted, 36% observed) across the study population, and 21% of infants randomized to the donor milk group were switched to preterm formula during the study. Therefore, this study lacked sufficient power to test its hypothesis.

B.6. Breastfeeding, Social, and Parental Effects on Neurodevelopmental Outcomes in ELBW Infants

There is concern expressed in the literature that breastfeeding is simply a surrogate for an enriched genetic and societal environment³³, and that the milk itself has no effect on neurologic development. Mothers who breastfeed are generally older, more affluent, and better educated than those who do not³⁴.

Although well-designed epidemiologic studies collect and adjust for information regarding household income and education, concern is raised that these variables alone cannot completely adjust for confounding between breastfeeding and intelligence, and that breastfeeding can therefore not be shown to have any effect on neurodevelopment. Donor human milk and the existing population of ELBW infants unable to receive maternal milk provide a unique opportunity to test the isolated effect of human milk, regardless of the mother's characteristics.

B.7. Maternal Milk and Growth and Mineral Status in VLBW infants:

It has been established that the feeding of *unfortified* maternal milk to ELBW infants results in nutritional deficits and poor growth during hospitalization and beyond³⁵⁻³⁷. ELBW infants require higher intakes of protein, energy, calcium, and phosphorous than can be supplied by maternal milk³⁸. Due to these recognized insufficiencies, it is standard practice to fortify human milk (donor or maternal) fed to ELBW infants with commercial human milk fortifiers, which increase the protein, energy, and mineral contents of the milk to levels that can support growth and adequate mineral status.

While milk from mothers delivering preterm does contain higher protein concentration than that of term mothers³⁹, the amount provided in even preterm milk is lower than that required for in-utero rates of growth⁴⁰. Calcium and phosphorus levels in human milk from both term and preterm mothers are inadequate for ELBW infants³⁵⁻³⁷.

Meta-analysis of comparisons of premature infants fed unfortified and fortified maternal milk shows that fortification results in better growth (weight, length, and head circumference), better bone mineral content, and more normal nitrogen balance than unfortified milk feeding⁴¹.

Comparisons have been made between growth and mineral status in preterm infants fed fortified maternal milk and those fed premature infant formulas. Infants fed fortified maternal milk have been repeatedly demonstrated to grow more slowly than those fed preterm formula^{14, 26, 42}. Interestingly, this slower rate of growth has not been associated otherwise with a disadvantaged nutritional status. Fortified-milk-fed infants have similar biochemical indices of nutritional status (calcium, phosphorus, serum urea nitrogen) as their preterm-formula-fed peers^{14, 26, 42}.

In a cohort of 463 infants with mean birthweight of 1300 g followed from birth through one year adjusted age, infants fed preterm formula (including DHA-enriched formula) weighed 500 g more than maternal milk fed infants at term corrected age, and were also longer and had larger head circumferences²⁶. However, by the adjusted age of nine months, weight, length, and head circumference were the same in both maternal milk and formula groups, indicating that the growth deficits experienced in human milk fed infants were transient. In addition, the human milk fed infants demonstrated better visual acuity than the DHA-enriched formula group, and a positive association between duration of human milk feeding and superior neurodevelopmental testing scores at 12 months. These findings suggest that the slower growth in the first nine months of age was not developmentally detrimental in the maternal milk fed group. The evidence is not clear, however. Recent evidence suggests that more rapid growth in infancy may lead to health disadvantages in adulthood⁴³, while faster growth in ELBW infants has previously been associated with lower risk of neurodevelopmental impairment⁴⁴.

The short-and long-term growth patterns of ELBW infants fed fortified donor human milk are unknown. Donor human milk typically is obtained from mothers delivering healthy term infants, and therefore has lower concentration of protein than milk from mothers delivering preterm during the first 4-6 weeks of lactation³⁹. Feeding of unfortified donor milk has been associated with slower growth than feeding of unfortified maternal milk^{36, 45}. Schanler et al, conducted a randomized trial of using either fortified donor milk or preterm formula as a supplement to maternal milk in ELBW infants. Infants fed fortified donor milk as a supplement to maternal milk grew more slowly than those fed preterm formula, although at similar rates to those fed a diet consisting solely of maternal milk³². All of these infants received at least 50% of their total diet as maternal milk during hospitalization. *Studies comparing the growth outcomes of a predominant (>80%) fortified donor milk diet to preterm formula or fortified maternal milk in ELBW infants are lacking.* To determine the safety and efficacy of donor milk, growth and nutritional outcomes need to be assessed.

All infants receiving human milk as a part of the proposed trial will receive fortified milk, and “maternal milk” or “donor milk” should be understood to refer to fortified milk.

B.8. Cost:Benefit Analysis:

Two reports of cost-benefit analyses of donor milk have been published, although all focus on benefits such as decreased NEC and sepsis, rather than neurodevelopmental outcomes. Using data regarding maternal milk published by Schanler et al in 1999²⁰, Wight estimated that the use of a solely human milk diet, maternal or donor, could save \$9669, due to decreases in length of stay, sepsis, and NEC. She goes on to estimate that

for every \$1 spent on donor milk, \$11 could be saved, assuming that donor milk is as effective as maternal milk. If donor milk were 50% as effective, a savings of \$6 for every \$1 spent is projected²⁵. Arnold published a cost:benefit analysis of donor milk using two models. Using March of Dimes NICU cost data and Schanler's data regarding differential outcomes and assuming that donor milk is as effective as maternal milk, she estimated that differences in length of stay and NEC rates could lead to cost savings of up to \$50,000 per infant fed donor milk. She also estimated that the cost to the state of Texas for excess NEC due to formula use (and therefore potentially targetable by donor milk use) was \$32,682,000⁴⁶. The potential lifelong neurodevelopmental benefit of a maternal milk diet for preterm infants, as measured by IQ points, is estimated at 5.16 points, according to a meta-analysis of 20 studies⁶. Dr. Vohr also estimated an effect size of 5.3 IQ points attributable to a sole maternal milk diet using the Glutamine Trial data⁹. The US federal government uses a lifetime cost of \$8800 per IQ point in cost benefit analyses of mercury and lead exposure⁴⁷. Given that a sole donor human milk diet for a healthy 1000 g infant costs approximately \$1200 over the course of the initial hospital stay, the cost of the milk is justified if a single IQ point is gained. Naturally, this difference can be argued to be insignificant on the individual level. However, across the entire population of ELBW infants, an increase in mean IQ of 1 point would represent significant benefit. An IQ difference of 5 points, however, would be significant with respect to life-long function at the individual level, and the cost of the milk is far outweighed by the additional savings of \$44,000.

B.9. Rationale and Significance for Proposed Studies:

Based on the body of strong evidence, preterm infants fed maternal milk during hospitalization have less short-term morbidity and superior neurodevelopmental outcomes compared to infants fed formula. Early deficits in growth of infants fed their own mothers' milk are transient and not sustained into childhood. It is unknown if the mechanism for the advantages of maternal milk feeding is biological, inherent in the milk itself, or social, stemming from genetic and economic advantages more common in breastfeeding families. Donor human milk provides a unique resource to study the effects of the milk itself (as distinct from socioeconomic and genetic confounders) on short and longer-term health and developmental outcomes in this vulnerable population. Donor milk provides a source of human milk for infants who historically would have been unable to receive it. Randomizing ELBW infants receiving no or minimal maternal milk to donor milk or preterm formula allows us to test the hypothesis that there is something specific about human milk itself that enhances health in these infants, regardless of maternal and family characteristics. This is the first US multi-center study, to our knowledge, to randomize a large number of ELGAN infants to donor milk or formula as the predominant diet, thus allowing robust comparison of outcomes between groups of infants fed these two diets.

C. Preliminary Studies

C.1. Mother's Milk Bank of Iowa (MMBI)

The Mother's Milk Bank of Iowa is a member of the Human Milk Banking Association of North America (HMBANA). HMBANA is the professional membership association for non-profit milk banks in the United States, Canada, Mexico and sets the standards and guidelines for donor milk banking in North America. MMBI dispensed its first donor milk to recipients at the University of Iowa Children's Hospital (UICH) NICU, in June 2003. As of October 2011, current milk usage is at an average of 72L per month. This usage has occurred primarily in the premature infant population, with highest usage in the ELBW group. Since inception, over 700 women have donated milk to the MMBI. The average donation per donor is 16.4 liters, with a range of 120 ml to 249 liters. All donor human milk to be used in this trial will be obtained from HMBANA member banks, with several banks involved.

C.2. Maternal and Donor Milk Use in the UICH NICU

Current nutritional practice in the UICH NICU is for all ELBW infants to be fed maternal milk or donor milk as the initial diet. No ELBW infant has been fed formula in the first month of life at UICH NICU since November 2003, six months after institution of the milk bank.

In a birth cohort spanning the first two years after institution of the milk bank (5/30/03-6/30/05), 183 infants <1271g at birth were cared for in the UICH, and have medical records available for review. These infants are

part of an ongoing nutritional database maintained by Susan Carlson, MCS, RD. 222 of 253 (88%) infants <1271 g survived to discharge, but full milk intake data were not available for 39. Details of this analysis can be found in Appendix A. To summarize pertinent findings:

- 92% of mothers of ELBW infants cared for at Iowa expressed milk for their infants, at least initially. 24% of mothers provided only minimal amounts of milk, averaging <11ml/kg/day throughout hospitalization, and all of these mothers had stopped providing milk before the infant had reached full enteral feeding. Therefore, 32% (8% + 24%) of our ELBW infants would have been eligible for inclusion in this trial.
- BSID II scores at 18-22mo were similar in a group of infants receiving a predominant donor milk diet and a maternal milk diet. Comparing the mean BSID MDI scores above to the published data by Vohr et al in a similar NICHD NRN cohort⁹, the mean MDI score in our maternal and donor-milk-fed infants is similar to the mean score in their maternal milk fed infants (87.3-NRN vs. 84 and 86-UICH, Table 1A, Appendix).
- Donor milk fed infants at Iowa grow similarly to maternal milk fed infants. Maternal and donor milk infants had similar discharge weights, (3240 g vs. 3147 g, p 0.87), and were discharged at similar postmenstrual age (40.5 weeks vs. 40.3 weeks, p 0.82). Using the growth charts generated by Fenton^{48, 49}, our donor and maternal milk infants had weight z-scores at discharge of -0.60 and -0.83.

C.3 Donor Milk Nutritional Information: Protein and fat content of donor milk from the Mother's Milk Bank of Iowa was measured in 130 batches of term milk from 37 pools (4L each, 520L total) and 66 batches of preterm milk from 18 pools (4L each, 264L total). Consistency between pools and batches within a pool was examined. Mean protein content of donor milk was lower (0.82g/dl) than what is reported for mother's own milk (1g/dl), but is relatively consistent across multiple pooled samples (See Appendix, Table 2A).

C.4. Current NRN Donor Milk Use Information: As of December 2011, several NRN centers use donor human milk in the ELBW population: Case, Cincinnati, Indiana (3 of 4 hospitals), Emory (1 of 3 hospitals), Stanford, Duke, Penn/CHoP, UCLA, Nationwide, and Iowa. Duration of use (in ELBWs) in cases of no ongoing maternal milk supply varies, from 14 days of use to the entire hospital stay. Several centers use donor milk until 1800 g, or 32-34 weeks, a similar time frame. Most centers that use donor milk get at least verbal consent and notify parents of its use. For the purposes of this study, consenting to the study would include consenting to donor milk use, although only 50% of subjects will receive donor milk. Donor milk is provided in 100ml frozen aliquots. **In most NRN centers one aliquot may be used to feed more than one infant. In the setting of this study, with all feedings being mixed centrally, this can be done safely and efficiently.** Records of donor milk use will be kept per Human Milk Banking Assoc of N. America standards for all study subjects. Donor milk could be used in the center-typical fashion for infants not enrolled in the study.

C.5 Current NRN Feeding Preparation Procedures / Feasibility of Study Design: To determine the feasibility of conducting this trial in the NRN, a survey was sent to all centers regarding facilities and personnel availability, as well as interest in participating. A brief summary of the results follows, and a more in-depth synthesis of the entire survey is included in Appendix B.

Of the 16 centers responding:

Facilities

- 14 centers have existing capability for blinding mixing of feedings
- 16 centers have one or more existing locations for mixing of study feedings
- 14 centers have existing personnel identified for diet mixing
- 15 centers have freezer space available to store donor milk

The majority of current NRN centers have facilities available for this trial.

C.6 Ethical Implications / Equipoise: Because maternal milk feeding has clearly been established as the gold standard, and conveys so many benefits, equipoise does not exist; a trial of randomization that includes maternal milk is not ethically feasible. However, as the benefits of donor milk compared to formula have not

been established, and both are used in the ELGAN population, it remains ethical to randomize to donor milk or formula infants whose mother’s milk is unavailable.

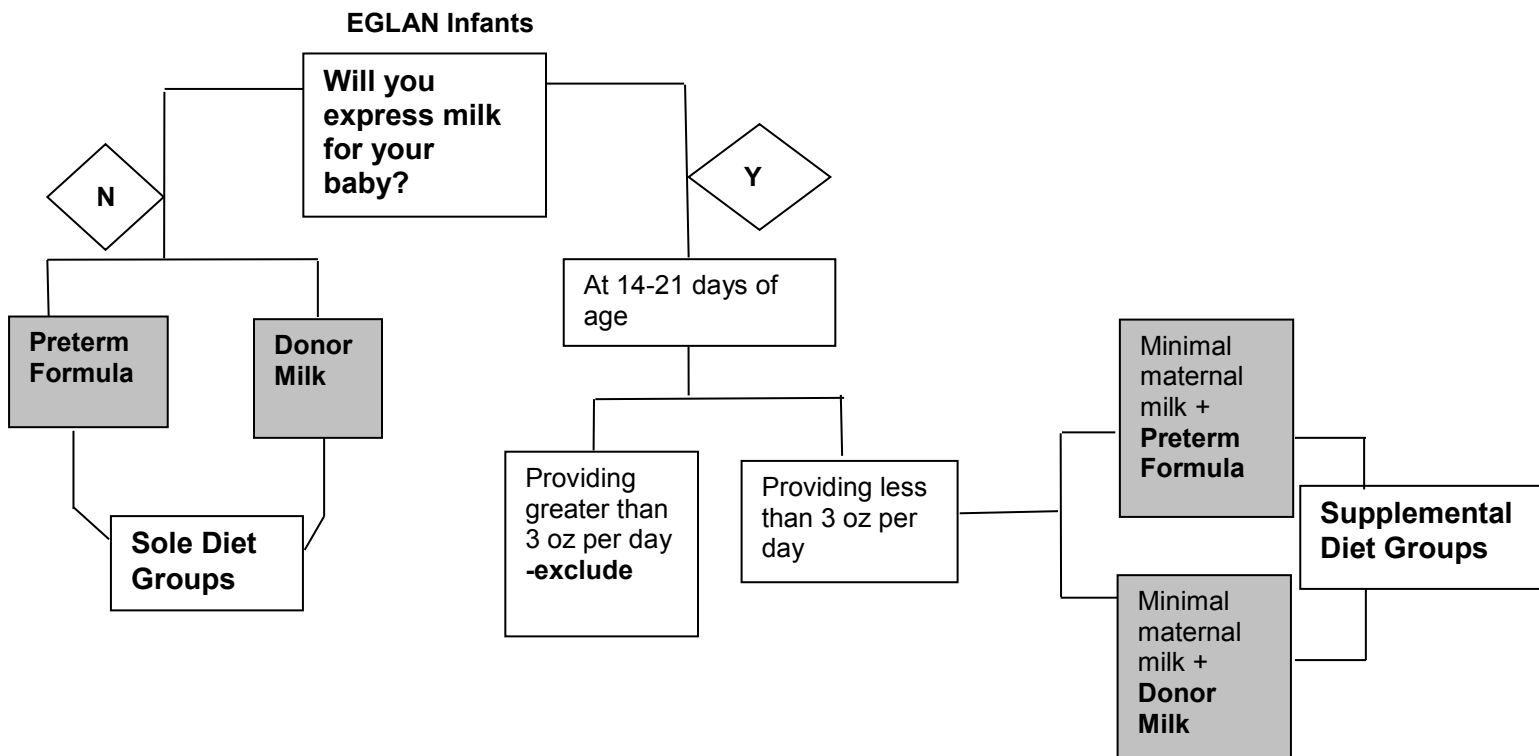
D. Research Design and Methods:

D.1 Study Design: We will carry out a multicenter randomized trial of fortified donor human milk vs. preterm formula for ELGAN infants receiving no or minimal maternal milk, to compare neurodevelopmental and health outcomes at 22-26 months.

D.1.2. Source Population: Premature infants of gestational age less than 29 0/7 weeks at birth will be recruited from a subset of the centers of the NICHD Neonatal Research Network (NRN). The centers of the NRN care for approximately 1800 ELBW infants annually.

D.1.3. Intervention and Control: Outcomes will be compared between infants randomized to be fed donor human milk vs. standard preterm formulas as the sole in-hospital diet if their mothers chose not to provide their own milk to their infants (no maternal milk) or are unable to provide at least 3 oz per day as mother’s milk at the time the infant is 14-21 days of age (minimal maternal milk).

Figure D.1.3.1: Randomization of Study Population, Feeding Group Schematic



D.1.4. Primary Outcome Variables: Neurodevelopmental outcome, as measured by scores on Bayley Scales of Infant Development III (BSID III). The study will be primarily powered to detect a difference of five points in the mean Cognitive Scale score between the donor milk and preterm formula groups, with subjects who died after randomization assigned the score of 54. The comparable scores on the Motor Scale and the Language Scale will serve as secondary outcomes.

D.1.5. Secondary Outcome Variables:

- In Hospital Morbidities – data will be collected from the GDB or separately if needed:
 - Death
 - Late Onset Sepsis/Meningitis
 - Length of TPN use
 - Length of initial hospital stay

- Necrotizing enterocolitis
- Bronchopulmonary dysplasia (BPD), defined as room air oxygen saturation of less than 90% at 36 weeks postmenstrual age using the NRN standard physiologic definition of BPD.
- Necrotizing enterocolitis or death
- BPD or death
- Growth outcomes:
 - *In-Hospital* growth parameters, including rate of weight gain, weight, length and head circumference at 36 weeks or discharge, whichever comes first. Weights will be obtained from hospital records, length and head circumference will be measured bi-weekly by study personnel. Weight, length and head circumference z-scores will be calculated based on gestational-age specific growth charts published by Fenton^{48, 49}.
 - *Follow-up* weight, length, and head circumference measurements obtained at 22-26 months of age.
- Follow-up Outcomes:
 - Number of hospital admissions between initial discharge and follow-up
 - Motor and Language scores on the BSID III
 - Cerebral Palsy
 - Neurodevelopmental Impairment (NDI), using current Follow-Up Study definition.
 - Profound Impairment, defined as BSID III Cognitive subscale score of <70
 - NDI or death
 - Profound Impairment or death

D.2 Study Protocol and Methods:

D.2.1. Eligibility: Infants who are less than 29 0/7 weeks gestation at birth and survive at least 12 hours will be eligible for inclusion. Infants may be inborn or admitted by transport prior to 7 days of age. Exclusion criteria will include chromosomal anomalies, congenital heart disease, congenital disorders known to interfere with feeding (gastroschisis, omphalocele, intestinal malformations/atresias, cystic fibrosis, etc), diagnosed intrauterine infections and other congenital disorders known to impair neurodevelopment, terminal illness, intestinal perforation or NEC prior to study consent sought. The study will be explained and consent sought at two points, depending upon mother's initial feeding choice:

1. **Sole Diet Group:** Infants will be eligible for the sole diet feeding protocol if the mother declines to provide breast milk for the baby.
2. **Supplemental Diet (minimal maternal milk) Group:** Infants whose mothers initially choose to provide breast milk and begin pumping will be re-screened for eligibility at least **weekly** until the infant is **21 days old**. If the mother stops expressing milk at any point prior to the infant's 21st day of life, her infant will be eligible for randomization. In addition, those whose mothers are providing less than 3 oz per day when the infant reaches **14-21 days of age (averaged over the previous five days)** will be eligible for randomization at this point. No infant will be randomized after reaching **21 days**.

The randomization age limit has been chosen based on preliminary data collected at Iowa, and by NRN experience gathered from collaborators. In our cohort, no mother that was providing less than 20% of the intake at this point went on to provide a significant amount of milk over the entire hospitalization, all infants of these mothers received less than 11ml/kg/day of maternal milk across their hospitalization. It is expected that mothers who are still providing small amounts of milk at three weeks will ultimately stop pumping shortly after this time point, and the majority of in-hospital diet will be study diet. This is supported by research regarding milk supply in exclusively pumping NICU mothers⁵⁰.

Based on data from the Glutamine Trial, approximately 25% of mothers of ELBW infants chose not to provide milk in the mid 1990s⁹. This percentage is likely lower in 2012, as breastfeeding rates are increasing across populations in the US⁵¹. An estimate of eligible non-maternal milk fed ELBW infants of 15%, and an estimate of 24% eligible minimal maternal milk fed infants will thus be used for estimating the time needed to enroll the required number of infants.

Special Considerations Regarding Supplemental Diet Group: The goal of the supplemental diet group is to capture infants whose mothers initially agree to provide milk, but ultimately do not develop a full milk supply or who ultimately choose to not provide milk. It is impossible to determine which mothers will fall into this category in the first days after birth. Study coordinators will need to use judgment and seek information from the nursing, lactation support, and medical teams to help determine which infants will be eligible for this arm of the trial. If mothers stop pumping prior to 21 days, they are eligible and should be approached. Mothers who are still providing small amounts of milk at 14-21 days will require additional fact-finding to determine eligibility. Maternal milk supply should be assessed, and if the mother is providing less than 3 oz per day averaged over the past five days, the infant may be eligible. However, communication with nursing and lactation staff about mother's habits will enhance assessment of eligibility. For instance, if mother is able to visit only rarely, but brings large amounts of milk and is known to be actively pumping large amounts, that infant is not a good candidate. Averaging over five days should ameliorate this problem. Nurses and lactation consultants often will know if mother has poor milk supply, and those women are the target population. Information regarding milk supply can also be sought directly from mothers if desired.

D.2.2. Subject Enrollment and Consent Procedure:

Sole Diet Group Enrollment: Soon after birth, mothers who have declined to provide breastmilk for their infants will be approached, the study will be explained, and consent will be sought. This meeting will occur within prior to the first 7 days of life, **ideally** prior to any enteral feeding. Infants eligible for the sole diet group may be fed according to center practice prior to randomization. Infants of mothers who choose not to provide breastmilk and consent to participate will be randomized to the donor milk or preterm formula groups. Infants whose mothers elect not to provide breastmilk and do not consent for the study will be fed according to center practice.

Supplemental Diet Group Enrollment: Enrollment will be reconsidered for mothers who initially start providing milk but subsequently stop pumping at any time prior to the infant being 21 days old, or if the average volume of maternal milk provided in the five days prior to day 14 or 21 is less than 3 oz per day. These mothers will be re-contacted and asked to consent to randomization of their infants to donor milk or formula for any feedings for which maternal milk is not available. Our preliminary single-center data analysis shows that **24.3% of mothers of ELBW infants at Iowa initially start providing milk but cease doing so before the infant reaches 21 days of age, or are providing only small amounts at this time.** Experience shows that these mothers cease milk production quite early. The ability to randomize infants whose mothers start to provide breast milk but stop very early is essential in our population, as very few mothers decline to provide milk entirely (7.6%), but a significant portion are able to provide milk in only small volumes and for only a short period (24.3%). The time to recruit a population of infants whose mothers do not attempt to provide milk (assuming NRN overall 15% of non-breastmilk feeding) would be prohibitive (234 eligible per year, 50% consent = 5.7 years to recruit) While the sole diet group will represent the no-milk and all-milk subgroups of the ELBW infants studied within the NRN observational study, the supplemental diet group infants will represent either the second-lowest milk quintile (randomized to formula) or the second-highest (randomized to donor milk) described in the secondary analysis of the Glutamine Trial ⁹. **During the time of monitoring for eligibility for the Supplemental Diet Group, infants will be fed according to center practice, using formula or donor human milk as a supplement to maternal milk if maternal milk is insufficient.**

D.2.3. Randomization: After parental consent is obtained, infants will be randomized through the Data Center at RTI (by phone) in a permuted block design. Randomized subjects will be stratified by center and within each center by birthweight ($\leq 750g$, $>750g$) and time of randomization (prior to first feeding for the sole diet group and after first feeding but up to 21 days of age for the supplemental group). Sole diet group infants will receive either donor human milk or preterm formula as the sole in-hospital diet from the time of randomization until close to discharge. Supplemental diet group infants will receive either donor milk or preterm formula as the supplement to their minimal maternal milk after randomization. Multiple gestation infants will be randomized individually.

D.2.4. Interim Data Monitoring: The standing NRN *Data Safety and Monitoring Committee (DSMC)* will review this protocol and will monitor the progress of the study when 25%, 50%, and 75% of trial subjects have reached status (death, discharge, transfer or 120 days of age, whichever comes earlier).

The charges of the DSMC are:

- Review study protocols, study instruments, and manuals of procedures
- Review preliminary study data at interim study points to ensure that neither randomized diet is leading to harm and to assess treatment efficacy.

Since the outcome is available only at 22-26 months follow up, the interim monitoring will focus on enrollment and safety issues. The specific safety events that will be monitored by the DSMC are death, NEC (Bell's Stage II or greater) or intestinal perforation, late-onset culture-proven sepsis or meningitis, or acquired viral infection proven by nucleic acid test, viral culture, or antibody titer test. Pocock bounds will be used to construct stopping rules for safety.

D.2.5. Feeding Protocols: Timing of the first enteral feeding as well as volume, frequency and mode of initial feeding will be determined by the treating neonatologist. Feedings may be advanced and caloric density changed according to each treating neonatologist's typical practice. **All infants will be initially fed Step One study diet (20 or 24 kcal/oz premature formula (per center preference) or unfortified donor milk), but timing of increasing caloric density and increasing volume will be dictated by typical center practices. Treating physicians may increase volume or caloric density of feedings at any time, but due to blinding, they will not know if the diet is donor human milk or preterm formula.**

D.2.5.1. Diet Dispensing For All Study Subjects: Feedings for all study subjects will be prepared and dispensed from a central location in each center, to enhance blinding and standardize mixing of donor milk diets. Diets will be mixed once a day, after daily rounds have been completed. Individual feeding volumes will be packaged in amber syringes. An extra feeding will be included in case of spillage or changes in feeding orders. Each patient's group of syringes will be delivered to the standard location for storing refrigerated feedings in the patient care area. Nurses will warm these syringes per unit standard practices, agitate them gently and use the syringe as the delivery device for bolus or pump-assisted intermittent feedings. The study diet provided in tinted syringes should not be decanted into other feeding delivery syringes, as this will unblind the nursing and medical staff. If the patient is being fed using a pump that requires a large bag to hold the milk/formula, amber bag shields will be provided and feedings prepared in the central location.

D.2.5.2. Preterm Formula Feeding Protocol: All infants randomized to preterm formula will initially receive feedings as either 20 kcal/oz or 24 kcal/oz premature formula, brand to be determined by individual center practice. This diet will be referred to as the Step One study diet. All infants should be advanced to the Step Two study diet by the treating team, at the time that they would typically add human milk fortifier to a known human milk diet. In centers that choose to use a 20 kcal/oz preparation for Step One, the Step Two diet will be 24 kcal/oz preterm formula. In centers that choose to use a 24 kcal/oz preparation for Step One, the Step Two diet will be a 24kcal/oz preparation, either the same preparation as Step One, or the high-protein 24kcal/oz preparation may be chosen if the standard preparation is used in Step One. Participating centers using the high-protein formula products (24 kcal/oz, 2.7 g/dl protein) may use these preparations for trial infants for Step One, Step Two, neither, or both. All infants must proceed from Step One to Step Two. If growth is inadequate and/or the patient requires fluid restriction, higher caloric density preterm formula may be used (27kcal/oz, 30kcal/oz) at the discretion of the treating physician, and these diets will be referred to as Step Three and Step Four diets. Standard recipes for all diet steps will be used in each center so that within a center, the diet steps are standardized across all subjects. If further specialized formulas are desired, more diet Steps can be created by centers.

D.2.5.3. Donor Human Milk Feeding Protocol: All infants receiving donor human milk from birth will initially receive unfortified donor milk as the Step One diet. Infants will be advanced to Step Two study diet at the time that the treating team would typically add human milk fortifier to a known human milk diet. All donor human milk will be fortified to 24 kcal/oz with commercial human milk fortifier (HMF) to make Step Two diet. Centers

will choose HMF products to be used for infants in this trial. The minimal target protein intake for infants in this trial on Step Two diet is 4 g/kg/day, combining enteral and parenteral protein intake. If a center's chosen HMF regimen does not provide adequate protein (2.8-3 g/dl), additional protein will need to be added as a supplement to standard HMF. Donor human milk should be considered to have 0.8-0.9 g/dl of protein for all protein calculations. **Step Two in the donor milk arm should consist of milk with 2.8-3.0 g/dl of protein, which provides 3.5-4.5 g/kg/day of protein intake to the infant.** (The ranges are due to the range of protein content of donor human milk and varying feeding volumes.) All infants must proceed to Step Two, with higher steps used if required or desired by treating team.

If growth is inadequate and/or fluid restriction is desirable, caloric density of the donor milk will be increased (27 kcal/oz, 30 kcal/oz), using Step Three and Four diets. Standardized recipes for Step Three and Step Four donor milk will be created by each center, and will use products available in each center. Minimal target protein content of Step 3 and 4 donor milk diets is 2.8-3.0 g/dl, and higher protein content may be used by centers as desired. Minimal target protein intake for infants fed Step Three or Step Four diets is 4 g/kg/d. If centers desire more than four diet steps, centers must create a standard set of recipes to be used uniformly across all study subjects for each diet step desired.

The donor milk arm protocol is designed to ensure similar protein intake in both arms of the study, as protein intake has been shown to be a rate-limiting nutrient for growth in ELBW infants^{38, 52-54}. Use of a similar scheme at Iowa to target 3.5-4 g protein/kg/day intake has resulted in equivalent growth across hospitalization in VLBW infants fed maternal milk, donor milk, and preterm formula (see Appendix A). Equalizing the protein content of the study diet arms should prevent significant differential growth between the donor milk and formula infants, which is advantageous for several reasons. Similar growth will decrease the likelihood of systematic unblinding, and should decrease the number of protocol violations due to poor growth, or at least distribute them equally between the study arms.

D.2.6. Oral Feeding Protocol: All oral feedings will be offered to the infant as **study diet**, in standard feeding bottles. Feedings will be delivered to the bedside in blinded syringes, and should be decanted into the bottle. If the infant does not finish the feeding by mouth, the remainder should be fed in the blinded syringe by NG or OG method of choice. Any feedings which are ordered as NG or OG feedings should be fed as study diet in the blinded syringe during this transition period. Study diet will be discontinued 1-2 weeks prior to anticipated discharge, or at the age of 120 days, whichever is sooner. For infants who are g-tube dependent and are expected to be discharged home with no oral feedings, study diet should be discontinued one to two weeks prior to anticipated discharge, to allow infant to demonstrate tolerance to discharge diet. Method of transition from study diet to discharge diet may be done per attending neonatologist preference, abrupt or gradual as desired.

D.2.7. Donor Milk Dispensing And Record Keeping Procedures: Donor human milk will be pasteurized and frozen in 100 ml aliquots. In most NRN centers using donor milk, a single 100ml aliquot can be used for several babies. In this study, diet mixing personnel will thaw several bottles a day at a single time point, depending on numbers of infants in the study and feeding orders. The Human Milk Banking Assoc. of North America requires that a log be kept of milk dispensed with the identifying number of the milk aliquot and the names of all recipients of that aliquot. These forms will be kept in the diet mixing area of each center, and mixing personnel will be required to maintain these records.

D.2.8. Special Considerations: Any infant for whom the feeding protocol is violated and the infant switched from study diet to open label formula will be analyzed in the original assignment group, according to intention-to-treat principles.

D.2.9. Feeding Intolerance and Poor Growth: Feeding intolerance, evidenced by abdominal distention and/or abnormal gastric residuals will be diagnosed and managed according to each center's typical practice. Study infants believed to have inadequate growth should have their study diet's caloric density and protein content increased.

D.2.10. Feeding Post-NEC: After an episode of NEC, re-feeding should commence at the discretion of the treating neonatologist. The study protocol will be modified slightly, to enhance study compliance. If a center typically uses a hydrolyzed or elemental formula to feed post-NEC infants, that may be specified, such that if an infant has been randomized to 'preterm formula', they will receive the hydrolyzed or elemental formula of the center's choice, but if they are randomized to 'donor milk', they will receive donor milk, at the caloric density of choice.

D.2.11. Provision of Study Diets: Preterm formula group infants will receive formula preparations currently used in their unit. Human milk fortifiers currently in use in treating centers will be used in this trial. Donor human milk will be shipped frozen to study centers from one of the milk banks of HMBANA. Donor milk will be kept frozen until needed and thawed and handled according to each unit's standard practice for handling frozen mother's milk. The study will pay the processing fees for donor milk dispensed directly to the milk banks.

D.2.12. Blinding: A blinded trial design will be used.

The following groups of people will be blinded to the diet group assignment (formula or donor milk):

- Parents
- All medical providers involved in making decisions regarding feeding (attending neonatologists, fellows, residents, NNPs, hospitalists, other MD providers)
- All medical providers involved in feeding assessments or who deliver feedings to the infant (bedside nurses, nurse managers)
- All clinicians otherwise involved in the care of the infant
- Neurodevelopmental assessors
- The study coordinator responsible for data collection
- The NRN center Principal Investigator for each center
- The MILK Trial subcommittee members

To enhance blinding of the treating medical teams, amber oral syringes will be used for feedings using syringes. Feedings that are dispensed in a bag for continuous infusion will also be provided in translucent colored containers, using amber bag shields as necessary. The diets are not distinguishable by sight within the small-bore naso/orogastric feeding tubes in use in this population.

D.3. Data Collected by Study Phase:

D.3.1. In-Hospital Phase:

Data will be abstracted from the infants' medical records by study personnel onto study data collection instruments. The Generic Database (GDB) form will be used as the source for all clinical outcome data. Other variables will be recorded on data forms created for this trial.

Potential Confounding Variables: The following variables have been associated with neurodevelopmental outcomes in ELBW infants, and will be collected for analysis as potential confounders:

- Maternal demographic variables: maternal age, maternal education level, maternal race/ethnicity, incidence of chorioamnionitis, reason for preterm delivery
- Infant demographic variables: gestational age at birth, birthweight, gender, small for gestational age status
- Infant neurologic and medical morbidity: incidence and severity of intracranial hemorrhage, incidence of periventricular leukomalacia, patent ductus arteriosus, early and late onset sepsis, bronchopulmonary dysplasia

All of the above variables are included in the Generic Database forms.

Nutritional/Feeding Variables:

- Age at first feeding
- Age when infant is tolerating 120 ml/kg/day of enteral intake (a GDB variable)
- Length of TPN use in days, (a GDB variable)

- Weights will be recorded weekly. Head circumference and length will be measured every 2 weeks by study personnel and recorded.
- Feeding intolerance incidence will also be monitored weekly, using the following question:
 - In the past 7 days, were feedings withheld for at least 24 hours continually, excluding feedings withheld for surgical or radiologic procedures?
- Maternal milk intake will be screened for in the Supplemental Diet Group thrice weekly: volume of maternal milk and volume of study diet will be recorded each Monday, Wednesday, and Friday until maternal milk supply is exhausted.
- Enteral intake prior to randomization will be recorded for all subjects in the following manner:
 - Sole Diet Group: all enteral intake of donor milk and/or preterm formula fed prior to randomization will be collected if infants were randomized prior to 2 days of age. If sole diet infants were randomized between day 2 and 7 of life, all enteral intake of donor milk and/or preterm formula will be collected for two days between day of life 1 and the day of randomization
 - Supplemental Diet Group: All enteral intake of maternal milk, donor milk, and/or preterm formula will be collected for two days per week (days of life 1-7, 8-14, and 15-21) until the time of randomization.

Adverse Events:

- NEC or intestinal perforation
- Late onset sepsis with positive blood culture
- Acquired viral infection (CMV, Hepatitis B, Hepatitis C, HIV, other viruses), proven by nucleic acid test, viral culture, or antibody titer test
- Death after randomization

D.3.2. Follow-Up Phase:

Neurodevelopmental Follow-up Visit: This visit will occur between 22 and 26 months' corrected age for all participants. All subjects in this study will also be enrolled in the Follow-Up Study, and will have a neurological examination and administration of the BSID III according to the Follow-Up Study protocol. All data used for outcomes in this study will be data currently collected as part of the Follow-Up Study. Examiners will be blinded as to feeding study treatment group. Examiners have been certified through the NRN as 'gold standard' examiners.

D.3.3. Data Management: Data will be collected using the stated forms, then entered into a Microsoft Access database, maintained by the study coordinators. The data entry software will perform range checks and consistency checks and errors will be corrected on the spot. The data will then be transmitted to the Data Center. The Data Center will perform the range and consistency checks done at data entry as well as more sophisticated machine checks designed to detect consistencies across forms. Edit errors detected at the Data Center will be sent electronically back to the study sites for reconciliation. The final database will be in the SAS format.

D.3.4. Performance Monitoring: Standard reports will be generated from the study database by the Data Center. These include:

- Monthly recruitment reports that provide the number of infants screened and enrolled
- Monthly reports detailing data received at the Data Center, missing data, adherence to study protocols and a variety of performance measures
- Periodic reports for the DSMC that detail data quality and protocol adherence, as well as adverse events, efficacy data and withdrawals or losses to follow-up
- Ad Hoc reports requested by the study investigators that do not disclose treatment group specific outcomes information (primary, secondary or any safety outcomes).

D.4. Sample Size and Power: The power calculations for this study are based on the most recently reported follow-up data from the NRN, data from the 2006 birth cohort.

Primary Outcome: Bayley Cognitive Scale Score: The primary outcome for this study is Cognitive scale score on the BSID III among all enrolled infants, with subjects who died assigned the lowest score of 54. Our primary analysis will compare the scores of infants who received donor milk to infants who received formula. Both survivors (who were evaluated), and enrolled infants who died will be included in the primary analysis. We will **power** the study to detect a 5 point difference in Cognitive scale score between infants who received donor milk and infants who received formula. Note that, given an assumed death rate of 15% between 12 hours and 18-22 months corrected age, and given that all deaths receive the lowest score, this translated into around a 6 point difference among survivors.

In the 2006 cohort, 1372 ELBW infants were enrolled in GDB, 839 of which were eligible for follow-up (<27 weeks gestation and survived). 92 inborn ELBW infants (7%) were transferred to other hospitals prior to discharge. The total number that would have been eligible for follow-up in this trial (survived, and < 1000g regardless of gestational age) was 1050.

We used data from the GDB for the cohort of babies born in 2006 who were under 1000 g birth weight and survived 12 hours, to conduct our sample size calculations using the 2006 Follow-Up study cohort, and BSID III data. Given the normed BSID III standard deviation (sd) of 15, we will conservatively assume an sd of 20 to reflect the higher variability in our data due to all deaths being assigned the lowest BSID III cognitive score. Statistical tests are assumed two-tailed with alpha level 0.05 and power is fixed at 80%. A follow-up rate of 90% is assumed. Based on these assumptions, we plan to enroll a total sample size of 670 into this trial. Simulations conducted using this sample size under the assumptions outlined above, show that using the analysis plan as detailed below, this sample size has more than 80% power to detect a statistically significant difference between the donor milk and formula groups for the mean BSID III cognitive score.

Time to Recruit and Conduct Study: *Assuming 90% follow-up rate (typical for NRN), 670 subjects enrolled are required. Assuming 1800 ELBW infants cared for in NRN centers per year, 87% surviving 12 hours (1566), 40% eligibility (15% non-breastfed, 25% minimally breastfed = 626 infants), 50% enrollment (313 infants), 90% follow-up (282 infants) it will take 29 months to recruit the sample, and 59 months to complete data collection.*

D.5. Statistical Analysis:

All analyses will be performed on an intent-to-treat basis. The primary outcome is BSID III cognitive scale mean difference. The primary analysis (and all analyses examining outcomes by treatment) will be adjusted for the stratification variables of birthweight, center, and time of randomization. Since our primary outcome is continuous, we will use linear regression to estimate the adjusted mean difference in BSID III cognitive scaled scores between the two treatment groups. Similarly, for secondary outcomes, we will use linear regression for continuous outcomes to obtain the adjusted mean difference in BSID III cognitive scaled scores across the two treatment groups, and robust Poisson regression for binary or categorical outcomes to obtain adjusted relative risk estimates for the treatment effect.

Planned subgroup analyses will be performed on the pure and mixed diet groups, as well as in the two different birth weight strata for both the primary outcome and all listed secondary outcomes. These analyses will be considered as exploratory, and will not be adjusted for multiple comparisons

Strengths of This Design: The randomized nature of this trial is ideal for comparing these two diets. This research is innovative, being the first multi-center trial in the US of randomization of fortified donor milk feeding in non-maternal milk fed ELBW infants. The multi-center nature of the study allows for testing of the two diets over a broad spectrum of the ELBW population in the United States.

Study Limitations: This study will have sufficient statistical power to detect a meaningful difference in BSID III Cognitive scale score at 22-26 mo. To detect a difference smaller than 5 points, however, would require a prohibitively large sample, yet may be clinically relevant, given the relative small expense of donor milk as an intervention. Loss to follow-up is a risk in any prospective study. We will over-enroll by 10% in both randomized feeding groups to ensure adequate sample size at study completion. The low baseline rate of purely non-

maternal milk fed infants (~15% across the NRN) is also a limitation, necessitating expansion of the study to minimal maternal milk fed infants.

Study Procedure Table:

Sole Diet groups – no maternal milk

Pre-Study		In Hospital Phase				Follow-Up Phase		
Screening	Study begins	Wt	Length	Feeding	Hospital Discharge GDB Outcomes collected	Dev	Growth	BSID
Consent			OFC	Intol		exam		III
Randomization		q week	X			X	X	X
Enteral intake prior to randomization collected		q 2 wks		X				
		8-17 weeks				22 - 26 mo adjusted age		

Supplemental Diet groups – minimal maternal milk

Pre-Study		In Hospital Phase					Follow-Up Phase							
Screening	Mother stops providing milk OR providing <3oz per day at 14-21 days	Consent/ Randomization Study begins	Weight	Length	Feeding	Maternal	Hospital Discharge GDB Outcomes collected	Dev	Growth	BSID				
Mother initially providing milk				OFC	Intol	Milk					exam		III	
Rescreen at least weekly until 21 days			q week	X		X					X			
Enteral intake prior to randomization collected for 2 d weekly between birth and randomization			q 2wks		X									
			♦				X	X	X					
		8-17 weeks					22 - 26 mo adjusted age							

* until infant is no longer receiving maternal milk

Appendix A: Donor and Maternal Milk Usage at the University of Iowa NICU

To determine the appropriate grouping of subjects for the proposed multi-center randomized clinical trial, extensive feeding data has been retrospectively collected and analyzed in these infants.

Infants cared for at UICH NICU in this time frame **received human milk (donor, maternal or both) feedings for an average of 63 days**, after which time formula feedings were started in 70% of the cohort. Timing of formula feedings was dependent solely on parental preference. 30% of this cohort received no formula during hospitalization, and were fed a sole human milk diet (maternal, donor or both).

Maternal Milk Use: Records for 183 infants born between 5/30/03 and 6/30/05 whose birthweight was less than 1271g, who survived to discharge were reviewed and maternal milk intake analyzed. 92.4% of mothers initially started pumping milk to feed their infants.

Of the 183 infants:

- 14 received no maternal milk during their stay (7.6%);
- 43 received some, but less than 11 ml/day of hospitalization (23.4%); In all 43 patients who received less than 11 ml/day of hospitalization, mothers stopped providing milk before the infants reached full enteral feeds.
- 126 (68.8%) received maternal milk past the time when they achieved full feeds, with 60% receiving maternal milk for at least 60 days or through discharge if length of stay was less than 60 days.
- 16 (8.6%) received maternal milk past the point of full feeds, not through hospital discharge or 60 days.

Therefore, in a sample of the VLBW population cared for at UICH NICU:

- 60% of infants receive sole maternal milk feedings for at least 60 days, or hospital discharge, if length of stay is less than 60 days.
- 40% receive a combination of donor milk, maternal milk, and formula, **with 32% receiving none or only minimal maternal milk.**

Donor Milk Use: Donor milk analysis was completed for 128 of the 183 subjects (all subjects born in 2003 and 2004), further analysis is ongoing in the 1/2005-6/2005 group.

- **70 (55%)** of infants in this cohort received donor milk. Full intake data is available on 55 of these patients, who fall into three categories:
- 9 patients (16%) received <33% of their total human milk intake as donor milk. In these patients, donor milk served as a bridge as maternal milk supply increased in the first weeks after birth. Infants in this group received an average of 61 days of human milk feeding.
- 4 patients (7%) received between 33 and 66% of their total human milk intake as donor milk. In these patients, donor milk was often used to extend the period of time that an infant would receive human milk prior to the introduction of formula, when maternal milk supply had failed in the first month of lactation. Infants in this group received an average of 58 days of human milk feeding.
- 42 patients (76%) received >66% of their total human milk intake as donor milk. 14 of these infants received no maternal milk. Infants in this group received an average of 52 days of human milk feeding.

Therefore, for the VLBW birth cohort 2003-2005 at UICH, 32% received no maternal milk or minimal milk and would have been eligible for randomization in the proposed trial.

Bayley Scales of Infant Development Scores in Donor Milk Fed and Maternal Milk Fed ELBW Infants in Iowa

Donor milk has been a therapy in common use in the NICU at the UI Children's Hospital since 2003. In 2003 and 2004 128 infants with birthweights less than 1000 g were admitted to the UICH NICU. As of June 1, 2008, Bayley Scales of Infant Development, version II scores are available on 57 of these patients. Version II was the standard test done in this time period, both at UICH and in the NRN.

BSID II Scores by Human Milk Intake and Type of Milk:

36 ELBW infants in the 2003-2004 cohort have both BSID II scores and complete milk intake data completed for analysis. 20 of these infants received donor milk: 4 received between <1% and 6% of all human milk as donor milk (42-134 days human milk), 1 received 61% donor milk (70 days human milk) and 15 received >70% donor milk (20-105 days human milk). The remaining 16 infants did not receive donor milk, and received between 14 and 134 days of maternal milk feedings. For comparison of BSID II scores, infants receiving <6% donor milk were added into the maternal milk group.

Table 1A. BSID II Scores, UICH NICU 2003-2004, by milk type n = 36

Milk Group	MDI (mean)	PDI (mean)
	% impaired	% impaired
Maternal (n = 20)	86 25% (4/16)	80 33% (5/15)
Donor (16)	84 33% (4/12)	79 33% (4/12)
p value	0.80	0.74

According to our preliminary findings in a limited group of VLBW infants, donor-milk-fed infants have similar BSID scores as those who are maternal-milk-fed. We have no formula-fed infants represented in this cohort for comparison, however. Comparing the mean BSID MDI scores above to the published data by Vohr, et al, in a similar NICHD NRN cohort⁹, the mean MDI score in our maternal and donor-milk-fed infants is similar to their mean in maternal milk fed infants (87.3-NRN vs. 84 and 86-UICH). *The mean MDI score for donor-milk-fed infants is higher than that reported in the formula-fed group in the Vohr cohort (84-UICH vs. 75.8-NRN), suggesting that this intervention may be beneficial.*

Donor Milk Nutritional Information:

Table 2A. Composition of Pooled Donor Human Milk

Component	Number of pools	Number of batches	Mean	S.D.	
				Between-pool	Within-pool
Term Milk					
Protein (g/L)	37	130	8.22	0.59	0.13
Fat (g/L)	37	130	39.0	3.51	1.60
Premature Milk*					
Protein (g/L)	18	66	9.11	0.76	0.18
Fat (g/L)	18	66	38.2	3.22	1.25

*containing term and premature milk in variable proportions

As we can see above, pooled donor human milk from the Mother’s Milk Bank of Iowa contains between 0.8 and 0.9 g/dl of protein, and 3.8-3.9 g/dl of fat. Other data about pooled donor human milk nutritional content is sparse. A recent article from Wojcik et al discussed the nutritional content of individual donor pools of milk collected for processing by Prolacta Bioscience (Monrovia, CA)⁵⁵. They found substantial variability in protein (0.7 – 2.1 g/dl, median 1.1g/dl), and fat (0.7-7 g/dl, mean 3.2 g/dl) content⁵⁵. However the median/mean values for protein and fat are relatively consistent with previously published norms for term human milk, most based on small numbers of subjects (see table 3A).

Table 3A. Term Human Milk Protein and Fat Content, donor and research samples

Author, year	# subjects	Protein, g/dl (mean)	Fat, g/dl, (mean)	Description
*Wojcik, 2009 ⁵⁵	273	1.16 (95% CI 1.13-1.2)	3.22 (95% CI 3.08-3.36)	Single donor pools collected for donation
*Drulis, 2008 (unpublished)	55 pools (~100 donors)	0.85 (sd 0.65)	3.85 (sd 3.3)	Multi-donor pools collected for donation
†Saint, 1984 ⁵⁶	9 (day 28 of lactation)	0.9	2.9	Single sample
†Hibberd, 1982 ⁵⁷	10 (day 36 of lactation)	1.4 (sd 0.26)	NA	Single sample
†Gross, 1981 ³⁹	14 (day 28 of lactation)	1.5	3.2	Milk collected from mothers pumping for own infants, 24 hr pool
*Schanler, 1980 ^{45, 58}	?not noted	1.6 (sd 0.18)	NA	Single donor "3 day" pools collected for donation
*Tyson, 1980 ⁴⁵	At least 30	1.09	2.21	Multi-donor pools collected for donation

*milk collected for processing and feeding to other infants

†milk collected for research analysis

Donor Milk and Growth at the University of Iowa NICU: A retrospective cohort study of infants weighing less than 1251 g born between 1/1/2003 and 6/1/2005 and cared for at the University of Iowa NICU was performed. Full enteral intake data was collected. Neonatal parameters and in-hospital growth were compared across three diet groups: full maternal milk, full donor milk, and full formula. Data is available for 150 of 180 infants. 68 met criteria for inclusion: 29 who received all maternal milk, 16 who received all donor milk, and 23 who received all formula. The remaining 82 infants received combinations of maternal milk and/or donor milk, and preterm formula. Formula infants were born at later gestational ages (EGA) than milk fed (29.1 vs. 26 weeks, $p < 0.001$). EGA adjusted lengths of stay were similar among groups ($p 0.19$). Maternal and donor milk infants had similar discharge weights, (3240 g vs. 3147 g, $p 0.87$), and were discharged at similar EGA (40.5 weeks vs. 40.3 weeks, $p 0.82$). Formula infants were heavier for EGA at discharge ($p 0.03$), but had similar EGA at discharge adjusted for birth EGA ($p 0.17$). Weight gain for four time periods, 0-14 days of age, 16-35 days, 36-56 days, and 57 days-term did not differ between groups ($p 0.25$, $p 0.41$, $p 0.91$, $p 0.05$). In our population of VLBW infants fed donor milk had similar growth patterns to those who were fed both a mother's milk and a formula diet. Our data show no evidence of systematic growth failure associated with a donor milk diet.

Appendix B:

Center Resources available for MILK trial participation

(Not all centers answered all questions, totals will not always add up to 18)

Question 1: "Will your site be able to participate?"

of centers able to participate: 14

of centers unable to participate: 1

Question 2: "Does your site have blinding capacity for the study via nutrition/dietary support or through the research staff?"

of centers with capability 14

unsure 3

Question 3: "Is your center currently ordering donor milk from a milk bank?"

centers using DM 10

centers not using DM 8

All centers answering yes were buying milk from HMBANA banks

Donor Milk Handling

At two centers, parents order and purchase milk privately, bring it to the NICU, where it is labeled for the baby and then used identically to maternal milk. At the rest of the centers, the milk handling procedures are very similar, starting with frozen milk delivered from milk banks, as we propose for this protocol. Reported lengths of acceptable use and thawing are all in line with HMBANA published guidelines

Question 5: "Does your center have a location to mix feedings in a blinded fashion?"

of centers with existing location 16

without 2

Question 6: "Does your center have personnel to mix feedings daily?"

of centers with personnel identified 14

without 4

Question 7: "Does your center have freezers in which to store donor milk?"

of centers with freezers 15

without 3

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