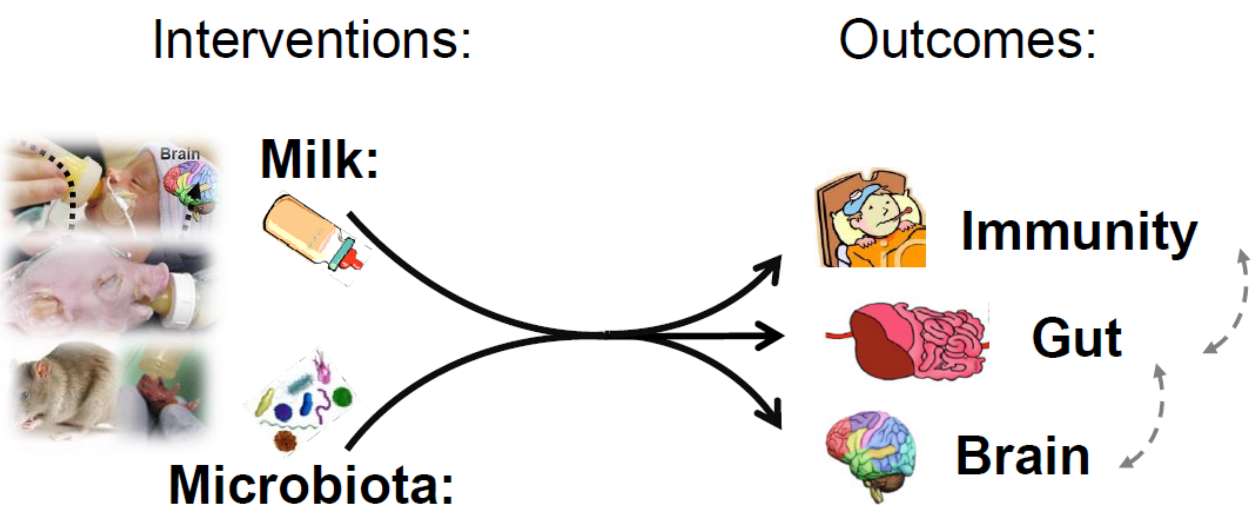
NEOMUNE research platform – work package synopses

(Updated May 2016)

NEOMUNE 2013-2018:



Contents

Target area:	WP 1 Infants												WP 2 Piglets					WP 3 Mice	
	1	2a	2b	2c	3a	3b	4a	4b	5	6a	6b	7	0	1	2	3	4	1	2
Preterm newborn							X	X	X	X	X	X	X		X	X	X		
Term newborn	X	X	X	X	X	X							X	X				X	X
Delivery & antibiotics		X	X					X						X	X			X	
Pro- & prebiotics					X			X	X		X				X		X		X
Feeding regime & PN	X	X						X		X						X			
Milk composition				X			X	X		X				X		X			
Formula composition						X											X		
Immunity & infection	X	X		X	X	X	X				X			X	X	X	X	X	X
Gut & microbiota	X		X	X	X	X	X		X	X	X		X	X	X	X	X	X	X
Brain & neurological							X						X	X	X	X	X	X	

See guidelines for synopsis on page 47

WP 1.1: Mode of delivery and gut microbiota in term infants

1. Related WPs, MG contact person: Synergies with WP 1.2b,1.3a,1.3b,1.6,2.2,3.1. MG contact: Dennis Nielsen

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim / sub-aims:
a) To document if delivery method influences infant gut microbiota colonization and composition, short term and more long term
b) If a) is verified, to test if cesarean delivery results in a less diverse gut microbiota with less gene richness compared to vaginal delivery
c) If a) is verified, to test if gut microbiota colonization, composition and gene richness is related to later obesity development, metabolic syndrome or impaired immunity.

4. Background and a central hypothesis:
It is well-known that caesarean delivery is correlated to increased risk of impaired immunity, obesity and metabolic syndrome. Whether the increased risks are due to pre-operative antibiotic use before caesarean section (WP1.2b), due to other maternal risk factors predisposing to caesarean section, to postnatal nutrition (e.g. extent of breast-feeding) or to the caesarean section per se is not explored in detail. Mode of birth (caesarean, vaginal) is therefore hypothesized to exert long term influences on the infant gut microbiota, leading to impaired immunity and to produce excess growth. This hypothesis is supported by a recent study using only a small sample size (Jakobsson et al.; 2013, n = 9 and 15 for caesarean section and vaginal delivery, respectively) and 454/FLX-based 16S rRNA gene amplicon sequencing. This study found that infants born by caesarean section have lower gut microbiota diversity and moderately lower levels of some Th1-associated chemokines (CXCL10, CXCL11). Moreover, the vulnerability in outcomes after caesarian delivery may be related to specific genotypes.
We hypothesize that delivery-associated gut colonization has long term effects on colonization and development of immunological and metabolic disorders. Subgroups of individuals, with specific genotypes, are more susceptible for altered gut microbiota patterns leading to impaired phenotypes.

5. Key analyses and methods:
Fecal samples from infants and mothers at 0, 4 and at 12 months after birth are frozen at -80 ^{o}C (Halmstad Hospital, n=150 caesarean, n=320 vaginal). Food patterns are recorded. Metagenomic analyses (complete genome sequencing and SNPs for polymorphisms) will show how the infant gut microbiota colonization and development is influenced by delivery method, maternal gut microbiota, food patterns and antibiotics during the first year of life and how this relates to specific human genotypes.

6. Expected results:
All vaginal delivered pairs are analyzed but no caesarean pairs are yet investigated. Within the coming year we will have an answer to the question if caesarean delivered children have a less diverse gut microbiota composition and lower gene richness and how this relates to weight development and immunity. In addition to microbial colonization of the gut, other factors such as less breast-feeding in mother-child pairs where caesarian delivery is performed or more overweight mothers asking for caesarean delivery possibly play a role here. Human DNA for candidate genes will be analyzed the coming 1.5 year and correlations to gut microbiota colonization, composition and gene richness will be carried out.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol	x																							
Sample collection	x	x																						
Metagenomics	x	x	x		x	x	x	x	x	x														
Metabolism/growth			x	x	x	x	x	x	x	x														
Immunity parameters					x	x	x	x	x	x														
Genotype (in planning)							x	x	x	x	x	x												
Publication(s)										x	x	x	x	x	x	x								

8. Estimated budget from NEOMUNE: 1.3 mio DKK

The amount covers metagenomics analyses in collaboration with BGI-Shenzhen for this and potential other NEOMUNE projects. Significant co-funding from BGI is required (to be negotiated) and further funding is being sought.

9. Estimated budget from elsewhere: 2.5-3.5 mio DKK

2013 ALF J Dahlgren 300,000 SEK, Region Halland 300,000 SEK, Svenska Läkarsällskapet 100,000 SEK
2014 ALF J Dahlgren 300,000 SEK, Region Halland 300,000 SEK, Frimurare 100,000 SEK
2015 Vetenskapsrådet J Dahlgren 700,000 SEK
2016 Vetenskapsrådet J Dahlgren 700,000 SEK
Co-funding/other sources 0.9-1.9 mio DKK

10. Additional comments:

- All sample collection is carried out by 2013 and the main limitation is sample analysis capacity. The study is partly sponsored from other sources, but NEOMUNE provides supporting funds for microbiome analyses at BGI-Shenzhen and DNA analyses in Copenhagen.
- End-point data (weight development, immunity) etc. will be collected until 3 or 5 years of age
- The project is relevant for the NEOMUNE study parts (infants, pigs, mice) that investigate birth methods/exposure to microorganisms at and after birth, exposure to antibiotics around birth and study effects on gut colonization and immunity.
- The project provides bridging between the -omics analytical capacity at BGI also with other projects in NEOMUNE, particularly the studies being performed in China (WPs 1.3, 1.6). BGI is involved also in WP 2.3 in piglets on the epigenetic characterization of the gut responses to the first feed and microbiota (0.7 mio DKK funds allocated).
- Method(s) for human genotyping not yet fully decided on. Might involve SNP chipping, exome sequencing and low pass whole genome sequencing.

WP 1.2a: Breast-feeding and infections in term infants

1. Related WPs, MG contact person: Synergies with WP 1.1, 1.2b, 1.2c, 1.3b, 1.4a. MG: Per Sangild

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
a) To examine the pattern of infections in an unselected cohort of Danish infants.
b) To investigate the influence of diet, especially breast feeding, on infections during the first year of life.
c) To investigate the influence of social status, number of siblings, use of day care and environmental smoking on infections during the first year of life.

4. Background and a central hypothesis:
The early diet may among other factors have a critical role for early and later immunity, gut microbiota and infection resistance. Most information is available from compromised infants, and information from a standard population of Danish term infants is needed. We therefore established an unselected population-based cohort with about 2500 mothers and children, born year 2010-12 in Odense (Odense Child Cohort, OCC, www.odense.dk/subsites2/OdenseBornekohorte). OCC gives a unique possibility to follow and register infections, and possible determinants of infections, during the first year of life and also in later childhood.
We hypothesize that infections during infancy is associated with diet (e.g. breastfeeding versus formula feeding), age at introduction of complementary food, and social variables such as parents education, number of siblings, parental smoking and use of day care.

5. Key analyses and methods:
Blood samples and questionnaire data are obtained from mothers during pregnancy and from the child at birth, 3 and 18 months of age. Clinical information includes mode of delivery, vaccinations, antibiotics use and medical and hospital visits. Precise data on breast-feeding are obtained from the mothers by weekly SMS contact and health nurse visits. The SMS technology will also be used to obtain information on infections at the individual level. Social information including social status of parent, parental smoking, number of siblings and use of day care is obtained from the Odense Municipality Warehouse Database. Statistical relationships among later infections, mode of delivery, diet and antibiotics use at birth are analyzed by multivariate regression analyses.

6. Expected results:
The infection pattern in term infants is mostly due to viral infections. The frequency of infections and hospital admissions depends on social factors including number of siblings, use of day care and parental smoking. Antibiotics use is mainly due to airway symptoms during the first year of life. Breastfeeding decreases the number of infections both in the first and second part of the first year of life.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol			x	x																				
Sample collection	x	x	x	x			x			x			x											
Analyses 1			x				x																	
Analyses 2			x				x			x														
Analyses 3											x		x											
Analyses 4												x					x							
Publication(s)										x			x				x				x			

8. Estimated budget from NEOMUNE: 0.8 mio DKK

9. Estimated budget from elsewhere: 5.0 mio DKK
Partly from the Municipality of Odense, Odense University Hospital and University of Southern Denmark.

10. Additional comments:

- OCC was established by Odense University Hospital and Odense Municipality in 2010. The two institutions support the continuous collection of data from the Cohort. Collected data from the OCC is stored in the OPEN (Odense Patient Exploratory Network) repository and can in the future be used by other researchers.
- Synergies with other NEOMUNE partners are expected in the area of microbiology and breast feeding in premature infants (WP1.1, 1.2b, 1.2c, 1.3b, 1.4a).

WP 1.2b: Maternal antibiotics and term infant gut colonization

1. Related WPs, MG contacts: Synergies with WP 1.1, 2.1, 2.2, 3.1. MG: Gorm Greisen, Dennis Nielsen

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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Nana Hyldig, PhD stud., Odense University Hospital, Nana.Hyldig@rsyd.dk (15%)
Shamrulazhar Shamzir Kamal, PhD stud., Dept. Food Science, Univ. Copenhagen, shamrul@food.ku.dk (35%)

3. Main aim and sub-aims:
To document if maternal antibiotics treatment during delivery affects term infant gut microbiota colonization and composition (short and long term). More specifically to document if maternal exposure to antibiotics (cefuroxim) during cesarean delivery influences a) the infant gut bacterial colonization and b) the gut microbiota antibiotic resistance. If influenced short term (10 days), then also long term effects (9 months) will be investigated.

4. Background and a central hypothesis:
Today it is recommended that mothers are given a single profylatic dose (1500 mg) of cefuroxim shortly before delivery by cesarean section. Cefuroxim readily passes from mother to infant before the umbilical cord is cut. Due to low renal function at birth, it is expected that cefuroxim will not be fully cleared from the infant until after the first 24 h of life. The influence of very early exposure to antibiotics (cefuroxim) on infant gut microbiota is not known. Cefuroxim is known to give rise to bacterial antibiotic resistance.
We hypothesize that early life exposure to antibiotics provided to the mother influences infant gut microbiota colonization (short and long term) and the prevalence of gut microbiota-associated antibiotic resistant bacteria.

5. Key analyses and methods:
A total of 40 mothers scheduled for elective cesarean section will be recruited for the study at OUH during winter/spring/summer 2014. The mothers will be randomized and 20 mothers will receive the standard treatment of a prophylatic dose of cefuroxim shortly before cesarean section (group 1) and 20 mothers will receive cefuroxim immediately after umbilical cord clamping (group 2). Blood will be sampled 2-3 times during 24 hours from the infants in group 1 for pharmacokinetic determination of cefuroxim clearance. Blood will be sampled on day 2-3 for gene expression of lymphocytes. Fecal content will be sampled 10 days and depending on initial gut microbiota analysis also 6 months after birth from both groups of infants.
a) Cefuroxim clearance (blood) will be determined by the clinical pharmacological department at Odense and Aarhus University Hospital
b) Gene expression of lymphocytes: Q-RTPCR (2-3 days samples)
c) Total fecal DNA will be extracted and gut bacterial composition determined by tagged 16S rRNA gene targeted Illumina (MiSeq) based sequencing
d) Presence and prevalence of key antibiotic resistance genes in fecal DNA will determined using targeted qPCR
e) Resistance against β-lactams is determined using a culture-based approach in collaboration with SSI

6. Expected results:

Pharmacokinetic determination of cefuroxim clearance will reveal how long time after birth the antibiotics remain in the infant body. Gut microbiota analysis will reveal if exposure to antibiotics very early in life influences GM colonization and the prevalence of antibiotic resistance gene markers among the GM members (as determined after 10 days). If gut microbiota is influenced after 10 days, information on more long term gut microbiota composition prevalence of antibiotic resistance gene markers (9 months).

Expected publications:

1. Cefuroxim clearance among term born infants (submitted and minor revision March 2016)
2. Very early life exposure to cefuroxim influences infant gut microbiota colonization, prevalence of antibiotic resistance gene markers, notably β-lactam resistance. Including gene expression of lymphocytes possibly influenced by early exposure to Cefuroxime.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol		x	x	x																				
Sample collection					x	x																		
Pharmacokinetics					x	x																		
GM composition					x	x																		
AB resistance genes and prevalence					x	x	x																	
Publication 1							x	x						x										
Publication 2								x	x						x									

8. Estimated budget from NEOMUNE:

OUH-based part of study (recruiting, sampling, pharmacokinetics): 0.2 mio DKK

GM analysis (composition and antibiotic resistance gene markers): 0.15 mio DKK

9. Estimated budget from elsewhere: 1.5 mio DKK

In house staff contributions from the participating institutions; PhD-student Shamrulazhar Shamzir Kamal is carrying out main part of GM analysis and is funded from other sources, MARA-grant.

10. Additional comments:

- If the main hypothesizes is proven and it is found that very early life exposure to cefuroxim influences gut microbiota colonization/composition, steps should be taken towards carrying out a new study including a larger cohort and with endpoints aiming to investigate the influence of the changed gut microbiota on long term immune system development.

WP 1.2c: Milk and immunity in children during chemotherapy

1. Related WPs, MG contact person: Synergies with WP 2.3, 1.6a. MG: Per Sangild

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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Tim Hansen, Biofiber, thh@damino.com (10%)

3. Main aim and sub-aims:
a) To test if a diet of bovine colostrum will improve immunity and gut function in children on chemotherapy.
b) To support the studies in children with piglet studies on different colostrum and chemotherapy regimens.

4. Background and a central hypothesis:
Pediatric Acute Lymphoblastic Leukaemia (ALL) is the most common form of childhood cancer. Cure rates are improving, but the intensity of treatment is limited by toxicity. The toxicity inherent to antineoplastic drugs is often the dose limiting factor rather than their actual antitumor properties or pharmacological effect. In the case of ALL, 2-5% of patients die from treatment related complications, mostly due to therapy-induced toxicity and immune suppression. All actively proliferating tissues are affected by antineoplastic drugs. The rapid proliferation of intestinal mucosal cells makes the gut particular vulnerable to chemotherapy affecting its absorptive, endocrinological, immunological and barrier functions.
Gastrointestinal toxicity induced by chemotherapy is likely to play a key role in the pathogenesis of treatment-related complications in chemotherapy treated children. Since the intestinal epithelium is in constant interaction with the gut microbiota it is essential for the maintenance of the immunological balance that the epithelial barrier is intact. Chemotherapy-induced toxicity may disturb this balance through damaging effects on the epithelium leading to translocation of bacterial components. This may result in both infections and systemic inflammatory responses with impact on post-chemotherapy immune recovery.
Treatment with broad-spectrum antibiotics is required to reduce the risk of infections, but it is largely unknown if dietary factors may improve recovery from the cancer itself (e.g. leukemia) and chemotherapy-induced gut and systemic complications. The first milk after birth, colostrum, is from nature designed to prepare the immune-compromised newborn mammal to adapt to life ex utero, including the rapid colonization with billions of bacteria along body epithelial surfaces. The child with ALL, further immune-compromised by chemotherapy treatment, is an extreme case of immune deficiency.
We hypothesize that bovine colostrum given shortly before and after chemotherapy may protect against gastrointestinal toxicity and thereby reduce associated complications such as infections and inflammation.

5. Key analyses and methods:
a) In the ALL patient studies, we investigate the effect of bovine colostrum on infections, gastrointestinal toxicity and systemic inflammation. The study is a randomized, double-blind placebo-controlled clinical study at the two hospital sites. Primary outcomes are days with fever, neutropenia and sepsis. Secondary outcomes are days given antibiotics and in intensive care, as well as clinical and paraclinical measures of gut toxicity and systemic inflammation (faecal calprotectin, blood citrulline, IgA, albumine, neutrophils, LPS). Finally, the faecal gut microbiota in children under chemotherapy is investigated.

b) In piglet studies, newborn piglets are treated with clinically-relevant doses of chemotherapy (e.g. doxorubicin) and immunity parameters (cytokines, inflammatory lesions, hematology) and gut functions (permeability, enzymes, histology, gut microbiota) are investigated. Subsequently, chemotherapy-treated piglets are fed with varying doses of bovine colostrum during chemotherapy and endpoints are measured.

6. Expected results:

We expect that ingestion of sufficient amounts of bovine colostrum will improve parameters of gut, structure and function and the clinical responses to chemotherapy in both children and piglets.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning of exp., ethical protocols	x																			
Part a) execution,Copenhagen/Odense			x	x	x	x	x	x	x	x	x	x								
Part b) execution, piglet studies	x	x	x	x																
Part a) lab analyses							x	x	x	x	x	x	x	x						
Part b) lab analyses				x	x	x														
Part a) publication phase												x	x	x	x	x				
Part b) publication phase												x	x	x	x	x				

8. Estimated budget from NEOMUNE: No specific NEOMUNE funds are allocated

9. Estimated budget from elsewhere:

4.5 mio DKK (university PhD scholarships, Rene Shen, Mathias Rathe, grant from Børnecancerfonden and the Odense University Hospital research fund, involvement of hospital personnel in Copenhagen and Odense). The industrial partner Biofiber Damino supports the work packages focusing on the use of bovine colostrum product in NEOMUNE (WP 1.3c, 2.3, 1.6a) with 1.0 mio DKK in total plus bovine colostral products.

10. Additional comments:

- The project was added to the NEOMUNE research platform 1st August 2013. The project contains scientific overlap and synergy with NEOMUNE goals because it aims to improve immunity and gut functions in an immune-compromised human patient population in early life. Further, the project has marked overlap with other NEOMUNE projects in involved personnel, hospital study sites, endpoints and the involvement of the same industrial partner (bovine colostrum supplier, Biofiber). NEOMUNE benefits by attracting synergistic scientific expertise and expanding the clinical implications of similar diet interventions.
- The project is subject to a special collaboration agreement between the participating NEOMUNE partners (Copenhagen University Hospital, Odense University Hospital, Biofiber, Univ. Copenhagen, University of Southern Denmark). This agreement, the partner institutions and the scientific goals and endpoints share overlaps with WP 1.6a.

WP 1.3a: Probiotics for term infants

1. Related WPs, MG contact person: Synergy with WP 1.1, 1.2a, 1.3b, 1.4b, 1.6, 2.2, 2.4, 4.3. MG: Per Sangild

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):

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3. Main aim and sub-aims:

To evaluate the sensitivity to infections after feeding with an infant formula containing probiotics as compared to an infant formula without probiotics.

Target population for intervention and control groups: Healthy full-term infants of mothers who could not or resigned completely from breast-feeding at age 21 ±7 days. Breast-fed term infants act as reference group. All groups, intervention, control and breast fed, are n = 200.

4. Background and a central hypothesis:

The main goal of infant formula development is to approach the composition and functionality of breast milk to give formula-fed infants as small a disadvantage as possible compared to breast-fed infants. Breast-fed infants have been shown to have a gut microbiota more dominated by bifidobacteria and lactobacilli compared to formula-fed infants, who have a more diverse microbiotia, containing *Bacteroides*, bifidobacteria, staphylococci, *Escherichia coli* and clostridia. These observed differences have been suggested to contribute to the lower incidence of infections, allergies and gastrointestinal disturbances in breast-fed compared with formula-fed infants, probably via a maturational effect on the gut and systemic immune systems. If this is the case, it seems reasonable to develop infant formulas to support the establishment of a microbiota, which resembles that of breast-fed infants, for example by adding probiotics.

We hypothesize that addition of probiotics will reduce infections in formula-fed infants, via improved composition of their gut microbiotia and immunity and in this way make them more similar to breast-fed infants.

5. Key analyses and methods:
The study is a randomized, double-blind, controlled trial with clinical recording of infections episodes (gastrointestinal and upper and lower respiratory infections).

6. Expected results:
Reduced incidence of infectious episodes (gastrointestinal and upper and lower respiratory infections) in infants fed with infant formula containing probiotics compared with standard formula.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol																								
Sample collection	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x						
Clinical data analysis																		x	x					
Possible microbiota analyses																		x	x	x				
Possible immunity biomarkers																x	x	x						
Publication(s)																						x		

8. Estimated budget from NEOMUNE:
5.0 mio DKK (financed entirely by ARLA Foods, no funds from DSF)

9. Estimated budget from elsewhere:
2.0 mio DKK (local doctors and hospital infrastructure). 1.0 mio DKK from synergy with NEOMUNE partners (fecal and plasma sample analyses)

10. Additional comments:

- The study outline is published at ClinicalTrials.com. The exact nature of the intervention and the details of the recorded endpoints are kept confidential to the public and to NEOMUNE partners not involved in the study until the results of the study will be published.
- This project is run entirely by the sponsoring industry partner but association with the NEOMUNE network is aimed to provide win-win at many levels. Scientific synergy with other NEOMUNE projects (see point 1) can be reached by sharing/collaborating on:
  1) Protocol formation for nutritional studies in Chinese hospital settings (WP 1.6a).
  2) Experience in performing clinical studies in China (WP 1.6a, 1.6b, 1.4b).
  3) Experience with Danish-Chinese partnerships, scientific, legal, ethical etc. (WP 1.6b, 1.4b, 4.3).
  4) Evidence for use of probiotics in newborn infants across the world (WP 1.5, 1.6b).
  5) Synergy and information from animal probiotic studies (WP 2.2, WP3.2).
  6) Possible shared analytical capacity for gut microbiota analyses (WP 1.1, BGI Shenzhen).
  7) Possible shared capacity for blood immunity analyses (WP 1.2a, SDU Denmark).
  8) Increased networking with Chinese partner institutions and stakeholders (WP 4.3).
  9) Joining the NEOMUNE scientific meetings for science and partnerships (WP 4.2).

WP 1.3b: Bioactive milk formula for term infants

1. Related WPs, MG contact person: Synergy to WP1.2a,1.3a,1.4b,1.6a,2.4,4.3. MG contact: Per Sangild

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
To evaluate effects on the immune system after feeding with an infant formula containing an improved Whey Protein Concentrate (WPC). Target population for intervention and control groups: Healthy full-term infants of mothers who could not or resigned completely from breast-feeding at infant age 21 ±7 days. Breast-fed term infants act as reference group. All groups, intervention, control and breast fed, are n = 200.

4. Background and a central hypothesis:
Specific milk proteins have been demonstrated to have antimicrobial activities and can prevent diarrhea in small children. It has also been shown that infants fed with formula with added WPC have reduced frequency of acute otitis media compared with infants fed with a standard formula.
We hypothesize that addition of an improved WPC will reduce infections in formula-fed infants, improve their health and make them more similar to breast-fed infants.

5. Key analyses and methods:
The study is a randomized, double-blind, controlled trial with clinical recording of infection episodes (gastrointestinal and upper and lower respiratory infections).

6. Expected results:
Reduce incidence of infectious episodes (gastrointestinal and upper and lower respiratory infections) in infants fed with infant formula containing an improved WPC compared to standard formula.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol																								
Sample collection	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x						
Clinical data analysis																		x	x					
Possible microbiota analyses																		x	x	x				
Possible immunity biomarkers																x	x	x						
Publication(s)																						x		

8. Estimated budget from NEOMUNE: 5.0 mio DKK
Financed entirely by ARLA Foods, no funds from DSF.

9. Estimated budget from elsewhere:
2.0 mio DKK (local doctors and hospital infrastructure). 1.0 mio DKK from synergy with NEOMUNE partners (faecal and plasma sample analyses).

10. Additional comments:

- The study outline is published at ClinicalTrials.com. The exact nature of the intervention and the details of the recorded endpoints are kept confidential to the public and to NEOMUNE partners not involved in the study until the results of the study will be published.
- This project is run entirely by the sponsoring industry partner but association with the NEOMUNE network is aimed to provide win-win at many levels. Scientific synergy with other NEOMUNE projects (see point 1) can be reached by sharing/collaborating on:
  1) Protocol formation for nutritional studies in Chinese hospital settings (WP 1.6a).
  2) Experience in performing clinical studies in China (WP 1.6a, 1.6b, 1.4b).
  3) Experience with Danish-Chinese partnerships, scientific, legal, ethical etc. (WP 1.6b, 1.4b, 4.3).
  4) Evidence for use of probiotics in newborn infants across the world (WP 1.5, 1.6b).
  5) Synergy and information from animal probiotic studies (WP 2.2, 3.2).
  6) Possible shared analytical capacity for gut microbiota analyses (WP 1.1, BGI Shenzhen).
  7) Possible shared capacity for blood immunity analyses (WP 1.2a, SDU Denmark).
  8) Increased networking with Chinese partner institutions and stakeholders (WP 4.3).
  9) Joining the NEOMUNE scientific meetings for science and partnerships (WP 4.2).

WP 1.4a: Donor human milk to preterm infants

1. Related WPs, MG contact person: Synergies with WP 1.4b, 1.6a, 1.7, 2.3, 2.4. MG contact: Per Sangild

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):

Diny van Zoeren, physician, Isala Clinics, Zwolle, d.van.zoeren@isala.nl (5%)
Elisabeth Kooi, Physician, University Medical Center Groningen, e.kooi@umcg.nl (10%)
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H. LaFeber, Prof., VU University Medical Center Amsterdam, hn.lafeber@vumc.nl (2.5%)
Ineke van Vliet, Physician, VU University Medical Center Amsterdam, I.vanvliet@vumc.nl (2.5%)
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Stefanie Kouwenhoven, PhD stud., VU Univ. Medical Center Amsterdam, s.kouwenhoven@vumc.nl (5%)
Viola Christmann, Physician, Sint Radboud Hospital Nijmegen, v.christmann@cukz.umcn.nl (10%)
Willemijn Corpeleijn, PhD stud, VU Univ. Medical Center Amsterdam, w.corpeleijn@vumc.nl (5%)

3. Main aim and sub-aims

Aim: To determine whether (supplemental) human donor milk has beneficial effects (in terms of reduction of infectious episodes and mortality) when compared to (supplemental) preterm formula during the first 10 days of life in very low birth weight (VLBW) infants.

Sub-aims: To determine if early use of donor milk results in a more diverse intestinal colonization, earlier attainment of full enteral feeding, reduced number of days on parenteral nutrition, similar growth rate, similar bone density and improved Bayley Scores of Infant Development III at 2 years, compared with infants fed preterm formula.

4. Background and a central hypothesis:

Feeding own mother's milk (OMM) to preterm neonates is thought to have important beneficial effects for VLBW infants when compared to formula feeding. Short-term effects seem to include a reduction in the incidence of sepsis and necrotizing enterocolitis (NEC). Neonatal sepsis, occurring in 20-60% of VLBW infants, is a major contributor to neonatal morbidity and mortality and compromised long term neurodevelopmental outcome. Unfortunately, it has proven to be very difficult to provide OMM within the first few hours and days of life as the onset of lactation is often delayed after preterm delivery.

We hypothesize that a diet completely consisting of human milk (OMM and/or donor milk) during the first 10 days of life reduces the incidence of sepsis/NEC and/or mortality in VLBW infants.

5. Key analyses and methods:

Blinded randomized controlled multicenter study, conducted in 5 Dutch hospitals. Infants with a birth weight <1500 grams will be included after obtaining informed consent. The intervention starts when the first enteral nutrition (MEF) is given according to the local protocol. If milk of the own mother is available this will always be used first in both groups. If milk of the own mother is not available, or the volume is not sufficient, infants in group A will receive donor milk and infants in group B will receive infant formula. The study intervention ends at day 10 of life and OMM or donor milk will not be fortified during these days, to avoid introduction of cow's milk protein before day 10 of life. Data on the primary and secondary endpoints will be collected until 60 d of age.

We consider a reduction in the combined incidence of serious infections and/or NEC and/or death from 40% in the control group to 25% in the donor milk group to be clinically relevant.

6. Expected results:

Data will be collected on the incidence of the combined outcome of serious late-onset infections

(sepsis/meningitis and NEC) and/or death occurring between age 72 hours and 60 days. Additionally, the composition of fecal microbiota (first stool and stool at days 10 and 30), time to full enteral feeding, days on parenteral nutrition, weekly growth rate (body weight, length and head circumference), bone density by ultra sound and Bayley Scores of Infant Development III at 2 years of age will be determined. We will determine differences in these outcomes between VLBW infants fed with a diet completely consisting of human milk and VLBW infants (partly) fed with formula during the first 10 days of life.

7. Estimated time frame

Task	2011				2012				2013				2014				2015				2016			
Planning, protocol		x	x	x	x																			
Sample collection						x	x	x	x	x	x	x	x	x	x									
Data analyses																x	x							
Analyses feces																		x	x					
Publication primary outcomes																					X			
Publication secondary outcomes																							X	X
Finish follow-up period																								X

8. Estimated budget from NEOMUNE: 1.0 mio DKK
Also used to support participation and consulting in WPs 1.4b, WP 1.6a and WP 1.6b.

9. Estimated budget from elsewhere: 0.8 mio €
Mead Johnson Nutritionals.

10. Additional comments:

- There is important scientific synergy to a number of other NEOMUNE projects on feeding preterm infants or preterm pigs (see section 1). As such, there is a possibility to make use of shared analytical capacity and/or knowledge sharing in the areas of gut microbiota, immunity, metabolism and brain-related endpoints. Possible analyses of samples within the NEOMUNE network will be determined after completion of the intervention studies.
- The Amsterdam group has intensive experience on nutrition research in preterm infants and in using pigs as models for infants. The leader of the Amsterdam group is a central opinion leader for nutrition in preterm infants via ESPGHAN. Central role in leading WP 1.4b.
- The Amsterdam group is important in planning the NEOMUNE intervention studies on preterm infants in China and is already leading infant nutrition studies in China.
- The Amsterdam group may be important as a training site for researchers from elsewhere in NEOMUNE, including WP 1.7.

WP 1.4b: Database of feeding preterm infants

1. Related WPs, MG contact person: Synergies with WP 1.4-6, MG contacts: Per Sangild, Gorm Greisen

2. Key involved personnel, institution and mail address (project leader + main study site underlined):

Aloka Patel, Rush Medical Center, Chicago, Aloka_Patel@rush.edu (2.5%)
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Gorm Greisen, Copenhagen University Hospital, Gorm.Greisen@regionh.dk (5%)
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Hung-Chih Lin, Children's Hospital of China Medical University, Taichung, d0373.cmuh@gmail.com (5%)
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Yanwei Liu, The First Affiliated Hospital of Sun Yat-sen Univ., zhaa97@163.com (10%)
Zhu Yanna, maternal and Child Health, Sun Yat-sen University, zhuyn3@mail.sysu.edu.cn (5%)

3. Main aim and sub-aims:

Aim: To know differences in nutrition practice and use of anti-/probiotics for preterm infants <1500 g.
Sub-aims: 1) to relate different clinical practices to disease and growth patterns; 2) to relate different practices to biological and cultural factors (in connection with WP 1.7); 3) to obtain baseline information on feeding practices and –biotics uses to help design future diet intervention studies in these hospitals.

4. Background and central hypotheses:

Feeding and gut colonization are believed to be two important factors for short and long term health of preterm infants. Feeding practices and use of anti- and probiotics for these patients vary widely around the world and the most beneficial regimen remains poorly understood. It is unknown to which extent the chosen feeding regimen is related to factors such as product availability, infant genetics and biology, clinical tradition or cultural elements. Our hypothesis is that 1) the time to achieve full enteral feeding (TFF, in this study set at 120 mL/kg/d) differs widely among hospitals, and variation in TFF is associated with type of diet and antibiotics use; 2) infants in units that achieve enteral feeding 120mL/kg/day earlier achieve higher body weight at the end of follow-up when corrected for GA and weight at birth; 3) differences in feeding practice is not significantly associated with differences in major neonatal morbidities.

5. Key analyses and methods:

Collection of retrospective and prospective data from 14 hospitals worldwide into a web-based database. The participating hospitals will enter the data of eligible infants from the 1st Jan. 2011 to the 15th Sept. 2013 retrospectively, and from 16th Sept. 2013 to 15th Aug. 2014 prospectively. Formation of the web-based database is led by Marita de Waard and Johannes van Goudoever. Marita and Yanqi Li will lead the communication with hospitals, collection of data, and analyses of the data.

6. Expected results:

The database collects data from preterm infants <1500 g from the first day of birth to 37 weeks corrected gestational age or discharge, whichever comes first, in the 17 participating hospitals. The data include maternal

data, volume, composition and days of parental nutrition, volume, day, and type of enteral nutrition, weekly demographic data, clinical data (e.g. NEC) and use of anti- and/or probiotics. Each parameter will be compared among hospitals to investigate whether they differ. Correlations among parameters are done to identify relationships between nutritional and –biotics practices and clinical outcomes (e.g. TFF, postnatal growth rate, antibiotics use, NEC). The observational data provide indirect evidence for the optimal feeding regimen and provide a basis for identifying the most important variables that affect clinical practices in different parts of the world. As such, the data are also important for doing nutrition and –biotics intervention studies at each hospital site.

7. Estimated time frame (edit task and indicate time by "x")

Task	2013				2014				2015				2016				2017			
Planning, database development	x	x	x	x																
Data entry			x	x	x	x	x	x	x											
Data analyses in each hospital							x	x	x	X										
Overall collective data analyses								x	x	x	x									
Publication(s), individual hospital*								x	x	x										
Collective overall publication(s)*														X	X					

* Data are allowed to be analyzed in individual hospitals for their internal use or publishing on local or international journals. To retain the novelty of the overall collective data, comparison of data among different hospitals is not allowed until after the publication of the overall collective data.

8. Estimated budget from NEOMUNE:
0,4 mio DKK

9. Estimated budget from elsewhere: PhD/post doc salaries (3 yrs, 30%, Marita, Yanqi): 0.9 mio DKK
Support from local hospital sites (man power to collect data, 16 x 5% salary): 0.5 mio DKK

10. Additional comments:

- The personnel who perform the data entry are provided by each participating hospitals or SYSU.
- The data base work is also instrumental in letting people come together and discuss the rationale for the clinical procedures taking place at different hospitals and in different countries.
- Regarding the authorship for future collective publications, we propose that we include everyone who contributes to the database work as the author. On the publication, the names of 1-3 junior authors (who contribute the most) are shown and the rest is shown as the Database group. An author list allowing 50 names with an alphabetical ordering is used to specify the Database group, and in this way every author can be indexed in pubmed.
- At each site a number of master's students will assist PhD's and Post docs in data collection.
- Base on this database work, we are discussing with SYSU about further collaborations to 1) follow-up the growth and brain development of infants recruited in this database from selected hospitals (e.g. FWCH) for up to two years; 2) investigate whether different feeding practices for preterm infants among different hospitals in east and west are related to cultural factors. These two side-projects are at very preliminary stage and depend on whether we can get extra budget. Zhu Yanna and her colleagues at SYSU aim for applying Chinese funds in 2014 in collaboration with NEOMUNE.

WP 1.5: Probiotics, feeding and NEC in preterm infants

1. WP (related WPs, MG contact): WPs 1.4b, 1.6a, 1.7; 2.2, 3.1, 3.2. MG contact: Gorm Greisen

2. Key involved personnel, institutions, mail address (project leader + main study site underlined):
Elisabeth Lyore, Physician, Neonatology, Copenhagen University Hospital, elisabeth.lyore@regionh.dk (10%)
Gorm Greisen, Prof., Neonatology, Copenhagen University Hospital, greisen@rh.dk (10%)
Irina Lambæk, Physician, Neonatology, Copenhagen University Hospital, irinakirill@hotmail.com (10%)
Karen Krogfelt, Prof., Dept. Microbiology, Statens Serum Institut, kak@ssi.dk (5%)
Sandra Meinich Petersen, Phd stud., Copenhagen University Hospital, sandrameinich@gmail.com (60%)

3. Main aim and sub-aims:
1. To examine the periodicity of the NEC rate at Copenhagen University Hospital
2. To describe the epidemiology of NEC at the national level
3. To describe the association between gut microbiota and NEC
4. To examine the effect of routine use of probiotics on a) rate of NEC and b) fecal microbiota
5. To evaluate the importance of naso-gastric tube biofilm on early upper GI colonization in preterm infants
6. To reexamine the Bell staging of necrotizing enterocolitis

4. Background and a central hypothesis:
NEC is one of the four major neonatal morbidities in preterm infants. NEC has high mortality and carries a high risk of long term consequences in the form of short bowel syndrome and neurological deficit. NEC is probably caused partly by too aggressive enteral feeding and bacterial overgrowth. Clinically, it is difficult to balance the risk of NEC with the nutritional needs of the small, preterm infant.
We hypothesize that:
a) The incidence and mortality of NEC is stable in absolute terms but decreasing when corrected for gestational age and weight at birth
b) The use of probiotics is associated with lower risk of NEC and probably with a 'better' gut microflora
c) The clinical staging system of Bell is suboptimal in describing the clinical presentations of NEC.

5. Key analyses and methods:
Collection of routinely recorded clinical data. Re-assessment of clinical X-rays. Access to local and national databases. Statistical analysis. DNA analysis of stool from cohorts of preterm infants from Copenhagen and Newcastle. Prospective study of preterm infants at high risk of NEC.

6. Expected results:
Confirmation or rejection of hypotheses (a-c)

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
1+2+4a. Data analysis				x	x	x																		
1+2+4a Publication													x											
3+4b DNA analysis														x										
3+4b Data analysis															x									
3+4b Publication														x		x								
5. Data collection					x	x	x																	
5. Data analysis								x																
5. Publication														x										
6. Analysis											x	x	x	x										
6. Publication															x		x							
Publication(s)											1		1	1	1	1	1							

8. Estimated budget from NEOMUNE:

0.8 mio DKK (0.33 MD PhD + tuition + annum), ressources moved to 1.6a.

9. Estimated budget from elsewhere:

0.7 mio DKK (analytical costs 0.3, contribution from senior researchers 0.4). Additional funds will be required to perform all the indicated tasks and further support will be applied for (e.g. task 5).

10. Additional comments:

- This work package has synergies with ongoing projects at Copenhagen University Hospital and Statens Serum Institute, as well as collects new data from patients at Copenhagen University Hospital.
- The depth of the gut microbiota analyses (various levels of conventional and/or molecular techniques) will be decided upon depending on the quality of the samples collected in relation to the hypothesis and the funding available at the time of sample collection. Collaboration with other partners will be added as judged appropriate (BGI Shenzhen, Newcastle, Univ. Copenhagen). Work not started initially due to lack of supplementary funding and now due to difficulties in getting permission from the data protection board. March16: still not started
- The data collected may influence the possible choice and the mode of probiotic interventions for preterm infants in WP 1.6b. No longer relevant.
- The key institution is part of WP 1.4b (data base project) and part of this WP will relate to observations collected for the overall international data base (NEC, probiotics use, antibiotics).

WP 1.6a: Minimal enteral colostrum for preterm infants

1. WP (related WPs, MG contact person): Synergies to WPs 1.4a,2.3. MG contacts: Per Sangild, Gorm Greisen

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
Elisabeth O. Lyore, Physician, Neonatology, Copenhagen University Hospital, elisabeth.lyore@regionh.dk (5%)
Gorm Greisen, Prof., Neonatology, Copenhagen University Hospital, Gorm.Greisen@regionh.dk (10%)
Gunnar Jørgensen, director, Biofiber (colostrum product), gja@damino.com (15%)
Hans van Goudoever, Prof. Vrei Univ. Amsterdam Medical Center, h.vangoudoever@vumc.nl (5%)
Per Sangild, Prof., Comparative Pediatrics and Nutrition, Univ. Copenhagen, psa@life.ku.dk (10%)
Ping Zhou, Baoan Maternal and Childrens Hospital, xianggalao@126.com (20%)
Rene Shen, PhD stud., Comparative Pediatrics and Nutrition, Univ. Copenhagen, rlsh@life.ku.dk
Sandra Meinich, PhD stud., Neonatology, Copenhagen Univ. Hospital, sandrameinich@gmail.com (30%)
Tim Hansen, Biofiber, thh@damino.com (5%)
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Yanqi Li, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, yli@life.ku.dk (10%)
Yiheng Dai, Neonatologist, Neonatal Dept., Foshan Women and Children's Hospital, Foshan, China (5%)

3. Main aim and sub-aims:
a) To investigate the safety, tolerability and preliminary effects of bovine colostrum, used as the first enteral diet for preterm infants at 1000-1800 g body weight.
b) To assess the feasibility of study procedures, including recruitment rates, parental consent, adherence, sample collection, clinical routines, observing adverse effects.
c) To facilitate the determination of the primary endpoint and sample size for a future larger randomized, controlled trial with bovine colostrum administration.
d) To perform a randomized, controlled trial investigating bovine colostrum versus donor milk or formula.

4. Background and a central hypothesis:
Minimal enteral nutrition (MEN) is a term that reflects the small volumes of milk fed to preterm infants just after birth. It is assumed that MEN feedings promote gut maturation, provide extra nutrients and energy and allow more rapid advancement to full enteral feeding (EN, e.g 120-160 ml/kg/d), and thus cessation of parenteral nutrition (PN). It remains unclear what milk diet is best when mother's own milk is not available. Mother´s milk is superior to infant formula in promoting feeding tolerance, body growth, intestinal function, and NEC resistance in preterm infants. Feeding with human donor milk is also believed to be beneficial, relative to formula, but pasteurized milk obtained from mothers later in lactation may be less beneficial, relative to the first mother´s milk, the so-called 'colostrum'. Human milk needs to be fortified with extra nutrients to support growth of preterm infants. Colostrum from cows contains some documented beneficial properties and its maturational and NEC-protective effects are documented in a series of studies in newborn, preterm pigs.
We hypothesize that bovine colostrum, used as MEN for preterm infants, is safe and helps to provide nutrients and improve gut maturation in preterm infants, when enough mother's milk is not available.

5. Key analyses and methods
Phase a+b: Pilot study to test safety/tolerability of bovine colostrum, feasibility of study procedures (n=10).
Phase c: Randomized controlled study, fully powered to detect differences in primary endpoints (n=20)
Phase a will be run both at Copenhagen University Hospital and at Foshan Woman's and Children's Hospital.
Diet interventions for a maximum of 14 days. Control group: Donor milk (Copenhagen) or infant formula (Foshan) supplemented with mother´s own milk. Intervention group: Bovine colostrum supplemented with mother´s milk (Copenhagen) or infant formula (Foshan) as needed.

6. Expected results

We expect to document whether bovine colostrum can be used as a beneficial first enteral diet for preterm infants that have limited or no access to mother´s own milk. Results include clinical neonatal outcomes (time to full feeding, feeding intolerance, combined incidence of serious infections/NEC, days of hospitalization, anthropometry data, days to regain birth weight, days on PN, and stool characteristics) as well as paraclinical outcomes (plasma citrulline, intestinal permeability, fecal microbiota composition and fermentation.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Exp. plans, ethical protocol phase a-c)	x	x	x																	
Phase a) execution, Copenhagen						x	x	x	x	x										
Phase a) execution, Foshan						x	x	x	x	x										
Laboratory analyses, Copenhagen							x	x	x	x										
Laboratory analyses, Foshan							x	x	x	x										
Publication from both phase a+b)										x	x	x								
Planning, phase c), ethical protocol									x	x										
Phase c) execution, Copenhagen											x	x	x	x						
Phase c) execution, Foshan											x	x	x	x						
Laboratory analyses, Copenhagen													x	x	x					
Laboratory analyses, Foshan													x	x	x					
Publication from phase c)																x	x			

8. Estimated budget from NEOMUNE:

Phase a) 0.6 mio DKK (PhD, post doc salaries, Yanqi Li, Sandra Meinich)
Phase b) 1.0 mio DKK (PhD, post doc salaries, Yanqi Li, Sandra Meinich, other personnel?)

9. Estimated budget from elsewhere:

Phase a+b) 0.6 mio DKK (Yanqi Li, PhD students, MSc students, hospital personnel). Co-financing.
Phase c) 1.0 mio DKK (Yanqi Li, PhD students, MSc students, hospital personnel). Co-financing.

10. Additional comments:

- Ethical approval of the studies was applied for in both Denmark and China and is approved for phase a). The exact planning of phase c) will depend on the results from phase a+b). If results from phase a) are not promising, then phase c) will be cancelled. Phase c will pave the way for a larger randomized, controlled trial (Phase d), fully proving the efficacy of bovine colostrum as the first enteral diet when mother´s milk is not available (n=100-200?). This possibility will be discussed with Chinese NEOMUNE partner hospitals in 2015.
- A large effort is involved in securing that the product, powered bovine colostrum, can meet current legislation for use as infant formula. The investigators work with Biofiber to secure this.
- Very close collaboration between Copenhagen University Hospital and Foshan Women's and Children's Hospital, China is required in this project. Baoan Maternal and Childrens Hospital was added to the protocol in 2015 due to low recruitment rates

WP 1.6b: Probiotics for preterm infants (Cancelled)

1. Related WPs, MG contact person: Synergy with WP 1.3a, 1.4b, 1.5, 2.2, 3.2. MG contact: Gorm Greisen

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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Jiaping Mei, Physician, Shenzhen Maternal and Children´s Hospital, mjp104478@gmail.com (5%)
Per Torp Sangild, Prof., Clinical and Experimental Nutrition, NEXS, Univ. Copenhagen, psa@life.ku.dk (5%)
Sandra Meinich Petersen, Physician, Copenhagen University Hospital, sandrameinich@gmail.com (20%)

3. Main aim and sub-aims:
To perform a randomized clinical trial in newborn infants with probiotics in China and possibly other countries.

4. Background and a central hypothesis:
Probiotics have been shown to reduce the incidence of necrotizing enterocolitis (NEC) and overall mortality in infants with birth <1500 g and/or gestational age <32 weeks. This has been demonstrated in systematic meta-analysis covering more than 15 randomized trials and more than 2,500 infants. Several large trials are at present awaiting conclusions. While probiotic use in clinical routine is far from universal, its use is increasing, and a standard large-scale placebo-controlled trial runs the risk of becoming ethically difficult within such a trial's lifetime.
However, there are several problems and questions that remain unanswered before use of probiotics can be recommended as part of standard clinical care for preterm infants. In previous studies, many different probiotic strains, or combinations of strains, have been used in many different concentrations. In most trials, probiotics have not been given during the first days of life and there may or may not be an advantage in allowing spontaneous bacterial colonization take place prior to introduction of probiotics. Finally, the interactions between probiotic effects and the timing, dose and type of antibiotics given to preterm infants have not been investigated. Clearly, this WP cannot answer all these important questions. The choice of intervention in this WP shall, after careful evaluation of a) already ongoing international trials, b) current practice at NEOMUNE hospitals (WP 4.1b), and c) supporting evidence from NEOMUNE animal model studies (WP 2.2 and WP 3.2), be built on the following hypotheses:
1. The clinical effects of 10^9 and 10^{10} CFU per day do not differ.
2. Initiation of probiotics administration on day one, prior to the spontaneous colonization of the gut, improves the clinical outcome.
3. The clinical effects of probiotics are more pronounced following use of antibiotics for preterm infants.

5. Key analyses and methods:
Primary outcome to be determined. Blinded allocation. 2 x 2 factorial design (dose and time). Pragmatic design. External monitoring.

6. Expected results:
See 4. – hypothesis

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
1. Exploring data base information				x	x	x	x	x												
2. Evaluation of pig model studies																				
3. Evaluation of mouse model study																				
4. Protocol formation																				
5. Organization of clinical study																				
(6. Clinical trial in preterm infants)																				
(7. Analyses of results)																				
8. Publication																				

8. Estimated budget from NEOMUNE:

Max. 2.0 mio DKK from NEOMUNE base funds for clinical studies in China (together with WP 1.4b/1.6a).
Possible industrial co-financing to be negotiated with potential partner(s) 0 mio.

9. Estimated budget from elsewhere:

3.0 mio DKK (university and hospital partners, internal staff and equipment).
Co-funding from other NEOMUNE projects (e.g. animal studies)
Failed to get support from industry

10. Additional comments:

- The WP depends significantly on the collaborative links among hospital partners formed as part of WP 1.4b. Considering the extensive NEOMUNE network in this area, results from WP 1.4b will help to gain ideal conditions for formulating a clinical trial that answers the most important question at the time of initiation.
- The WP is linked with animal model WPs to maximize basic knowledge transfer to this WP before the project plans are fixed (probiotics time, dose and interaction with antibiotics).
- The total scale and nature of proposed clinical trial depends heavily on sponsoring from industry partner(s). Possible partners still being negotiated.
- While the costs of the planning phase for this project (see above Gantt diagram) are mainly covered by funds from elsewhere in NEOMUNE, some funds may be allocated also from this project.
- This work packed was cancelled in 2014 due to difficulties in consensus between university and industry partners on optimal interventions and because of difficulties in having network in China fully established. Allocated NEOMUNE central funds to be allocated for remaining work in china (WP 1.4a) and internationally (e.g. Karolinska, Newcastle)

WP 1.7: Ethical, social and cultural processes of translational research

1. WP (related WPs, MG contact persons): WP 1.4b,1.5,1.6; MG: Gorm Greisen and Per Sangild

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
With the overall purpose of exploring the ethical, social and cultural processes of translational research in NEOMUNE this work package sets out to map the moral reasonings and organizational frameworks that are mobilized and affect possibilities for translation between animals and infants in experimental and clinical research settings in Denmark, Holland and China. The following research question is guiding the enquiry: What moral reasonings are mobilized and what social responsibilities are negotiated in NEOMUNE activities and how do these reasonings and negotiations form the translational activities of NEOMUNE?

4. Background and a central hypothesis:
Dialogue between experimental animal studies and clinical studies in Europe and China is a critical component of achieving both scientific and clinical goals in NEOMUNE. However, prior social science studies on translational medicine have shown that the relationship between laboratory and clinic is often complicated by unexpected results, epistemic disagreements and differences in experimental cultures of biologists and clinicians trying to make sense of cross-species comparisons. In NEOMUNE the international context may further complicate the translational processes.
We hypothesize that different sets of norms and practices shape translational processes and set out to study how this happens in NEOMUNE, focusing on the interface between clinical and laboratory studies in Denmark. This study will enable analyses of the moral reasoning and social responsibilities negotiated in the translational activities of NEOMUNE.

5. Key analyses and methods:
Overall WP 1.7 takes a qualitative approach to the scientific activities in NEOMUNE, drawing methodologically and theoretically on anthropology, sociology and public health. The study of moral reasonings and social responsibilities actualized in NEOMUNE will be undertaken trough multi-sited ethnographic fieldwork where NEOUMUNE activities are situated. This is primarily:

- NEOMUNE project meetings
- The Danish perinatal pig laboratory at the University of Copenhagen (5 weeks)
- Neonatal intensive care units at hospitals in Denmark (5 weeks)
- The data collection methods are participant observations and open-ended semi-structured interviews (approx. 20) primarily with researchers and clinicians involved in NEOMUNE

6. Expected results:
By an investigation of the collaborations between the experimental pig studies and randomized clinical trials in NEOMUNE, WP 1.7 will enable analyses of how negotiations and trade-offs between clinical, scientific, economic and political rationales take place. The WP will result in three scientific publications and the facilitation of cross-disciplinary reflection on the concept and practice of translational medicine thereby contributing to the social robustness of the NEOMUNE centre.

7. Estimated time frame

Task	2013				2014				2015				2016			
Planning, protocol	x	x														
Fieldwork (data collection)		x	x	x	x	x										
Modelling a biological system: a sensitive human patient					x	x				x						
Bovine colostrum – the familiar, foreign and natural										x	x	x				
Managing multiple responsibilities												x	x	x		
Publication(s)													x	x	x	

8. Estimated budget from NEOMUNE: 1.6 mio DKK
PhD scholarship for Mie S. Dam.

9. Estimated budget from elsewhere:
0.5 mio DKK by co-financing from collaborating partners with salaries paid from elsewhere.

10. Additional comments:

- NEOMUNE contains different interfaces between species, nations, scientific disciplines and public and private partnerships, more than may be covered in one Ph.D. project.
- While WP 1.7 takes a specific focus on the relationship between animal laboratory and human clinic, it only covers the role of industry and the general public in relation to this specific part of the translational processes.
- We will attempt over the course of the project to apply for supplementary funding that will enable analyses of many other aspects and allow maximal translation from basic biological research, over clinical studies to applied effects at the societal and commercial levels.
- The PhD stud. in this project will be based formally at Copenhagen Univ. Hospital, but the daily work place will be at the work place of the main supervisor, Centre for Medical Science and Technology Studies.
- As the NEOMUNE project unfolds with studies in Netherlands and China and around the world, the WP may also incorporate separate analyses of the international translational and cultural dimensions of NEOMUNE topics. These parts are predicted to require additional funding and personnel.

WP 2.0: Development of a preterm pig brain model

1. Related WPs, MG contact person: Synergies with WPs 1.4a, 2.1-2.4, 3.1. MG contact: Thomas Thymann

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):

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3. Main aim and sub-aims:

The primary aim is to develop a clinically-relevant preterm pig brain model of preterm human infants. We will identify and describe functional and structural neurodevelopmental differences between preterm- and term-delivered piglets e.g. by using a series of *in vivo* methodologies and approaches traditionally used by neonatologists, chosen to maximize the translational value to preterm human infants. We also aim to relate any neurobehavioral and –functional deficits and/or delays with various relevant brain and gut structural, cellular and molecular endpoints within the first 4 weeks of life. Of these, the most sensitive endpoints will serve as key outcome parameters in subsequent interventions.

4. Background and a central hypothesis:

The pig is emerging as a valuable animal model of humans in biomedical research related to both neuroscience and to gastrointestinal diseases. Our group has >10 yrs experience with preterm pig models of gastrointestinal disorders such as necrotizing enterocolitis (NEC), a prevalent disease in preterm delivered infants which is associated with an increased risk of suboptimal neurodevelopment. Humans and pigs are thought to share similar brain growth and development patterns. A growing awareness of the important interplay between the early milk diet, the gut and the brain, and their possible interdependence during postnatal development, has prompted us to expand our research to investigations of brain maturation in the preterm pig model. This preterm pig model may be used to develop diet and feeding regimens that optimize brain development in the most vulnerable newborn infants. *We hypothesize that prematurity in pigs is associated with functional and structural neurodevelopment deficits and delays that reflect those observed in preterm infants.*

5. Key analyses and methods:

A comparison of newborn caesarean-delivered preterm (~90% gestation) and term pigs initially reared in our neonatal intensive care unit (NICU) and subsequently (after 12 d) transferred to our pediatric unit for longer-term rearing (26 d). We assess brain function, structure and development at various levels and at all stages of

postnatal development. The following brain related endpoints are assessed for their suitability for further use in the preterm pig model. Functional: Basic motor functions (eye lid opening, first walk/stand), total activity in home cage, balance and coordination assessments, general and specific object exploratory interest and locomotion in an open field arena, longitudinal basic electro-cortical activity by electroencephalography (EEG) and cognition assessed by learning performance in a novel poke-reward test and short term-memory in a novel object recognition test. Gut microbiota analysis using MiSeq-based tag-encoded 16S rRNA gene targeted high throughput amplicon sequencing. Structural: blood brain barrier maturation, concentration and/or localization of neurotransmitters, receptors and trophic factors (e.g. brain derived neurotrophic factor (BDNF) and Sonic Hedgehog. We also apply a qPCR array of 84 neurogenesis-pathway related genes (targeting aspects related to neuronal migration, cell differentiation, synaptic functions, growth factors & cytokines, apoptosis, signal transduction and transcription factors) in selected brain regions in a subset of the piglets, followed by a more targeted approach using a high through put microfluidic q-PCR system on samples from all piglets. Brain weight and structural magnetic resonance imaging (volumetric analyses and diffusivity of large white matter tracts) and stereology for an ontogenetic description of brain development in preterm *vs.* term delivered piglets.

6. Expected results:

We will develop a preterm pig model with a high translational value that will be used in subsequent intervention studies. We expect this work to facilitate advancements in pediatric research within the field by providing a novel diet-sensitive model of brain development and maturation in weak newborns. An estimated 5 publications will result from WP 2.0.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocols	x	x	x																	
Study execution and sample collection		x	x	x		x														
Activity, locomotion, balance			x	x	x	x														
General movement analyses		x	x	x	x	x														
EEG brain analyses		x	x	x	x															
Cognition tests			x	x	x	x	x													
Blood brain barrier		x	x	x	x	x	x	x	x											
qPCR assay					x	x	x	x												
Sonic Hedgehog , BDNF					x	x														
MRI, volumetric, myelination	x		x		x	x		x												
Publication 1						x	x	x												
Publication 2+3						x	x	x	x	x	x	x								
Publication 4								x	x	x	x	x	x							
Publication 5+6										x	x	x	x	x	x					

8. Estimated budget from NEOMUNE: 2.6 mio DKK

Mainly derived from contributions from ARLA Food Ingredients and Danone allocated for 2013 and 2014.
Some co-financing from NEOMUNE base funds are expected (max 1.0 mio DKK)

9. Estimated budget from elsewhere:

2.0 mio DKK (co-financing from numerous collaborating institutions and partners, sample analyses etc.)

10. Additional comments:

- Development of the preterm pig brain model is a high-risk, high-priority NEOMUNE study part. Maximal effort is put into this project during the first 1½-2 years because it forms a foundation for later parts of NEOMUNE (e.g. WPs 2.3, 2.4 and some human studies).
- Refinement of the model continues beyond the initial test phase.

WP 2.1: Germ-free birth in preterm pigs (Cancelled)

1. Related WPs, MG contact person: Synergies with WPs 1.1, 1.2b, 1.5, 2.1-4,3.1. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
The main aim is to determine if delayed microbial colonization after preterm birth, has effects (beneficial or detrimental) on gut-, immunity and brain development.

4. Background and a central hypothesis:
Timing of the first exposure to gut microbes is believed to be important for maturation of gut, immunity and brain. The links between microbiota and gut, brain and immunity may be direct or indirect, but the recent finding that gut microbial colonization affects brain maturation and motoric control in mouse pups supports direct links. It is very important to document these fundamental effects in an animal model that more closely resemble human infants. This effect of early colonization could be particularly important after preterm birth when immunity, gut and brain are vulnerable to insults due to immaturity.
We hypothesize that immunity, gut and brain functions are dependent on timing of microbial colonization after birth (immediate versus delayed).

5. Key analyses and methods:
Preterm pigs are derived by cesarean section. Half of the newborn pigs are kept in conventional incubators while the other half is kept in germ-free isolators for up to 7 days before they are conventionalized. Key analyses include open field test as well as structural, functional, microbiological and immunological parameters of the developing gut and brain. Specific endpoints include immunological parameters (goblet/leucocyte cell counts, tissue gene expression and protein content of cytokines), gut parameters (digestive enzymes, cell cycle, histology, microbiota composition, short chain fatty acids) and brain parameters (morphology, blood brain barrier)

6. Expected results:
We expect to consolidate the notion that the timing of microbial colonization impact on gut and brain development. This project provides important mechanistic information for further clinically-relevant studies. Artificial rearing under germ-free conditions cannot be expected to be of direct translational value. However, germ-free rearing during the initial 7 days after birth enables us to determine whether late colonization is beneficial with regards to clinical and organ functional endpoints. Provided that we can substantiate the beneficial effects of delayed colonization, it creates the scientific rationale for studying how colonization can be delayed/inhibited under more clinically relevant conventional conditions.
Predicted publication: The effects of delayed microbial colonization and early life enteral nutrition on gut and brain development.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol					x	x	x	x	x	x	x	x								
Sample collection																				
Behaviour																				
Microbiota comp. and SCFA																				
Goblet/leucocyte cell count																				
Mucus production																				
Cytokines																				
Histology																				
Blood brain barrier proteins																				
Publication(s)																				

8. Estimated budget from NEOMUNE: 1.8 mio DKK

9. Estimated budget from elsewhere: 0.3 mio DKK
Self-financing (i.e. university funded salaries) and collaboration with SPF Vejen, Denmark (intellectual and practical support from a commercial supplier of germ free pigs).

10. Additional comments:

- Germ free experiments are considered high-risk as contamination of the isolator can occur after even the smallest breach. We collaborate with experienced personnel to minimize this risk. The model may be refined after the first series of experiments, but is unlikely to become a routine experimental animal model. In the event that the experimental periods of earlier WP's within WP2 are extended, WP2.1 will be postponed.
- The protocol may be supplemented also with a diet-intervention, formula versus colostrum under both germ free and conventional conditions.
- Due to expanded contents of studies in WP 2.2-2.4, we have decided to cancel this WP. Funds allocated among other WPs in pigs to increase output from these WPs.

WP 2.2: Anti- and probiotics in preterm and term pigs

1. Related WPs, MG contact: Synergy with WP 1.3a, 1.4b, 1.5, 1.6b, WP3. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
The main aims are: 1) to assess the effect of route of administration of antibiotics (oral vs. systemic), 2) probiotic intervention to preterm neonatal piglets given as a fecal transplant from a healthy donor, 3) antibiotic and probiotic effects in neonatal piglets born at term.

4. Background and a central hypothesis:
Postnatal gut colonization starts immediately after birth and is influenced by environmental bacteria. Probiotics supplementation may be a way to manipulate the early colonization to prevent gut disease. Addition of probiotics to milk feedings has been speculated to promote colonization of beneficial gut bacteria, suppress pathogens and stimulate immune development. However, the current level of evidence limits widespread use of probiotics, especially for vulnerable newborn infants as their potential positive effects are currently not predictable enough. We speculate that the unpredictable effects of probiotics are in part due to the highly variable use of neonatal antibiotics that may hamper the probiotic effect. Also the timing of probiotics inoculation after birth may influence how well they establish and display their beneficial effects. A major factor that influences the gut microbiota is the use of antibiotics. Antibiotics are essential to prevent and treat infections, especially for the weakest newborn infants. Preventive antibiotics treatment around birth may prevent neonatal infections, but the effects on immune, gut and brain maturation are not well known. Both positive and negative effects have been demonstrated. The interaction between antibiotics and probiotics in newborn (preterm and term) infants remains unknown despite its potential high importance for clinical outcome for infants. *We hypothesize that antibiotics will initially benefit the immature immunity, gut, and brain via the reduced bacterial load, and that subsequent probiotics used at the optimal time, dose and strain combination will improve maturation.*

5. Key analyses and methods:
In experiment 1, preterm piglets are derived by cesarean section and reared in incubators. All pigs are provided

with total parenteral nutrition for 2-3 days via the umbilical artery. After this they are gradually transitioned to full enteral nutrition with milk formula. During the entire period we prevent NEC out break by providing antibiotics (either oral or systemic administration).
In experiment 2, we collect colon content from healthy suckling piglets, and supply preterm newborn piglets with daily doses of this material. This fecal transplant is assumed to exert probiotic effects in the newborn intestine. Endpoints include clinical assessment, growth velocity, diarrhea scoring and NEC symptoms. Tissue samples and samples from the gut luminal content are collected and analyzed (proinflammatory cytokines, gene expression, mucosal digestive function, microbial composition, SCFA, metabolomics, antibiotic resistance).
In Experiment 3, antibiotic intervention is studied in term pigs in collaboration with Aarhus University, Foulum, based on experiences from experiment 1.
In Experiment 4, antibiotics and probiotics intervention are studied in term suckling piglets in collaboration with Aarhus University, Foulum. Clinical and laboratory endpoints as mentioned above.

6. Expected results:

We expect to determine how profound manipulation of the gastrointestinal microbiota during early postnatal colonization can impact on gut and brain development. Secondly we expect to determine if probiotics given as fecal transplant influences early clinical and paraclinical parameters.

7. Estimated time frame

Task for exp 1, 2, 3, 4 (see point 5)	2013				2014				2015				2016				2017			
Planning, protocol			1			2				3		4								
Sample collection				1	1		2	2			3	3	4	4						
Clinical/ behaviour				1	1		2	2			3	3	4	4						
Gut tissue analyses						1	1		2	2			3	3	4	4				
Microbial comp						1	1		2	2			3	3	4	4				
Brain endpoints (preterm)						1	1													
Publication(s)								1	1	1	2	2	2		3	3	3	4	4	4

8. Estimated budget from NEOMUNE:

2.5 mio DKK, derived from the KU-SCIENCE NEOMUNE budget (Thomas Thymann).
0.5 mio DKK derived from the AU NEOMUNE budget (Charlotte Lauridsen).

9. Estimated budget from elsewhere:

0.5 mio DKK is expected by self-financing (i.e. university funded salaries) plus industrial co-financing
1.0 mio, post doc stipend for Dr. Lena Martin from German Research council

10. Additional comments:

- This WP relates closely to WP2.1 and WP3 on the experimental side. All these WPs represent profound experimental manipulation of the gut flora just after birth. Relative to WP2.1 (germ free conditions), WP2.2 represents a more clinically relevant model. All the animal studies are meant to rely on clinically relevant situations for infants (e.g. WPs 1.2b, 1.4b, 1.5) and the possible interventions for infants (WPs 1.3a,1.6b).
- It will be a challenge to choose the optimal product(s), timing and doses of both antibiotics and probiotics. Clearly it is impossible to test all clinically-relevant combinations. We will focus on products, timing of administration and doses that are currently used in the neonatal clinics around the world. The results from WP 1.4b (data base work) will help to determine this.

WP 2.3: Enteral and parenteral feeding for preterm pigs

1. Related WPs, MG contact person: Synergies with WPs 1.4,2.1-4,3.1. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
The aim is to identify optimal feeding regimens (enteral and/or parenteral) within the first weeks after birth using the preterm pig model, i.e., when to feed, how much to feed, and which diet? Focus is on the transition to natural, unmodified enteral milk diets, such as bovine colostrum, human donor milk and porcine amniotic fluid. To identify the optimal timing, nature and amount of parenteral nutrition is an associated goal.

4. Background and a central hypothesis:
The first milk after birth promotes intestinal growth, bacterial colonization and immune maturation in weak newborns. Enteral feeding may however predispose to harmful inflammatory lesions, especially using large feeding volumes and suboptimal diets. Thus parenteral nutrition is crucial to promote adequate growth and development before full transition to enteral nutrition can be implemented. For both enteral and parenteral nutrition, there is much debate about the optimal diet composition, time and amount of feeding for preterm infants. In this project we investigate some *intact natural perinatal diets* and how these should be fed in association with parenteral nutrition to promote optimal immunity, gut and brain maturation. Newborn, caesarean-delivered preterm pigs on parenteral nutrition are fed graded doses (minimal enteral nutrition, MEN) during the first one or two weeks after birth. Each of the experimental diets will be compared with a corresponding diet of preterm infant formula, or no feeding at all (total parenteral nutrition, TPN). Separate studies on the nature of parenteral nutrition may be added (particularly related to lipid fractions).
We hypothesize that feeding small amounts of colostrum, human milk or amniotic fluid, as enteral adjuncts to parenteral nutrition within the first week, improve immunity, gut and brain functions in preterm neonates.

5. Key analyses and methods:
Study 1: Preterm pigs are fed total parenteral nutrition or bovine colostrum as minimal enteral nutrition for 5 days and then transitioned to a bovine milk formula for up to 26 days
Study 2: Preterm pigs fed 10-60 mL/kg/d bovine colostrum for 5 days are compared with pigs fed formula.
Study 3: Preterm pigs fed porcine amniotic fluid are compared with pigs not fed any enteral diet for 5 days.
Study 4: Preterm pigs are fed human donor milk, bovine colostrum or formula for 12 days
Study 5: Preterm pigs fed total parenteral nutrition with improved fatty acid composition.

Human milk is obtained from a local donor bank, amniotic fluid from caesarean section on sows, bovine colostrum from the company Biofiber Damino, also delivering product for corresponding infant studies.
Manipulations of the composition of the parenteral nutrition will be done in collaboration with Fresenius Kabi.
Immunity endpoints: Immune cells, genes, proteins (bovine IgG in plasma/feces), inflammation, NEC.
Gut: Histology, digestive enzymes, tight junction proteins, permeability, FISH microbial analyses of tissue and high throughput sequencing fecal analysis, food passage. Brain: Wet weight, BdNF, total activity levels, open field test, activity test.

6. Expected results:

The results will document whether bovine colostrum and human donor milk is better that preterm infant formula in improving growth and development of preterm pigs as models for infants. It will also provide information about apparent safe and efficacious volume of feeding. Finally, the results will help to improve the regimen for parenteral nutrition as a life-saving therapy for preterm infants necessary during the gradual transition from parenteral to enteral nutrition after birth. This information will be important for interpretation of the corresponding infant studies (WP 1.4a, 1.6a). The results will also show preliminary evidence for possible use of amniotic fluid in preterm infants. If beneficial for growth and development, this may lead to pilot studies on feeding human amniotic fluid to preterm infants as part of NEOMUNE.

Some predicted publications:

1) Minimal enteral nutrition with human donor milk or bovine colostrum in preterm pigs
2) Minimal enteral nutrition with amniotic fluid improves growth and development in preterm pigs
3) Parenteral fatty acids influences immunity and brain outcomes in preterm pigs

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning of experiments, protocols	x																			
Study 1 execution	x	x	x	x	x	x														
Study 2 execution	x	x	x																	
Study 3 + 4 execution	x	x	x				x	x	x											
Study 1-4 immunity analyses				x	x	x	x	x												
Study 1-4 gut analyses				x	x	x	x	x												
Study 1-4 brain analyses			x	x	x	x	x	x												
Study 5 execution													x	x	x					
Study 5 analyses															x	x	x			
Publications									x	x	x							x	x	

8. Estimated budget from NEOMUNE:

2.0 mio DKK (costs for pig experiments, analyses, immunity, allocated from Thomas Thymann funds)

9. Estimated budget from elsewhere:

1.8 mio DKK. PhD stipends for Rene Shen and Stine Petersen, post doc salary for Mette Ø
2.0 mio DKK predicted from industry partners (products, direct funds, BioFiber, Fresenius Kabi, Medela).

10. Additional comments:

- The results serve as proof-of-concept for pilot infant studies with bovine colostrum, and to support possible pilot intervention studies with human amniotic fluid for human infants (WP 1.6a). The experiments will also support scientific evidence for use of human donor milk (WP 1.4a and WP1.4b).
- The work related to the composition of parenteral nutrition will be run with the industrial partner Fresenius Kabi and run in collaboration with CNRC Houston (Prof. Burrin), Aarhus University (Ass. Prof. Charlotte Lauridsen), Univ. Hong Kong (Ass. Prof. Jetty Lee) which all have activities on the dietary fatty acid composition on immunity, gut and brain endpoints. Fresenius Kabi has provided 150.000 Euros co-funding for Houston/Copenhagen studies.

WP 2.4: Bioactive formula diets for preterm pigs

1. Related WPs, MG contact person: Synergy with WPs 1.3b, 1.4a, 2.1-2.3. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
Afrouz Abbaspour, PhD stud, Karolinska Institute, afrouz.abbaspour@ki.se (5%)
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Thomas Thymann, Ass. Prof., Comparative Pediatrics and Nutrition, Univ. Copenhagen, ttn@life.ku.dk (20%)
Yanqi Li, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, yli@life.ku.dk (15%)

3. Main aim and sub-aims:
The benefits of breast-feeding may be explained by absorption of milk bioactives present in natural milk. We aim to use the established preterm pig model (WP 2.0), coupled with the experience from WPs 2.1-2.3, to test the effects of selected compounds and milk diets provided by the NEOMUNE partners ARLA FI and Danone. The compounds will be selected based on their potential effects on immunity, gut and brain endpoints.

4. Background and a central hypothesis:
While specific milk components have long been hypothesized to have important immune effects, firm evidence of effects in sensitive newborn infants is not available. Using diet- and microbiota-sensitive preterm pigs, we investigate how milk formulas enriched with selected milk bioactives affect immune, gut and brain maturation. It is not possible to investigate all relevant milk bioactive components in pigs. The choice and number of interventions will rely on further discussions with our partners, as guided by both scientific rationale, the model development phase (as described in WP2.0) and financial constraints. Specifically, for the brain functional and structural endpoints, the exact nature/timing of the functional tests and their structural correlates will be defined in WP2.0 studies (the model development phase).
We hypothesize that feeding milk formula diets containing specific bioactive components will improve immunity, gut and brain maturation, as tested in the preterm piglet model.

5. Key analyses and methods:
Preterm pigs are derived by cesarean section and provided parenteral nutrition and minimal enteral nutrition with either formula or formula enriched with bioactive compounds. Following 5-10 days they are gradually weaned off parenteral support. After transition to full enteral nutrition both groups are bolus-fed up until day 22. On postpartum days 4 and 9, all pigs are subjected to an open field test to document motor skills and explorative behavior. Additionally, cognitive function is assessed in a T-maze system during the last week of the experiment. The extent, to which microbial composition and other endpoints related to gastrointestinal function will be studied, depends on the nature of the selected bioactive compounds. Likewise, the exact choice of endpoints related to brain function will be determined when the model development phase (WP2.0) has been completed.

6. Expected results:
We expect to test the effect of one or two bioactive compounds/diets from each of the partners ARLA FI and Danone. The results will provide the best available evidence for the effects of these selected compounds/diets in a preterm neonatal animal model. We expect to produce three to four scientific manuscripts, one for each of

the tested compounds.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol							x	x												
Sample collection									x	x	x	x								
Open field test									x	x	x	x								
Cognitive test									x	x	x	x								
Clinical assessment									x	x	x	x								
Blood brain barrier													x	x	x	x				
Brain and gut histology													x	x	x	x				
Gut microbiota													x	x	x	x				
SCFA													x	x	x	x				
Cytokines													x	x	x	x				
Gut digestive function													x	x	x	x				
Publication(s)																	x	x	x	x

8. Estimated budget from NEOMUNE: 3.2 mio DKK

- The budget for WP2.4 is mainly derived from our industrial partners (ARLA FI and Danone)
- Remaining parts will be derived from the DSF funds of NEOMUNE (Thomas Thymann).

9. Estimated budget from elsewhere:

0.6 mio DKK is supplied as university self-financing, 1.7 mio DKK from industrial sources (e.g. ARLA Foods amba), 1.0 mio DKK from personnel and product resources supplied by our industrial partners. ARLA Foods Ingredients have provided 1.5 mio DKK extra (2015) for additional WPC study within NEOMUNE.

10. Additional comments:

- The exact choice of endpoints in WP 2.4 will have to await the results of the model development phase in WP 2.0. The industrial partners will be given priority for further use of the preterm pig brain model beyond the described project phase, according to separate contracts.
- While brain endpoints are the main priority in this WP, also gut and immunity endpoints will be recorded if this is relevant and economically feasible. Contribution from ARLA Foods FI will in part support these analyses for interventions of interest for this company.
- This WP may expand over the course of NEOMUNE as new ingredients/products become relevant for tests from new/existing university/industrial partners.

WP 3.1: Antibiotics in newborn mice

1. Related WPs, MG contact: Synergies with WPs 1.1,1.2b,1.4b,1.5,2.2,3.2. MG contact: Hanne Frøkiær

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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Thomas Thymann, Ass. Prof., Comparative Pediatrics and Nutrition, Univ. Copenhagen, ttn@life.ku.dk (5%)

3. Main aim and sub-aims:
To obtain a thorough understanding of the influence of the microbiota on early events in immune development of importance for establishment of long term immunity. This will be done by treatment of different antibiotics (e.g. Ampicillin, Gentamycin, Metronidazol and to a less extent Vancomycin) and at different perinatal periods. The studies will provide key basic knowledge to support and guide the pig and human infant studies.
Sub-aims are:
a) To evaluate variations in early hematopoietic events after antibiotics-induced variation in gut microbiota.
b) To evaluate how variations in early hematopoietic events affect the establishment of adaptive immunity.
c) To establish an influenza challenge model to test long term immunity effects of perinatal antibiotics.
d) Evaluate how variations in early hematopoietic events affect propensity to infectious diseases later in life.
e) To identify key genes/molecules/cells that can be used as markers in pig and human infant studies.
f) To evaluate how variations in early hematopoietic events affect specific parameters in brain development.

4. Background and a central hypothesis:
We and others have previously demonstrated that the microbiota plays a key role in establishment of early hematopoietic events, i.e. differentiation of HSC into myeloid derived suppressor cells and other neutrophil-like cells, of importance for development of adaptive immunity and accordingly may influence long-term immunity, e.g. resistance towards viral infections and development of autoimmune diseases. In addition, a diverse microbiota accelerates the development/maturation of the gut epithelium and the kinetics of the epithelial maturation may be determined by the composition of the gut microbiota postpartum, which in turn may be a key determinant for development of mucosal and systemic immunity. Despite many studies of the effects of antibiotic treatment in early life, the effects on the very early events in immunological maturation have not been addressed.
We hypothesize that peri-/postnatal treatment with antibiotics will decrease the diversity of the microbiota and this will impact the early hematopoiesis and, accordingly, maturation of the immune system causing a weak or skewed immune system with less resistance towards infections and higher risk of autoimmune disease development.

5. Key analyses and methods:
Perinatal antibiotics treatment of dams and offspring mice: To eradicate major populations of the gut microbiota, dams are treated with e.g. vancomycin, gentamycin or ampicillin during gestation until few days after birth or treatment is initiated few days postpartum.
Flow cytometry: to investigate the proportion and composition of CD11b+ cells in spleen during the first weeks of life.
Microscopy: to assess the efflux of differentiating HSC from liver and influx of cells to spleen and other organs.
RT-PCR: to assess effects of microbiota on maturation of gut epithelium and cell migration in liver and spleen

(up- and down regulation of chemokines, specific enzyme markers such as arginase, elastase).
16s sequencing and RT-PCR of gut contents.
ELISA: e.g. cytokine/chemokine measurement of ex vivo stimulated spleen cells to assess the responsiveness to microbial stimuli.
Western blotting: e.g. assessment of enzyme (elastase, myloid peroxidase) production in cells.
Optical Projection Tomography Scanning: to assess localization of HSC in liver, spleen and gut and to identify cells in different organs expressing specific proteins, e.g. Cxcl2R.

6. Expected results:
a) Establishment of mechanisms involved in the early maturation of the mucosal and systemic immunity.
b) Demonstration of a link between perinatal events involving the microbiota and early hemapoietic events and resistance towards e.g. viral infection later in life (microbial resistance?).
c) Establishment of an influenza challenge mouse model.
d) Identification of key markers of early life immune maturation (e.g. specific cell populations in blood, +/- expression of certain genes, e.g. in the brain.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol			x	x																
Sample collection				x	x	x	x	x	x	x	x									
Flow cytometry of spleenocytes				x	x	x	x	x	x	x										
OPT of spleen, liver, gut, brain					x	x	x	x	x	x										
16s sequensing/rtPCR gut microbiota						x	x	x	x	x										
rtPCR, microscopy, ELISA etc					x	x	x	x	x	x	x	x								
Publication work(a)												x	x							
Publication work (b+c)											x	x	x							
Publication work (d)												x	x	x						

8. Estimated budget from NEOMUNE: 3.5 mil DKK
Running costs, phd student, lab/animal tech.

9. Estimated budget from elsewhere: 1.5 mill DKK
Salaries Hanne Frøkiær, Axel Kornerup, Stine Metzdorff, Dina Malling, Dennis Nielsen, equipment.

10. Additional comments:

- This work may provide important knowledge to support pig studies, particularly for longer time-frame outcomes that the pig model may not be able to focus on, and might also lead to insight of value for preterm infant treatment/feeding.

WP 3.2: Probiotics in newborn mice

1. Related WPs, MG contact: Synergy with WPs 1.3a, 1.4b, 1.5, 1.6b, 2.2, 3.1. MG contact: Hanne Frøkiær

2. Key involved personnel, their institution,mail address (project leader + main study site underlined):
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Gorm Greisen, Prof., Neonatalogy, Copenhagen Univ. Hospital, Gorm.Greisen@regionh.dk (5%)

3. Main aim and sub-aims:
We aim to establish whether administration of probiotic bacteria peri- and postnatally will accelerate maturation of the gut epithelium and affect early hemapoiesis.
Sub-aims are:
a) To identify relevant probiotic bacteria or mixtures hereof.
b) To test whether administration of probiotics may compensate for effects of antibiotics treatment on development of the immune system.

4. Background and a central hypothesis:
If perinatal antibiotic treatment of mice as anticipated shows to compromise immune development due to a decrease in the diversity of the microbiota and/or the absence of specific genera, one way to compensate may be administration of one or more probiotic strains during antibiotics treatment.
We hypothesize that administration of probiotic bacteria from birth, especially concomitant with antibiotic treatment may lead to a faster maturation of GI epithelium and well-balanced maturation of immunity.

5. Key analyses and methods:
Perinatal antibiotics and probiotics treatment of dams and offspring mice.
To eradicate major populations of the gut microbiota, dams are treated with an antibiotics, identified in WP3.1 as a potent manipulator of immune maturation, perinatally, and probiotics are administered concomitantly.
Flow cytometry: to investigate the proportion and composition of CD11b+ cells in spleen during the first weeks of life.
Microscopy: to assess the efflux of differentiating HSC.from liver and influx of cells to spleen and other organs.
RT-PCR: assess effects of microbiota on maturation of gut epithelium and cell migration in liver and spleen (up- and down regulation of chemokines, specific enzyme markers such as arginase, elastase).
16s sequencing and RT-PCR of gut contents
ELISA: e.g. cytokine/chemokine measurement of ex vivo stimulated spleen cells to assess the responsiveness to microbial stumuli.
Western blotting: e.g. assessment of enzyme (elastase, myloid peroxidase) production in cells.
Optical Projection Tomography Scanning: to assess localization of HSC in liver, spleen and gut and to identify cells in different organs expressing specific proteins, e.g. Cxcl2R.

6. Expected results:
To establishment if probiotic administration early in life improves immune development in antibiotic treated mice pups.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol													x											
Sample collection														x	x									
Flow cytometry														x	x									
OPT-scanning														x	x									
16s seq/rtPCR															x	x								
rtPCR, microscopy, etc															x	x								
Publication work																		x	x					

8. Estimated budget from NEOMUNE:

1.2 mio DKK

9. Estimated budget from elsewhere:

0.4 mio DKK. Salaries and equipment.

10. Additional comments:

- These activities will be based on results from WP 3.1. Therefore, protocols and planning are initiated late in project.

WP 4.1: NEOMUNE administration and dissemination

1. Related WPs, MG contact person: Synergies with WPs 4 and WP 1.7. MG contact: Stine Bering, Per Sangild

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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Yanqi Li, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, yli@life.ku.dk (15%, AG)

GB = Governing Board; HP = Head of Project; MG = Management Group; AG = Administrative group; SAB = Scientific Advisory Board

3. Main aim and sub-aims:
Main aim: To develop effective management and leadership of NEOMUNE over the 6-year project period.
a) To maintain effective and open leadership via the Management Group (MG) and the Head of Project (HP).
b) To obtain maximal inspiration and guidance from the Governing Board (GB) – the strategic advisory board.
c) To obtain maximal inspiration and guidance from the Scientific advisory Board (SAB).
d) To secure good working collaboration with associated industrial partners.
e) To secure the framework for relationships, in part by appropriate scientific, legal and ethical contracts.
f) To secure optimal and timely scientific reporting to the Danish Research Councils.
g) To secure optimal and timely financial reporting to the Danish Research Councils.
h) To secure adequate communication to NEOMUNE participants and to the public (e.g. NEOMUNE website).
i) To solve unforeseen scientific and/or managerial conflicts and challenges in NEOMUNE.
j) To apply for funding that aims to expand the present activities and extend NEOMUNE beyond 2018.

4. Background and a central hypothesis:
The scientific and administrative leadership in NEOMUNE is divided among partners, affiliated partners and collaborators, as indicated in the original application. While NEOMUNE may provide the main funding for some projects, for others it only constitutes a small part of the total project expenses. NEOMUNE is a dynamic research platform that incorporates and integrates with new synergistic projects as these become apparent. The NEOMUNE research platform aims to expand the total number of interventions, sample analyses and research outputs from studies that are not entirely financed by NEOMUNE.

The NEOMUNE Management Group (MG) is the main leadership group and members represent the main work areas from infants (GG, VC), pigs (TT), and mice (HF), to the milk and microbiota analyses (DN). The HP (PS) is supported by academic administrators (SB, MC, YL) forming the administrative group (AG) to secure daily administrative matters incl. finances. A Governing Board (GB) is formed to enable to the greatest possible long-term, strategic impact of the project ("strategic advisory board"). The GB will give advice on major strategic decisions regarding a) project leadership and partner structure, b) long-term financial considerations, c) ethical and legal challenges, d) infrastructure, and e) legal contracts among universities, hospitals, and industries. A Scientific Advisory Board (SAB) is formed to provide critical inputs into the scientific quality of NEOMUNE at all work package levels. Based on an annual progress report by the MG, the GB and SAB are invited to give critical comments to the research at all levels (rationale, protocols, analyses, interpretation, publication, societal effect, and leadership structure).

Leadership philosophy in NEOMUNE: To maintain creativity, enthusiasm and results orientation, research groups are given the freedom to work within the given framework defined by NEOMUNE and the MG. The projects that are financed to the largest degree by NEOMUNE have the greatest responsibility to adhere closely to NEOMUNE strategies. In close connection with the GB, SAB and MG, the HP will assist in adapting research plans to obtain the best possible NEOMUNE outcome. We are aware of both potentials and limitations of working closely with industry partners in NEOMUNE. The working relationship is guided by separate Part Project Agreements with each industrial partner on specific parts of the project. Just as important, it is based on respect for the complementary values of university, hospital and industry partners.

We hypothesize that pro-active management and leadership, good communication and adequate trust-building among university, hospital and industry partners in NEOMUNE lead to lasting research and societal outcome.

5. Key analyses and methods:

a) Frequent meetings within the MG, with agendas published at the NEOMUNE website and minutes published at a closed collaborator website, and direct information flow to cluster leaders for dissemination at cluster group meetings.
b) Contact with the SAB and GB at least once annually.
c) Formation of legal contracts among partners.
d) Initiate the annual scientific meetings.
e) Scientific reports to the Danish Research Councils.
f) Financial reports to the Danish Research Councils.
g) Maintenance of the NEOMUNE website.

6. Expected results:

a) High research output and societal implications of NEOMUNE.
b) A reasonable degree of satisfaction among NEOMUNE participants, collaborators and institutions.
c) A long term impact of the collaborations in NEOMUNE, beyond the project period.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
MG meetings	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MG meeting with GB		x	x	x		x				x				x				x				x		
SAB contact		x				x				x				x				x				x		
AG meetings				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Scientific report to DSF						x				x				x				x				x		
Financial report to DSF						x				x				x				x				x		
Platform contract sign		x																						
Sub-project contracts			x	x	x	x	x	x																

8. Estimated budget from NEOMUNE: 3.0 mio DKK

20-30% salaries for academic administrator, administrative post doc, secretary and HP.

9. Estimated budget from elsewhere: None

10. Additional comments:
- The total budget for WP 4.1 should be seen in connection with the budgets for WPs 4.2+4.3

WP 4.2: Scientific meetings and results dissemination

1. Related WPs, MG contact person: Synergies with WPs 4. MG contact: Stine Bering

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
Dennis Nielsen, Ass. Prof., Dept. Food Science, Univ. Copenhagen, dn@life.ku.dk (10%, MG)
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Hanne Frøkiær, Prof., Veterinary Disease Biology, KU-SUND, Univ. Copenhagen, hafr@sund.ku.dk (10%, MG)
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Vibeke Brix Christensen, MD, Copenhagen University Hospital, brixchr@dadlnet.dk (10%, MG)
Yanqi Li, post doc, Clinical and Experimental Nutrition, Univ. Copenhagen, yli@life.ku.dk (15%)

3. Main aim and sub-aims:
a) To secure effective exchange of scientific ideas within the NEOMUNE fields and help steer the subprojects.
b) To secure effective results dissemination (scientific and/or societal) from NEOMUNE projects, press contact.
c) To maximize synergy among individuals, groups and institutions/companies/hospitals within NEOMUNE.

4. Background and a central hypothesis:
NEOMUNE will seek international exposure and impact by hosting a common meeting for central partners and stake holders once per year. Most often this will be held as a satellite meeting to the annual congress of one of two relevant European scientific societies (ESPR or ESPGHAN). In addition to this, a larger NEOMUNE scientific platform meeting will be arranged by the MG to present all active NEOMUNE research activities and results. This will involve all partners, collaborators, research leaders, young investigators and representatives of GB and SAB, aimed to evaluate the overall research. All university-, hospital- and industry-based key personnel, and the SAB, are expected to join at least one main platform meeting per year.
In addition to the general scientific assemblies, sub-meetings are held for clusters of projects (NEOMUNE subgroups, see section 7 below)) within NEOMUNE. The MG takes the initiative to formation of such clusters and each group is responsible for reporting back and forth to the MG on both scientific and administrative issues. Furthermore, these clusters are responsible for a newsletter update every three months to involved partners.
Scientific results, both those that confirm or reject NEOMUNE hypotheses, will published in international, recognized journals with relevant review competence and readership. Where relevant, we will aim to co-publish results on the same intervention from different study levels of evidence (infants, pigs, rats, isolated cells). We also seek to co-publish natural and social science results in the same scientific (review) papers. The NEOMUNE website will play a central role for results presentation, using public open access, and web sections closed to the public.
We hypothesize that a minimum two personal meeting encounters for key researchers, and one meeting encounter for the other researchers, are necessary for NEOMUNE project coherence and research output.
Subgroups of project clusters are encouraged to arrange separate meetings as required and report to the MG.

5. Key analyses and methods:
a) Effective platform meeting organization initiated by the MG.
b) Effective cluster meeting organization initiated by cluster leaders referring to MG.

c) Evaluation of meetings by the MG and the cluster leaders.
d) Appropriate and adequate use of the NEOMUNE website.
e) Coordination, management, and evaluation of public press related to NEOMUNE.

6. Expected results:
Effective and enjoyable meetings that maximize time and money resources to increase research output. Adequate and effective communication of NEOMUNE results to the scientific community and to the public.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Congress related meetings		x				x	x			x				x				x				x		
Scientific platform meeting			x	x		x		x			x				x				x				x	
*Sub-group meetings:																								
Infant probiotics (GG)		x		x		x		x		x		x		x		x		x		x		x		x
Infant birth, AB, GM (DN)		x		x		x		x		x		x		x		x		x		x		x		x
Pig +Mice AB/PB/diet (TT,HF)		x		x		x		x		x		x		x		x		x		x		x		x
Piglet gut-brain (AA)		x		x		x		x		x		x		x		x		x		x		x		x

* The indicated frequency of sub-group meetings is shown only as a guideline for expected meeting intervals

8. Estimated budget from NEOMUNE:
0.5 mio DKK. Mainly to cover platform meeting expenses and invitation to GB and SAB members.

9. Estimated budget from elsewhere:
0.5 mio DKK. Local administrative support not funded by NEOMUNE.

10. Additional comments:

- The NEOMUNE research platform consists of a large, diverse number of core participants from hospitals, universities and industry. As such, it cannot be expected that the platform meetings will be able to cover all aspects of the research. Focus is placed on forming scientific cluster groups in NEOMUNE, and corresponding cluster meetings. Clusters are dynamic and are meant to connect research areas that are relatively closely connected in scientific methodology and research questions.

WP 4.3: Training and education

1. Related WPs, MG contact person: Synergies with WP4.1, 4.2. MG contact: Per Sangild, Gorm Greisen

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
Elin Skytte, personal coach, Comp. Pediatrics and Nutrition, Univ. Copenhagen, els@life.ku.dk (5%)
Gorm Greisen, Prof., Neonatology, Copenhagen University Hospital, Gorm.Greisen@regionh.dk (5%, MG)
Per Sangild, Prof., Comp. Pediatrics and Nutrition, Univ. Copenhagen, psa@life.ku.dk (5%, MG)
Stine Bering, Comp. Pediatrics and Nutrition, Univ. Copenhagen, sbs@life.ku.dk (5%,)
Yanqi Li, post doc, Clinical and Experimental Nutrition, Univ. Copenhagen, yli@life.ku.dk (15%)
Zhu Yanna, Ass. Prof., School of Public Health, Sun Yat-sen University (5%)

3. Main aim and sub-aims:
a) To provide appropriate scientific training for young researchers (PhDs, post docs) in NEOMUNE.
b) To provide appropriate leadership training for young researchers (PhDs, post docs) in NEOMUNE.
c) To provide possibility for personal counseling and career support for all researchers in NEOMUNE.

4. Background and a central hypothesis:
NEOMUNE facilitates training for many young investigators across wide scientific disciplines, and in new collaborative networks that hopefully exceed the NEOMUNE project period. NEOMUNE makes deliberate attempts to place younger researchers, with limited training in leadership positions where they develop leadership skills (e.g. as project leaders, or as part of MG and NEOMUNE subgroups). Careful supervision is required and/or personal counseling is the natural responsibility of a well-functioning research team.
Besides the PhD students specifically sponsored by the NEOMUNE funds, the project will create training possibilities for a number of other PhD students and young researchers. To obtain and maintain enthusiasm for biological research, it is important that young scientists are able to put their own scientific method and understanding into a greater methodological and scientific perspective. Working with scientists from other fields (even across the domains of natural, social and human science) and across countries, make it clear that the basis of scientific epistemology differ widely. Alone, or with other university partners, NEOMUNE will seek to establish short term courses on career development and personal integrity in work life, especially directed towards younger research staff. PhD students will be encouraged to participate in the 6 ECTS PhD course in "Food, Medicine and Philosophy in East and West" (http://www.courseinfo.life.ku.dk/ Kurser/phd_fmp.aspx; lead by PS) and in a 2 ECTS course in Pediatric Research (lead by GG). These courses will train students to know the potentials and limitations of research methods directed towards infants and children. The courses will seek to place maximal focus on aspects that are relevant for NEOMUNE.
We hypothesise that careful attention to the widely differing career and personal goals of younger researchers, and the need for international work exchange, will enable NEOMUNE to foster mature researchers and good leaders with personal integrity.

5. Key analyses and methods:
a) A PhD course in Pediatric Research will be offered to PhD students each year of the project.
b) A PhD course in Food, Medicine and Philosophy in East and West is offered every other year.
c) The younger investigators will gather separately in young investigator subgroups.
d) Younger investigators will be offered day-course in work methods and career development.
e) A large attempt will be made to exchange researchers among different parts of NEOMUNE, from Asia to Europe and US, from basic science to clinical practice, from academia to industry.

6. Expected results:

- Development of senior researchers that become not only good researcher but also good research leaders
- Development of senior researchers that communicate well across scientific fields and work cultures
- Development of senior researchers that have good of integrity in work life, and good work-private balance
- Increased overall productivity of the work and international impact

7. Estimated time frame and plans for international personnel exchange

Task	2013				2014				2015				2016				2017				2018			
Pediatr. Res. course	x				x				x				x				x				x			
FMP PhD course				x								x								x				
China personnel to DK	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DK personnel to China	x	x		x				x				x				x				x				
Europe personnel to DK						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DK personnel to Europe			x				x				x				x				x				x	
US personnel to DK		x				x				x				x				x				x		
DK personnel to US				x				x				x				x				x				x

8. Estimated budget from NEOMUNE:

0.25 mio DKK. Specific exchange programs, even if directly related to NEOMUNE, will have to seek additional funding

9. Estimated budget from elsewhere:

0.5 mio DKK. Funding sought from external sources and internal university co-funding. Until now the educational program has received funding from C Van Foundation (Daloon) to facilitate student exchange (0.1 mio DKK), USA Pediatric Research Foundation (0.05 mio DKK), Carlsberg Foundation (0.5 mio DKK PhD course in China) and ARLA Foods amba (1.0 mio DKK for PhD course in China)

10. Additional comments:

- Basic science researchers in NEOMUNE are encouraged to become familiar with clinical routines at hospital partners in NEOMUNE.
- Clinical staff related to NEOMUNE hospital departments are encouraged to become familiar with the scientific routines of basic science partners in NEOMUNE.
- The exchange of researchers among different NEOMUNE labs constitutes an additional important training activity. Specific university-university collaboration agreements will be sought between specific partners (e.g. Univ. Copenhagen, Sun Yat-sen University).
- NEOMUNE researchers are encouraged to take part in academic teaching activities when this is NEOMUNE-relevant and possible within the set time as defined by the research plan.

NEOMUNE research platform – work package synopses

Guidelines to synopses:
The purpose of each synopsis (maximum 2 pages) is to give internal participants and the public a brief overview of the contents in each subproject in the NEOMUNE research platform. In addition, the purpose is to identify relevant synergies among different subprojects. The synopses are dynamic and will be edited annually, as new research potentials and unforeseen challenges develop. As they serve as more detailed project plans, each synopsis has one or more supporting documents (e.g. research protocols, ethical approval protocols, PhD study plans, analytical protocols, NEOMUNE Part Project Agreements etc.). The work package coordinator is the responsible person for these supporting documents. The synopses contain only non-confidential information and will be available to the public and other NEOMUNE participants via the NEOMUNE website.

1. A brief title to show the intervention area and the species (infants, pigs, mice, other), and should as far as possible follow the structure of the original NEOMUNE application. Indicate which other WPs this sub-project acts to support and relate to. Indicate the person in the NEOMUNE Management Group (MG) that is your contact person. 1-2 lines.

2. List key academic personnel (alphabetically by first name) involved in project supervision, execution, analyses and publication (name, title, institution, mail address). Indicate the approximate time allocation from each participant over the project period (proportion of their full-time work allocated to the project - given in parenthesis). This shows the involvement of each listed participant from a minor supervisory/consultancy role (e.g. 5-10%), to major work allocation of that person´s work time to that work package (e.g. 30-100%). 3-10 lines.

3. What are the main aim and the sub-aims? Which question(s) shall the sub-project answer? Be specific and focused, and give preferably aims that are measurable. 1-5 lines.

4. Brief scientific background, ending with a central scientific (biological) hypothesis that forms the basis for this sub-project. 5-8 lines.

5. Key (biological) endpoints and analytical methods used to obtain results (including use of specific laboratory equipment if necessary). 5-8 lines.

6. Expected results from the work package project. If possible, include the working title(s) for predicted publications from this subproject. 10-25 lines.

7. Estimate the time frame (put “x” into boxes) for execution of different parts of the sub-project (e.g. planning, sample collection, various analytical tasks, and publication). Be specific by editing the row text to suit the specific task. There may be more time periods for the same task. Add additional lines to the diagram if necessary. 7-15 lines.

8. The NEOMUNE funds pre-allocated to this sub-project (excl. overheads), as indicated in the application, or estimated from negotiations among the specific participant(s) and the NEOMUNE head of project or Management Group. 1 line.

9. A crude estimate of required additional co-funds (expenses not covered by NEOMUNE/ NEOMUNE partners). This estimate arises from predicted total use of personnel, analyses and publication to complete the sub-project. This is especially informative for subprojects for which NEOMUNE only cover a (small) part of the projects costs. 2-5 lines.

10. Indicate any special background or work conditions for the described work package sub-project, relevant to know for other NEOMUNE participants, the public, or the NEOMUNE Management Group, Governing Board (Strategic Advisory Board) or Scientific Advisory Board. Identify special work conditions, particular uncertainties or specific potentials for future development of this subproject. 5-10 lines.