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 a big 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 3000 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														
Analyses 1			x				x																	
Analyses 2			x				x																	
Analyses 3											x													
Analyses 4												x												
Publication(s)										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.

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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NN, midwife and clinical pharmacologist, Odense University Hospital, xxx@kkk.dk (18%)

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 (6 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 autumn/winter of 2013/2014. 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. 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 Aarhus University Hospital.
b) Total fecal DNA will be extracted and gut bacterial composition determined by tagged 16S rRNA gene targeted Illumina (MiSeq) based sequencing.
c) Presence and prevalence of key antibiotic resistance genes in total fecal DNA will determined using targeted qPCR.

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 (6 months).

Expected publications:

1) Very early life exposure to cefuroxim influences infant gut microbiota colonization.
2) Very early life exposure to cefuroxim increases gut microbiota prevalence of antibiotic resistance gene markers.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol		x	x																					
Sample collection				x	x																			
Pharmacokinetics				x	x																			
GM composition				x	x																			
Antibiotic resistance gene markers					x	x																		
Publication 1						x	x																	
Publication 2							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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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														
Part b) execution, piglet studies	x	x	x	x																
Part a) lab analyses							x	x	x											
Part b) lab analyses				x	x	x														
Part a) publication phase									x	x	x									
Part b) publication phase						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). This agreement, the partner institutions and the scientific goals and endpoints share overlaps with WP 1.6a.