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PROGRAMME CONFERENCE IN THE STRATEGIC AREAS

Infection Biology
Clinical Biomarkers

Abstracts of lectures and posters

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Contents

The abstracts are arranged in the order as the projects are presented at the conference (except for the KeyNote lectures which are placed first here), with all abstracts for the oral presentations first, and then all abstracts for the posters

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ABSTRACTS - ORAL PRESENTATIONS

KEYNOTE:

Translational Infection Biology: Cholera – from basic research to licensed vaccines

Jan Holmgren, University of Gothenburg

The theme of this talk is similar to that of session 6 *"Commercialisation without giving up research"* or perhaps more adequately *"From basic research to medical products"*.

I will briefly describe my experiences from several projects where our early basic research has prompted us to collaborate with industry towards development of such diverse commercial products as (a) vaccines for infectious diseases, (b) biomarkers for cancer, and (c) anti-inflammatory immune therapies for e.g. diabetes and atherosclerosis. In some instances these efforts have resulted in commercialized international products and in other cases not or not yet. We have been working with both small-size biotech companies and with "big pharma" and I will discuss some "pros and cons" experiences of each type of interaction.

My main focus will be on how our early basic research on the mechanisms of disease and immunity in cholera was translationally developed into the internationally widely licensed oral cholera vaccine Dukoral, and how this in turn has paved the way for efforts to develop related mucosal vaccines. One example of the latter is the development led by A-M Svennerholm in collaboration with a Swedish industrial partner (Scandinavian Biopharma) of an oral vaccine against diarrhea caused by enterotoxigenic E. coli (ETEC) and being partly pursued within the SSF Infection Biology program (see session 4). Another example, also being part of our SSF project, is the development, in collaboration with partners in India and Bangladesh, of 2nd generation oral cholera vaccines - "Hillchol" and "Hillchol-B" - with good prospects of being licensed in 2018 and 2021, respectively. Further details about the latter project is provided below.

The Hillchol and Hillchol-B oral cholera vaccines. *There are currently three WHO prequalified vaccines for cholera in the market: Dukoral ® based on inactivated whole bacteria plus recombinantly produced cholera toxin B subunit (rCTB) and two whole-cell only vaccines, Shanchol® and recently Euvichol®, based on the same strains and procedures as Dukoral. All three vaccines are complex to manufacture due to multiple strain composition and two different methods of inactivation, which adds to overall cost of vaccination. To overcome this barrier, we (Michael Lebens, Stefan Karlsson and JH) could recently by genetic engineering generate a novel Vibrio cholerae vaccine strain (MS1568) which stably expresses approximately 50% each of Ogawa and Inaba LPS and using a single inactivation method prepare a single-strain so-called Hikojima serotype vaccine with equivalent preclinical oral immunogenicity and efficacy against different V. cholerae O1 serotypes and biotypes as the WHO prequalified OCVs [Karlsson S et al. PLoS One 2014]. The Hilleman Laboratories in India in collaboration with us, Incepta Vaccines Ltd and icddr,b have now produced the Hikojima MS1568 vaccine at cGMP industrial scale for clinical testing. Phase 1 studies have shown excellent safety and immune responses and age-descending phase 2 studies likely to result in licensure in Bangladesh are in progress. The optimized strategy, process improvement, and collaboration of credible institutions give promise of a soon available affordable, easy to manufacture and best-in-class novel OCV, which can markedly increase global vaccine access for use especially in the control of endemic cholera. Work is also underway between us and Hilleman Labs to develop a further improved Hillchol-B vaccine, initially for use primarily in cholera outbreaks and in travelers, in which the whole-cell Hikojima vaccine is being combined with inexpensively produced rCTB in a heat-stable enterocoated tablet or capsule formulation. The final formulation will be decided later in 2018 for clinical studies aiming at licensure initiated in 2019.*

KEYNOTE:
Biomarkers - Versatile Tools in Clinical Research - from bench to clinical applications

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Blood coagulation is the essential process to prevent excessive blood loss upon an injury through fibrin formation (hemostasis) but can also contribute to occlusion of vessels (thrombosis). In addition, thrombus formation is not the only consequence of coagulation activation since components of the coagulation system also acts on cells and tissues to promote cellular activation and inflammatory responses. Excessive activation of the coagulation process is involved in the pathogenesis of several of our common diseases. The molecular mechanisms behind these observations are still elusive. Tissue factor (TF) and coagulation factor VIIa form a proteolytically active complex, which functions as the main trigger of blood coagulation and also activates cell signaling. My research is focused on TF expression and signaling pathways and their relation to biological functions as well as to the clinical relevance in cardiovascular diseases (CVD) and cancer. In these diseases TF constitutes a prognotic new biomarker.
The acute cardiovascular event and stroke following thrombus formation are primary causes of morbidity and mortality of patients with CVD and atrial fibrillation. In these diseases biomarkers have the potential to further the development towards the goal of personalized medical treatments. In large global populations of patients with CVD and AF we have taken a multimarker approach in terms of risk stratification and treatments and to identify disease mechanisms and potential therapeutic targets. Novel results of some of these biomarkers will be discussed.

BIOBANK-BASED EVALUATION AND IMPLEMENTATION OF NEW CERVICAL SCREENING TESTS

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Grant: Biomarkers 2013 RB13-0011

The aim of the project is to establish whether any new biomarker, or combinations of such, are superior to the currently used best practice in cervical screening which is molecular virus testing (human papillomavirus (HPV)-test) with secondary screening of HPV-positive women using cytology triage. Evaluation of new biomarkers will be done through exploitation of the Swedish register and biobank infrastructure. A state-of-the-science biobank, collected within a population-based cervical screening program, will be followed for clinical outcomes and original screening test results through linkages with comprehensive registries. This study base will be used to evaluate both commercially available biomarkers, and biomarkers under clinical development. In addition, we will perform a comprehensive new analysis of genetic markers, RNA transcription and key regulatory proteins. We will compare whether the omics-derived biomarkers contribute any additional predictive ability over and above the performance of the biomarkers that are on, or close to, market. The identification of study cases and controls through register linkages and retrieval of corresponding samples has been completed. The first biomarker to be evaluated is methylation of HPV and human DNA. There are commercial tests using methylation patterns to predict CIN3/carcinoma in situ or invasive cervical cancer risk and a substantial body of literature supporting that methylation patterns will be a useful biomarker likely to become used in the screening program. Illumina has recently developed a DNA methylation array that simultaneously analyses 840000 CpGs that will be tested. Other biomarkers under consideration include whole genome sequencing, HPV-DNA genotypes (commercial tests), HPV variants and subtypes, mRNA profiles (Transcriptomics) and protein expression patterns, oncogenic mutations and HLA exome sequencing in human DNA. In conclusion the long-term goal of the project is to tailor screening intensity to each individual according to her biomarker-determined risk of disease – thereby improving public health as well as furthering Swedish competiveness in biomarker research.

Host-virus interaction in development of cervical cancer

Ulf Gyllensten, Matts Olovsson and Erik Wilander, Uppsala University

Cervical cancer is the third most common cancer in women worldwide and is caused by infection by oncogenic types of human papillomavirus (HPV). Only some of the infections lead to cancer, indicating that viral and host factors interact in determining the outcome of an infection. We have formed a translational research group including preclinical and clinical scientists that are working together to: a) develop a new diagnostic tool for predicting the malignant potential of a HPV infection, using the sequence profile of the infecting HPV type and the genetic profile of host genes, and b) determine the ability of this diagnostic tool to identify women with prestages of cervical cancer by performing a randomised study using self-sampling at home for collection of samples. The present screening system for cervical cancer in Sweden costs over 400 MSEK and has reduced the cancer burden by 50%, but further reduction is not possible without a different strategy and diagnostic test. Vaccination has been introduced in young girls but is only efficient in those previously not exposed to HPV. It is necessary to develop a new diagnostic test and increase the coverage of the screening to achieve a further reduction of the cervical cancer incidence. Modelling of the health-economic benefits of self-sampling and a new diagnostic test for malignant potential, shows that this will result in savings in the order of 200 MSEK per year, excluding a better quality of life for individual women.

(Abstract from original proposal in absence of an updated one)

Predicting and monitoring aggressive prostate cancer

Pernilla Wikström[1], Marene Landström[1], Anders Widmark[2], Jan-Erik Damber[3] and co-workers. [1]Department of Medical biosciences, Umeå University. [2]Department of Radiation sciences, Umeå University. [3]Department of Urology, University of Gothenburg.

Background: Prostate cancer (PC) is the most common cancer in Swedish men and also the most common reason to cancer-related deaths in Sweden today. Diagnosis of PC builds upon detection of prostate specific antigen (PSA) in serum followed by histological examination of prostate biopsies. Prognosis is judged from tumor grade, stage, and the PSA level. Patients with low to intermediate risk PC are treated by surgery or radiation with intentions to cure. Patients with advanced PC are treated with androgen deprivation therapy that with time leads to castration-resistance and incurable disease. Diagnostic and prognostic tools used today are, however, unsatisfactory resulting in over-treatment of patients with indolent tumors, while patients with aggressive PC are inadequately treated. A battery of drugs is available for treatment of patients with castration-resistant PC (CRPC), but no biomarkers are used for therapeutic choice or to complement PSA when monitoring response.

Hypotheses: Heterogeneous mechanisms behind PC and CRPC exist that need to be taken into account when selecting the most suitable therapy for an individual patient. Biomarkers for advanced PC should be primarily searched for and monitored within metastases or, as a proxy for the whole tumor burden, in liquid biopsies such as plasma, circulating tumor cells (CTCs), platelets, exosomes, or free circulating DNA.

General strategy: The project is a collaborative effort between researchers in Umeå and Gothenburg with the the main goal to improve PC prognostication and therapeutics based on biomarker predictions and monitoring. The research plan focus on finding biomarkers for identification of progressive PC at an early, curable stage as well as biomarkers for therapy selection and monitoring of metastatic disease. Biobank samples are available from retrospective and prospective collections of tissue and liquid biopsies obtained during disease progression and therapy. In biomarker discovery studies, genomic, transcriptomic, proteomic, and metabolomic technologies are used, in parallel with hypothesis-driven approaches. For validation studies, specific RT-PCR or antibody based methodologies are used. Expected results are that patients with aggressive PC will be treated with the most suitable therapy given in time to prolong survival, while patients with indolent PC can be safely left untreated.

Specific aims: i) To identify biomarkers in biobank samples associated with progressive PC and to validate promising biomarkers in separate cohorts, ii) To establish and validate robust biomarker assays for clinical use, iii) To validate biomarkers in prospective cohorts and biomarker driven clinical trials.

Clinical biomarkers for ROS-based anticancer therapy

Thomas Helleday[1], Craig Wheelock[2] and Jeffrey Yachnin[3]
Presenter: Ulrika Warpman Berglund[1]

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It is well established that cancer cells are ubiquitously characterized by increased aerobic glycolysis (Warburg effect) and an imbalance in the redox system, leading to high levels of reactive oxygen species (ROS). Many cancers have upregulated levels of MTH1 and rely on MTH1 activity to prevent conversion of ROS into lethal DNA damage. Non-transformed cells do not need MTH1 activity and MTH1-/- mice live and grow old, likely owing to the controlled redox balance and low ROS level in normal cells. These data led us to suggest a novel concept for treatment of cancer; MTH1 inhibition.

We have identified first-in class, potent MTH1 inhibitors that reduce cancer survival by enhancing ROS-induced lethal DNA damage. In order to identify target groups and predict efficacy, resistance and safety of this treatment, biomarkers are required. The aims of this project are; 1) validating biomarkers for target engagement (Poster: Christina Kalderén et al), 2) identifying biomarkers for efficacy of ROS induced DNA damage in cancer, 3) identifying companion biomarkers to predict responders of MTH1 inhibitor therapy (Poster: Kumar et al), 4) identifying resistance pathway to MTH1 inhibitors and associated biomarkers, 5) identifying safety biomarkers and 6) validating candidate biomarkers in clinical trials.

Candidate biomarkers have been identified by using cellular thermal shift assay (CETSA), proteomics, metabolomics, genetic screening, modified Comet assay etc. Briefly, a dose-response related target engagement and elevated levels of oxidised nucleotides as well as DNA damage response proteins were observed both in vitro in cancer cells and in tumours from MTH1 inhibitor-treated animals. Polymerase kappa has been shown to be a potential responder biomarker, supporting the mode of action of the MTH1 inhibitor. Our MTH1 inhibitors have been further optimised and a clinical candidate, Karonudib, developed. The application to initiate a clinical phase I study has been approved by the Medical Products Agency and the Ethical Committee. Next, the potential clinical biomarkers will be investigated in clinical samples from cancer patients treated with Karonudib.

Human hantavirus infections: New insights into pathogenesis and possibilities for treatment

Ljunggren HG, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, Michaëlsson J, Malmberg KJ, Mohamed N, Gupta S, Tischler ND, Sundström KB, Gupta S, Saskia Scholz[1], Baharom F, Rankin G, Maleki K, Vangeti S, Pourazar J, Discacciati A, Höijer J, Bottai M, Rasmuson J, Blomberg A, Connolly Anderssen AM, Kerkman P, Forsell M, Smed-Sörenssen AS, Björkström NK, Ahlm C, Klingström J and collaborators

From the Karolinska Institutet, Stockholm, and Umeå University, Umeå, Sweden

Aim: The present project that aims towards better understanding the pathogenesis of human hantavirus infections, and to find new treatment strategies for the diseases caused by these infections.

Background: In humans, hantaviruses cause two severe type of diseases: hantavirus pulmonary syndrome (HPS) in the Americas and hemorrhagic fever with renal syndrome (HFRS) in Eurasia including Sweden, with case-fatality rates of up to 50% and up to 10%, respectively. As of today, no specific treatment of these diseases, or FDA-approved vaccines to prevent them, exists. Only symptomatic treatment is available.

Findings: We have found that human hantavirus infection is associated with exceptionally strong cytotoxic lymphocyte responses, increased vascular permeability, hyper-inflammation and shock. Together, this indicates that HPS/HFRS likely are immune-mediated diseases. However, the detailed mechanisms behind the effects of hantavirus-induced diseases are largely unknown, hampering the development of specific treatments. In the latter respect, we recently had a breakthrough in our laboratory. Results indicate that the massive immune activation and associated sepsis-like condition observed in hantavirus infected patients could ultimately be caused by (i) a failure of cytotoxic immune cells to efficiently eliminate virus-infected cells, due to hantavirus-mediated inhibition of apoptosis in the infected cells (ii) leading to a subsequent massive activation of cytotoxic immune cells, (iii) that, at least partly, could be driven by hantavirus-mediated induction of IL-15 expression on surface of infected cells.

Implications: These findings open up for a new view of human hantavirus infection. Severe hantavirus infections resembles familial hemophagocytic lymphohistiocytosis (FHL). FHL is similarly characterized by a massive cellular immune activation due to an inability to eliminate virus-infected cells because of inherited deficiencies in genes encoding perforin or proteins involved in the degranulation machinery of cytotoxic lymphocytes. Interestingly, FHL has a specific, effective treatment - the HLH-94/HLH-2004 protocol combining the use of atoposide and steroids. This suggests the possibility that this immunochemotherapy protocol, as well as the possibility of using anti-IL-15 mAbs, might provide a treatment option also for HPS/HFRS.

SSF Rambidrag SB12-0003

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Immunomodulation of host-microbe interactions in infections caused by commensal pathogens - MOHICAN

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MOHICAN is a multidisciplinary project targeting molecular mechanisms through which the major global pathogen *Streptococcus pneumoniae* interacts with the host immune defense system, with the goal to identify and test means to modulate these interactions to the benefit of the host. The central issue is to understand how a microorganism such as the pneumococcus can colonize the upper airways of healthy children but also be the most common bacterial cause of community acquired pneumonia, sepsis and meningitis, diseases estimated to kill about 2 million people (primarily small children and elderly) annually. The MOHICAN team gathers a unique blend of expertise including infection biology, super resolution imaging, cytometry, organic and synthetic chemistry, and structural biology from Karolinska Institutet (KI), Royal School of Technology (KTH), and Umeå University. The project is divided into 3 work packages. In work package 1 we elucidate bacterial (pneumococcal) components affecting disease development and mechanisms modulating immune cell activation. In work package 2 we study bacterial immune cell interaction after exogenously added modulating compounds such as vitamin D, cinobufagin, and sialic acid, and the role of hydrogen peroxide in pneumococcal host interaction. In work package 3 we use different approaches to modulate bacterial susceptibility to antimicrobial substances and aim at identifying novel antimicrobial compounds.

Chemical Probes to Study and Block Bacterial Virulence

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In the current research program a chemistry based multidisciplinary approach is used to explore molecular mechanisms of bacterial virulence. We focus on the opportunistic gram-negative pathogen *Pseudomonas aeruginosa* which is a ubiquitous bacterium commonly resistant to many antibiotics and highly prominent in healthcare-associated infections where it can cause life-threatening infections. The ultimate goal is to generate knowledge and small organic molecules that will lead to highly specific antibacterial regimens that counteract virulence mechanisms and minimize the risk for development of resistance. The research thus addresses the grand challenges dealing with global health. Specifically, we target two virulence mechanisms, type III secretion (T3S) and Twin arginine translocation (Tat) in P. aeruginosa. We employ screening based approaches to identify small molecule inhibitors of Tat, the T3S needle tip protein PcrV, and the ADP-ribose transferase activity of the T3S toxins ExoS and ExoT, and use them as chemical probes to study bacterial virulence and as starting points for drug discovery and development.

Biomarker profiles in CVD – The ABC risk score project

Biomarker profiles for risk stratification and personalized treatment in atherosclerosis and atrial fibrillation – the ABC risk scores project.

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In an aging population, myocardial infarction and stroke are the main causes of death, sudden deterioration of health and need for health care. The aims of the research program are to show that information on Age, Biomarkers indicating disease pathophysiology and/or organ dysfunction and Cardiovascular events history provides the key information to guide personalized treatment in patients with atherosclerotic disease or atrial fibrillation (AF). The project is based in Uppsala Clinical Research Center, which provides all resources needed for clinical trials, registry, statistics, bioinformatics, biobanking, and biochemical analyses. Biobanks include samples from 70 000 patients with acute and stable coronary artery disease (CAD) and AF, population based cohorts and national quality registers. The work is divided into 6 work packages.
We have identified the most important biomarker predictors for myocardial infarction, stroke, and bleeding during antithrombotic treatments. Based on these biomarkers the first versions of ABC risk scores have been developed and validated internally and externally in large cohorts of patients with AF and CAD (WP1 and 4). The new biomarker scores based on four to five variables performed better than conventional scores and seemed to provide tools useful as decision support on antithrombotic treatments in these disorders. The design and planning of a prospective randomized clinical trial in AF patients on the utility of the ABC-scores is ongoing (WP5).
Use of new technologies in search of novel biomarkers that will improve the understanding of key pathophysiological processes, identify new targets for drug treatment and give an added value to ABC risk scores is simultaneously pursued according to plan. We have developed novel methods for circulating microparticles and arrays for microRNAs. We are also exploring a wide array of additional potential biomarkers in CAD and AF by applying state-of-the-art genomic and proteomic technologies in the SciLifeLab platforms and in collaboration with several biotechnology companies (WP2).
We are also evaluating if the novel biomarker profiles from WP1 and 2 are applicable in the general population to screen for high-risk individuals prone to develop stroke or myocardial infarction (WP3).
Recently we started WP6 by planning the integration of the ABC-score tools in the electronic patient records and laboratory systems in collaboration with the industrial and health care partners.

Non-Invasive, Point-Of-Care, Biomarkers of Asthma: An overview of the ChAMP project.
A project within the SSF-action **"***Biomarkers 2013***"**

Sven-Erik Dahlén, The Institute of Environmental Medicine, and CfA (The Centre for Allergy Research), Karolinska Institutet, Stockholm, Sweden.

Background: Asthma and allergies affect 25% of the Swedish population, represent a significant cost to society (>100 billion SEK per year), and an unmet need in terms of effective treatments, in particular for severe asthma. There are currently few established clinical diagnostic biomarkers that identify sub-phenotypes of asthma and thereby long-term prognosis or choice of treatment to prevent the major losses of production hours that are caused by asthma. ***In order to realise the vision of biomarker-driven precision medicine, there is a great need to define markers that reliably monitor clinically relevant mechanisms in asthma and related diseases***.

Objective: The aim of the ChAMP (CfA highlights Asthma Markers of Phenotype) consortium is therefore to utilise material collected from a unique portfolio of some 25 well-phenotyped clinical and epidemiological cohorts and to measure a broad range of biomarkers with different omics technologies. The project is focused on ***biomarkers in non-invasive matrices such as exhaled breath ("Breathomics"), urine, saliva, and blood.*** Samples from healthy controls and subjects with mild-to-moderate or severe asthma, have been collected at baseline, after spontaneous or induced worsening of asthma, as well as after controlled therapeutic interventions such as oral glucocorticosteroids.

Some key findings: 1) The profile of lipid mediators in urine can be used to identify Th2-driven asthma but also other specific sub-phenotypes (see also poster abstract by Kolmert et al) such as mast cell-dependent asthma with high urinary levels of prostaglandin (PG) D_2**.** 2) YKL-40 and other chitinases in blood reflect severe steroid-resistant, non-Th2-driven airway disease (James et al AJRCCM 2016). 3) Periostin levels in blood relate both to Th2-inflammation and airway remodelling. 4) Affinity proteomic screen of blood plasma identified about 50 differentially expressed markers in severe asthma (see poster abstract by Mikus et al). 5) Initial application of the Somalogics™ technology to collections of exhaled particles from the small peripheral airways with the Pexa-methodology supports the identification of specific signatures that differ between asthma, COPD and healthy subjects (see poster abstract by Viklund et al). 6) Fluctuation analysis of daily recordings of lung function distinguishes three different clusters among patients with severe asthma, correlating also with specific biomarker expressions and meaningful differences in clinical outcomes.

Next steps: 1) Biomarker discoveries in particular ChAMP cohorts will be validated both in other cohorts and in ongoing controlled interventional trials 2) Relations between different biomarker molecules (e.g. proteins, lipid mediators, etc) will be further analysed by the use of different system biology approaches 3) Usefulness of particular biomarkers and biomarker patterns in the different investigated matrices will be assessed taking into account also kinetics and clinical utility

Biomarkers predicting joint inflammation

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Inflammation in cartilaginous joints occurs in many common diseases such as rheumatoid arthritis (RA), osteoarthritis (OA) and psoriasis arthritis (PsA). It is known that antibodies to modified proteins (anti citrullinated protein antibodies and rheumatoid factors) are useful for predicting and classifying RA. It has, however, been difficult to identify specific and useful biomarkers derived from the joint inflammation itself. We have focused on the immune response to joint cartilage proteins, in particular type II collagen (CII). This is directed to exposed triple helical structures, as well as to structures modified e.g. by citrullination. The response is connected with the onset of the RA and provides very sensitive serum biomarkers for joint inflammation.

The three main goals of the project are:
• To develop a multiplex antibody diagnostic kit with posttranslationally modified and triple helical CII epitopes. The purpose is to allow diagnosis of RA before disease onset and to help in determination of the type of therapy. The assay will also be used to select RA patients suitable for vaccination.
• To develop a diagnostic kit to identify T cells specific for glycosylated CII. It will be used to predict and monitor vaccination effects of pre-RA and RA patients.
• To extend the antibody kit with modified CII epitopes useful for diagnosing all joint inflammatory diseases, including common diseases like OA and PsA.

We have so far identified a set of novel biomarkers specific for established RA and have some promising biomarkers, which could be used to predict disease protection and disease severity.

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Identification of drug targets in infectious diseases*

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In order to cause invasive infection, bacterial pathogens have to breach protective barriers of the host by exploiting port of entries, establish an infection within the surrounding tissue, and in severe cases, eventually become systemic. A better understanding of this complex chain of events requires novel conceptual approaches that take the dynamics of these processes into account. In this application we aimed to address the molecular mechanisms involved at each stage of the infectious process. To this end we focused on skin and pulmonary infection (ports of entry), deep tissue infection (establishment of infection), and sepsis (systemic infection). Our work has resulted in a couple of targets which efficiently are able to clear an infection under *in vitro* and *in vivo* conditions. Future work will show whether our findings can be used to establish novel antimicrobial therapies.

*This work was supported by a grant from The Swedish Foundation for Strategic Research (*Synergy Grant in Infection Biology*)

Molecular Interplay between man and *Plasmodium falciparum*

Collaborating research groups *Mats Wahlgren, MTC, Karolinska Institutet, Stockholm, Sweden and IngMarie Nilsson, Department of Biochemistry and Biophysics, Stockholm University, Sweden.*

Collaborative partners *Ola Blixt, University of Copenhagen, Copenhagen, Denmark, Nicolai Bovin, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation*

We study the molecular interplay between *P. falciparum* infected red blood cells and the human host in order to develop treatment for severe malaria of strategic importance for Swedish industry. The goals are to identify Plasmodium falciparum proteins involved in adhesion, study protein translocation over the red-cell membrane and unveil carbohydrate receptors and generate anti-adhesive neo-conjugates.

There are 225 million cases of clinical malaria globally each year and more than 1.2 million deaths from the disease – primarily among children living in Africa. Indeed, half of the world's population is at risk of becoming infected with malaria. Malaria has been found to be present in 108 countries and territories in the world. The highest number of malaria cases and deaths occur in sub-Saharan Africa where the disease accounts for 20% of all childhood deaths. In Asia, Latin America, and to a lesser extent the Middle East, malaria is also a serious public health problem. Malaria is the largest global killer of children with a death rate of one child every 30 seconds.

We have assembled an outstanding group of collaborators that are all world-leading in their own fields. with different types of relevant complementary scientific expertise.

1) The Wahlgren group in exploring the pathogenesis of malaria, including major discoveries in cellbiology, immunochemistry and molecular biology. Board member and co-founder of Dialforette AB, now Modus therapeuticus ab.

2) The Nilsson group has extensive experience of experimental cell membranes, it is in fact one of the strongest research milieus in the world in the this field.

3) The Blixt group is one of the few in the world who has available carbohydrate arrays and who has now further developed them to include bead-arrays. Outstanding and important player in this consortium.

4) The Bovin group works on the carbohydrate-chemical aspects of the project. Experience in synthesis of oligosaccharides, neoglycoconjugates, study of specificity for lectins and anti-carbohydrate antibodies and carbohydrate analysis. Outstanding experience of carbohydrate chemistry since the 1970-ies, in particular blood group A which is at the centre stage of this application.

We have over the first years identified malarial adhesins (RIFINS) and host derived receptors, performed a clinical trial and defined the structure of (PFEMP1).

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Integrated Pathophysiology of Infection

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The basal state of any organ is far from static, and during infection, the situation becomes exponentially complex. Local deviations from native tissue physiology and histology combined with systemic responses and distant organ activities act in concert to clear the infection. To study the integrated pathophysiology of infection, we apply our intravital model of pyelonephritis. This model offers superior spatial and temporal control of infection, as infection is initiated by microinfusion of uropathogenic **E. coli** directly into individual tubules in the kidney cortex. Using optical imaging techniques such as two-photon and confocal microscopy, we visualize the progression of infection in the living tissue at cellular and molecular resolution. We have mapped the sentinel leukocyte populations in the kidneys of both mice and rats, and are currently studying the recruitment dynamics toward the infection site. Additionally, we have identified molecular signaling candidates responsible for the rapid epithelial-endothelial crosstalk leading to coagulation in local peri-tubular capillaries, an essential host response protecting from systemic spread and sepsis.

From the microbes' perspective, altered tissue homeostasis requires adaptation that profoundly affects the bacterial lifestyle, as exemplified by the switch from planktonic state to biofilm. Our research teams have developed a class of optoelectronic tools, denoted luminescent conjugated oligothiophenes (LCOs), which we utilize for optotracing in real-time of disease-associated molecular targets. These molecular chameleons produce specific signals when they bind to curli and cellulose, two major constituents of the endogenously produced extracellular matrix defining bacterial biofilms. In addition to generating a more comprehensive and coherent view of biofilm-related infection pathogenesis **in vitro** as well as **in vivo,** we now utilize our emerging technology as the basis for development of biofilm sensor devices. Collectively, we foresee an exciting future of the project from both of the academic and societal perspectives.

Novel Neuroblastoma Biomarkers of Clinical Relevance, a Jan 2017 update (The NNBCR consortium).

Tommy Martinsson[1], Bengt Hallberg[2], John-Inge Johnsen[3], Chandrasekhar Kanduri[2], Per Kogner[3], Erik Larsson-Lekholm[2], Ruth Palmer[2] - www.nnbcr.se

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Background: Neuroblastoma (NB) – NB is a cancer of early childhood that arises from the developing sympathetic nervous system. NB displays a broad clinical heterogeneity ranging from patients with spontaneously regressing tumors to those displaying an aggressive metastatic disease with tumors prone to resistant relapses despite intensive multimodal therapy. It is vital to be able to identify patients that need aggressive therapy and patients that will thrive without in order to minimize side-effects from treatment in this group. Aggressive NB tumors often exhibit near-diploid/tetraploid karyotypes and chromosomal rearrangements, such as MYCN-amplification, deletion of 11q and gain of 17q. A limited number of recurrent gene mutations have been identified, using new whole genome sequencing strategy, including alterations in ALK, ARID1A/1B, ATRX and ALK. Thus, it is of great importance to characterize biomarkers for stratification and treatment NB.

Research being done in the NNBCR consortium:

◆ All NB patients are sampled for biological material at diagnosis and repeatedly during treatment at the DNA/RNA level for a battery of clinically relevant biomarkers, including genomic pattern, whole genome sequencing and detailed analysis of e.g. ALK, MYCN, RAS, NF1, ATRX, NBAT-1, WIP1 and others as yet identified.

◆ Biomarkers for use in assessment of patient response as well as identification of relapse will be available for clinical use in NB patients within national and international NB studies as carried out by VSTB, NOPHO, SIOPEN and other clinical collaborative groups.

◆ Novel biomarkers suitable for therapeutic use have been identified, and are undergoing further validation and development. Validated biomarkers will be incorporated into clinical stratification and tailoring of clinical therapy. Detailed biological information will be used for early use of targeted therapies and implementation of experimental trials at relapse and/or resistant disease.

◆ Specific therapeutic targets e.g. NBAT-1, Wip1, RAS, ALK/ERK5 are under development and/or in early clinical phases. Identification of the Rho-Rac pathway as a druggable neuroblastoma target, Development of novel anti-inflammatory neuroblastoma therapy, Selection of novel targeted therapies for precision medicine. The ALK ligands –FAM150A/B have been identified by us recently and represent a putative target for the future.

◆ Knowledge obtained within this project will be implemented in translational efforts for other diseases including other childhood tumors included in the national biobank (BTB, Barntumörbanken) and the national registry and specifically lung cancer (ALK-targeted therapies), breast and ovarian cancer (Wip1-targeted therapies) and tumors of the CNS, in particular medulloblastoma (Wip1 and NBAT-1 targeted therapies).

New biomarkers in early diagnosis and treatment of Alzheimer´s disease

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Alzheimer´s disease (AD) is the most common form of neurodegenerative brain disorder that poses a great challenge for the healthcare system and society for the steep increase all over the world as the population ages. Due to the lack of current available preventive or disease modifying treatment there is a strong urge to understand the complex pathophysiological AD disease processes in order to identify new early diagnostic biomarkers as well as effective targets for new drug candidate interventions. Our project has strong translational and multidisciplinary approach which cover research from in-silicon modelling, including chemistry development of molecular probes to validation of in-vitro tracer binding and autoradiography experiments in human autopsy brain tissues, in-vivo animal models to in vivo positron emission tomography (PET) and magnet resonance imaging (MRI) in subjects at risk and patients at different stages and subtypes of AD,
The project has a strong focus on developing new PET imaging tracers for visualizing in brain the different AD proteinopathies including beta amyloid peptides from oligomeric, protofibrils to fibril aggregates, tau deposition and α□□nicotinic acetylcholine receptors related to non-neuronal glia cell activation (astrocytosis, microglia activation).

By using modern in silico methods we have so far identified several core and surface sites for the beta amyloid fibrils and obtain insight into basic mechanisms and properties and been able to calculate affinity data for several binding sites on the beta amyloid fibril which have been in agreement with our obtained in vitro binding data for various amyloid tracers in autopsy AD brain tissue.

Our second approach is the α7 nicotinic receptor (α7 nAChR) modulating cholinergic transmission in brain and considered as an indirect amyloid beta-receptor and richly present in AD astrocytes. Since the protein structure is available the in silico studies have been focussed on developing new compounds binding to the α7 nAChR and suitable as PET tracers. The conformational state of the receptor seems to determine the binding affinity of different compounds. Several compounds have been selected from the theoretical modelling and are presently synthesized and experimental tested.

Due to the still lack of detailed structure of the tau protein complex the silico modelling has so far been some exploratory work while the focus is on characterization both in vitro and in vivo of the different chemical classes of tau tracers that so far have been developed. In an on-going in vivo PET study in patients with mild cognitive impairment and AD a head to head comparison is performed for the first time of two different classes of Tau PET tracers in order to able in the same individual compare the binding properties and measured regionals tau deposition in brain. A new synthesis procedure for ^{11}C-THK5351 been developed allowing PET studies combined with the tau PET tracer ^{11}C-PBB3 on the same day in the same patient.

An important aim for new diagnostic imaging biomarkers is to be able to predict AD, discriminate from other dementia disorders as well as enrich the patient population suitable for clinical trials for preventive/disease modifying therapies. Different subtypes of AD based upon pattern of atrophy measured by MRI has already been defined and studies are presently on-going to translate these observations also to prodromal and presymtomatic stages of AD.

Gastrointestinal virus infections and next generation of mucosal vaccines

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The overall aim of this project is to acquire a detailed understanding of the interaction between norovirus (NoV) with host cells to allow identification of molecular targets that can prevent virus cell entry. This information will then serve as the basis for developing prototype mucosal vaccines against NoV infection and to discover new anti-viral drugs and diagnostic tools for characterization of emerging strains. Because human NoV cannot be studied in experimental models and the virus has just recently been propagated in vitro we have taken several complementing approaches. Firstly, the virus-host cell interaction was studied by total internal reflection fluorescent microscopy (TIRFM) and the ability of monoclonal antibodies generated against NoV VLPs to inhibit this interaction was studied in detail. A linear peptide array analysis identified unique binding sites on the NoV VLP and these were selected for vaccine development. Secondly, a novel combination of lipid nanoparticle and targeted fusion protein based on the CTA1-DD platform proved efficient for induction of protective immunity. The dendritic cell targeting element was further optimized using single-chain anti-CD103 antibodies that replaced the DD-element. Thirdly, a mouse model using murine NoV was developed to allow for detailed investigations of the protective mechanisms following mucosal immunizations. In particular, we evaluated the contribution of CD4 T cell and antibody-mediated immunity to protective immunity. Finally, culture techniques to propagate human NoV ex vivo have been acquired that will allow us to evaluate whether the selected vaccine targets can prevent NoV infection in vivo.

SSF Framework Project for Research on Novel Biomarkers of Clinical Relevance

BIO IBD – A multi-modal national study to identify BIOmarkers for diagnosis, therapy response and disease progression in IBD

Presenter: J. Halfvarson[1]

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Background and Aim
Inflammatory bowel disease (IBD), comprising the two predominant forms Crohn's disease and ulcerative colitis, is a chronic disease of unknown origin characterized by relapsing symptoms, such as diarrhoea, abdominal pain, and weight loss. There is no reliable diagnostic or prognostic marker and treatment is expensive and lacks therapeutic precision, which reduces efficacy and safety.

The overarching objective of the framework project is to 1) Identify novel biomarkers for diagnosis, therapy response and disease course prediction in IBD; 2) Pave the way for future exploitation of these novel biomarkers in collaboration with the Swedish biomedical industrial sector.

Material and Methods
Biological material has been collected from treatment naïve patients at diagnosis (n>450) and from patients at initiation of biological therapy (n>250). These cohorts have then been followed over time and samples collected repeatedly as well as from symptomatic non-IBD patients (n>50) and healthy controls (n=47), constituting the discovery cohort. Clinical information is captured using study-specific CRFs and data entered into an established database. Analyses within the discovery phase will be initiated March 2017, followed by a validation phase, in which the biological variation of the identified predictors will be assessed within healthy controls and their presence in IBD patients confirmed.

The microbial content in faecal samples and mucosal biopsies will be sequenced, using the Illumina MiSeq platform (SciLifeLab) and the GA-map™ dysbiosis test. A panel of faecal markers (ECP, EPX, CgA, CgB, secretoneurin and calprotectin) will be assessed by mainly in-house ELISA- and RIA-techniques. Surface cell receptors and cytokine expression of in vitro cultured cells, supplemented with anti-TNF antibodies, will be obtained by flow cytometry, multi-plex ELISA and the Meso Scale Discovery platform. Genotyping will be performed by Illumina chips (Global Screening Arrays), DNA methylation data will be produced by Illumina chips (MethylationEPIC) for EWAS data and the Sequenom EpiTYPER methodology for targeted DNA methylation typing. Transcriptomics (mRNA) analyses will be carried out by using commercially available microarrays (Affymetrix Human Tarnscriptome Array HTA) and/or RNA-seq protocols. Targeted protein profiles will be generated by Olink's Proseek Multiplex panels, SciLifeLab.

The initial tentative strategy for biomarker identification will be based on a three-step process i) multivariate analysis on single predictors; ii) unsupervised clustering algorithms and matrix factorialization to explore data sets and pinpoint redundant or synonymous elements and iii) reverse engineering the process to evaluate its prediction power by means of machine learning algorithms.

Conclusions
The use of strategic national cohorts, repeated large scale molecular profiling technologies and integrated analyses will enable identification of novel panels of biomarkers within IBD. Collaboration with corporate partners, support from Innovation centres and Holding companies at involved Universities ensure efficient transfer of the findings into commercial and future clinical use.

Host-parasite interactions and vaccine development: ETEC and *V. cholerae*

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Vibrio cholerae and enterotoxigenic *Escherichia coli* (ETEC) are major causes of diarrheal illness and death in endemic areas, and ETEC infection is also a leading cause of illness in travellers to such areas. The main goals for this project are 1) to further elucidate the molecular epidemiology of these important pathogens and their interactions with the infected host and 2) to contribute to the development of an effective specific oral vaccine against ETEC disease as well as to an improved, affordable oral cholera vaccine for global use.

The following research areas are addressed: I. Molecular epidemiologic studies of *V. cholerae* and ETEC; II. Molecular studies of host-pathogen interactions; III. Genome-wide studies of human susceptibility and immune response genes for cholera and ETEC; IV. Development of new or improved vaccines against cholera and ETEC and the development of effective oral-mucosal vaccine adjuvants.

Important results obtained so far include the generation of phylogenetic trees for both *V. cholerae* and ETEC. For ETEC, several lineages encompassing strains isolated worldwide and being stable during several decades have been identified. The sequencing analyses have also resulted in identification of novel ETEC colonization factors and new alleles of the heat-stable ETEC toxin. For *V. cholerae*, evidence for environmental rather than host-immunity mediated selection leading to serotype (Ogawa/Inaba) shifts in cholera epidemics has been obtained and critical mutations in the wbeT gene determining serotype have been identified. The studies have also included establishment of novel models and methods for identifying host receptors for bacterial attachment and colonization, for allowing comparisons in mouse, piglet and human systems of host-pathogen cross-talk during infection, and for population studies of human susceptibility and immune response genes in Bangladesh. These results elucidate important aspects of ETEC and cholera infection biology.

A novel single-component oral cholera vaccine (OCV) based on the creation of a stable recombinant “Hikojima” strain expressing both Ogawa and Inaba O (LPS) antigens has been developed during the project period. The vaccine which has been further co-developed and manufactured under GMP conditions with the Hilleman Laboratories, India has now entered clinical testing in Bangladesh with prospects of licensure 2018/19. A next generation OCV with the Hikojima whole-cells combined with entero-coated toxoid (rCTB) is in advanced preclinical development for clinical testing and a promising novel oral-mucosal adjuvant (mmCT) has also been developed.

A novel multicomponent oral recombinant ETEC vaccine (ETVAX) has been developed in collaboration with Scandinavian Biopharma, Stockholm. The vaccine has been tested in different Phase I trials in Sweden with excellent results: ETVAX was shown to be safe, to induce significant mucosal (intestinal) immune responses against all primary vaccine antigens, and to induce long-lasting immunological memory. The vaccine is now being tested in extensive collaborative Phase1/Phase2 trials in descending age groups, including young infants, in Bangladesh. A large Phase 2B study will soon begin to test immunogenicity and protective efficacy of ETVAX in European travelers to Africa and to try to find immunological correlates of protection.
The project has closely followed the project plan and fostered extensive international as well as local and national collaboration, largely cross-disciplinary in nature, and also active collaboration with national and international vaccine industry.

Outreach strategy for generating international press coverage for a research article

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Swedish Medical Nanoscience Center, Karolinska Institutet

The expectations on researchers disseminate their results to the press and to the general public is increasing all the time. Grant proposals and job adverts specifically ask for evidence of various outreach activities. The challenge for modern researchers is how to fit effective outreach into an already busy schedule. I describe the results of my outreach strategy and how we implement this in the Swedish Medical Nanoscience Center at Karolinska Institutet. In this strategy, each researcher from the center contributes to the outreach efforts which are coordinated by one person, who is also responsible for research. This is important as it effectively crowdsources the effort of the outreach while maintaining a consistent vision and goal. Using the strategy we have been featured on National Television and in global news outlets such as IFLScience, Out of the Box Science, the Huffington Post and Business Insider. All of this outreach activity is done in parallel with the ongoing research at the center and it is now considered to be one of our core activities.

ABSTRACTS – POSTERS

Poster 1

BIOBANK-BASED EVALUATION AND IMPLEMENTATION OF NEW CERVICAL SCREENING TESTS

See Abstract for Oral Presentation

Poster 3

Evaluation of components in TGFβ signal transduction pathway as potential biomarkers for prostate cancer

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High levels of transforming growth factor β (TGFβ) in blood in patients with prostate cancer is known to be correlated with aggressive, metastatic disease and poor outcome. We have established *in vitro* models using human androgen-independent prostate cancer cells, to study the molecular mechanisms whereby TGFβ regulates invasion. We have found that the membrane-bound TGFβ Type I receptor (TβRI) undergoes proteolytic cleavage in a manner regulated by TGFβ, resulting in shedding of its extracellular domain (ECD). The RING E3 ligase tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6), which is known to induce Lys^{63}-linked polyubiquitination of its substrates, interacts with a consensus motif present in TβRI. Ligand-induced heteroligomerization of the receptor-complex causes also activation of the catalytic activity of TRAF6, leading to a cascade of intracellular events summarized as non-canonical TGFβ signals. We have reported that TβRI undergoes a second cleavage, resulting in generation of an intracellular domain (ICD) which is liberated from the cell membrane. The nuclear translocation of TβRI-ICD is regulated by TRAF6 and the endocytotic adaptor protein APPL1, and the nuclear TβRI-ICD-APPL1 complex promotes expression of proinvasive genes such as *MMP2* and *MMP9*, thereby regulating invasiveness of the cancer cells. We have observed a strong correlation between a high Gleason Score (used to grade aggressiveness of prostate cancer) and TβRI-ICD-APPL1 complexes, using in situ PLA, in human prostate cancer tissues, indicating the potential usefulness of this complex as a novel biomarker for aggressive prostate cancer. Activation of PI3K-pathway is another arm of the non-canonical TGFβ-signaling pathway, known to promote survival and therapy resistance of cancer cells. We have explored the molecular mechanisms for this pathway and have identified TRAF6 as an important regulator for Lys63-linked polyubiquitination of the regulatory subunit in the PI3K pathway. We have found a strong correlation between Lys^{63}-linked polyubiqutination of the PI3K regulatory component, and high Gleason score, using in situ PLA. Future studies are needed to investigate the usefulness of this event in prostate cancer tissues as a potential novel biomarker.

In collaboration with the Drug Discovery Platform at SciLifeLab, we are in process of generating novel antibodies for detection of the shedded TβRI-ECD and TβRI-ICD, in body fluids and tissues. These novel antibodies will be important for establishment of sensitive and specific methods, for detection of oncogenic components of the TGFβ signaling pathway in patients with prostate cancer to better predict prognosis.

Poster 4

Liquid biopsies for monitoring disease and selecting therapy for patients with metastatic prostate cancer

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Background: Metastatic prostate cancer is treated with androgen deprivation therapy but inevitably relapses in a castration-resistant form. Novel therapies for castration resistant prostate cancer (CRPC) have been introduced in the clinic with possibilities for individualized treatment plans. Best practice of those expensive drugs requires predictive biomarker monitoring. Biomarkers are needed that can aid clinicians in selecting the most suitable therapy in individual patients, and also for monitoring therapy response and resistance over time. The aim of the present study is to evaluate i) the potential of liquid biopsies as sources of biomarkers, reflecting the phenotype of metastases, and ii) if liquid biopsies can be used to predict therapy response in patients with metastatic CRPC.

Method: Liquid biomarkers in the form of circulating tumor cells (CTC), platelets, and plasma were isolated from patient blood samples, in order to extract liquid biomarkers. Matched tissue biopsies from bone metastases were sampled in a subset of patients, during surgery for spinal cord compression. Transcript biomarker levels in the liquid biopsies were analyzed using a designed RT-PCR panel for analysis of 44 cancer-related genes, or by RT-PCR analysis of single transcripts.

Results: Biomarker levels were analyzed in matched pairs of CTCs and metastasis biopsies from 17 CRPC patients. In 10 cases (59%) there was a significant correlation between gene expression profiles in CTCs and metastatic tissue. Interestingly, individual genes contributed differently to this correlation, highlighting a potential for optimization of the panel to reflect metastasis expression profiles. In a parallel study, a specific set of prostate cancer-derived biomarkers were analyzed in the platelet fraction obtained from 50 patients receiving either docetaxel (n=24) or abiraterone (n=26) therapy. Patient response to abiraterone therapy was possible to predict from platelet levels of specific cancer-derived transcripts, based on a significant association between biomarkers and short progression-free and/or overall survival.

Conclusions: This study shows that biomarkers residing in CTCs and in platelets can be used to identify cancer characteristics and enable prediction of outcome after therapy in CRPC patients. The assays presented here may further be developed to support personalize treatment decisions in the clinic.

Future directions: Our study supports the use of ”liquid biopsies” in therapy stratification. From the current results, the RT-PCR mRNA panel will be refined to include the most informative genes. In parallel, a gene sequencing panel will be developed to enable analysis of biomarkers in cell free DNA (cfDNA) in plasma. The potential of CTC- and platelet-residing biomarkers, and mutations in cfDNA, as treatment-predictive biomarkers will then be retrospectively evaluated in patients during treatments for CRPC, from whom liquid biopsies are currently collected. Specifically, their predictive value will be evaluated in an on-going phase II biomarker study (AbiCab) where patients are randomized to either Abiraterone or Cabazitaxel therapy and then crossed-over at progression.

Poster 5

Protein profiles for prostate cancer detection and prognosis

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Background. Prostate cancer (PC) is the most common cause to cancer-related death among Swedish men. Diagnosis of PC is made by detection of raised PSA levels in serum followed by histological examination of randomly taken prostate needle biopsies. Prognosis is judged from tumor grade, stage, and the PSA level. Diagnostic and prognostic tools used today are, however, unsatisfactory resulting in over-treatment of patients with indolent tumors, while patients with aggressive PC are inadequately treated. The main goal of the project is to improve PC prognostication by developing assays for clinical use based on prognostic protein profiles in tissue and/or plasma.

Specific aims are to i) identify proteins in biobank samples associated with progressive PC, ii) validate prognostic proteins/profiles in separate cohorts, iii) validate biomarkers in prospective set-ups.

Methods: For biomarker discovery studies, transcriptomic and proteomic technologies were used, in parallel with hypothesis-driven approaches. For validation studies, antibody based methodologies were used.

Results: Transcriptomic data from whole genome expression analysis (Illumina and Affymetrix) was integrated with proteomic data from LC-MS/MS analysis of clinical biobank samples and animal models. Biomarker discovery studies using Proseek Multiplex Assays (Olink) are ongoing. Proteins with potential prognostic capacity were identified, based on increased levels during disease progression and enrichment in PC compared to other cancers. So far, 281 tissue markers were taken further for validation in plasma using an antibody bead array platform (SciLifeLab). Validation of interesting plasma proteins in relation to patient outcome will follow in separate plasma cohorts. Specifically, the pro-form of neuropeptide Y (pro-NPY) was identified by LC-MS/MS in high risk PC and verified by immunohistochemical analysis as a poor prognostic marker (Iglesias-Gato *et al*, Eur Urol 2016). A sandwich assay for pro-NPY were developed, and pro-NPY levels plasma levels were analysed in 98 patients at diagnosis and found to increase with disease stage and decrease with therapy. Moreover, a prognostic profile indicating immunoevasion in advanced PC was identified, including loss of MHC class I expression and increased tumor infiltration of immunosuppressive macrophages in patients with poor prognosis (Bovinder-Ylitalo *et al*, Eur Urol 2016; Lundholm *et al,* Sci Rep 2015). A prospective follow-up study is ongoing, were the immune profile of peripheral blood is analyzed in relation to disease stage at diagnosis using flow cytometry. Preliminary results indicate suppressed T cell and co-stimulatory activity in patients with advanced disease. Another focus has been on identifying diagnostic and prognostic proteins in the tumor surroundings, i.e. in “Tumor Instructed Normal Tissues” (TINT). In animal models, the presence of a tumor effected expression pattern and immune cell infiltration in substantial parts of the tumor-bearing organ (Adamo *et al*, PloS One 2015), and the magnitude of selected TINT factors were related to poor prognosis in patients (Nilsson *et al,* PloS One 2015; Halin *et al*, Sci Rep 2016). A broad discovery study of prognostic TINT proteins in clinical samples using LC-MS/MS has been initiated.

Conclusion: Protein profiles have been identified that may contribute with prognostic information if measured in plasma and/or prostate tissue biopsies from PC patients.

Poster 6

Cellular target engagement of Karonudib as a potential clinical biomarker

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Therapeutic efficacy as well as off target effects are dependent on drug binding to its target(s). Monitoring drug binding in live cells or tissue has been challenging and recently a new technology has been described that enable measurement of target engagement in live material. The technology of CETSA (Cellular Thermal Shift Assay) is based on ligand-induced thermal stabilization of the target protein in live cells, cell lysate or tissue. Treatment of cell or tissue with target specific ligands will increase the thermodynamic stability and the melting point of the target protein which can be measured with established biochemical methods (1).

The MTH1 protein (human MutT homologue) sanitizes oxidized dNTP pools to prevent incorporation of damaged bases during DNA replication (2). Karonudib (TH1579) is described as a selective MTH1 inhibitor that induces elevated 8-oxo-dG levels *in vitro and in vivo,* DNA damage signaling and reduces tumor growth (3). Cellular target engagement has been investigated for Karonudib in live cells and tumor tissue in order to measure MTH1 binding kinetics and dose dependency *in vivo* and *in vitro.* Here we demonstrate that Karonudib engages MTH1 and stabilizes the protein in tumor grafts and PDX models in a dose dependent matter that correlate with drug concentration in plasma and tumor.

To investigate the target selectivity of Karonudib a combination of CETSA and LC-MS/MS has been used to determine the thermal profile of the proteome in drug treated live cells (4). Ten proteins were shown to be significantly stabilized by the drug, either direct or through down-stream signaling. MTH1 was detected as the major target demonstrating a Tm-shift of 13 °C in both live cells and cell lysate indicating that there is a direct interaction between Karonudib and MTH1.

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Poster 7

Polymerase kappa determine the sensitivity of MTH1 inhibitors to cisplatin resistant cell

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Acquired or intrinsic resistance is one the main reason for an overall decrease in survival of cancer patients treated with cisplatin in different types of cancer. Cisplatin kill cancer cells by various mechanisms, but mainly through oxidative stress and formation of inter- and intra-stand crosslinks of DNA. Different types of DNA repair protein, Translesion polymerase (TLS) including Polymerase kappa (Pol κ) are involved in repair of ICL (Interstrand crosslinking) DNA lesions. We observed high expression levels of MTH1 and Pol κ in cisplatin resistant bladder cancer cells in comparison to parental cells. Due to its low proofreading activity Pol κ can incorporate 8-oxodGTP into DNA. The MTH1 protein (Nudix hydrolase- NUDT1) sanitizes oxidized dNTP pools to prevent incorporation of damaged bases during DNA replication. Recently, we have made MTH1 inhibitors that damage the DNA and induce cancer specific cell death through incorporation of more oxidized dNTPs. We found cisplatin resistant bladder cancer cells (NTUB1/P) were more sensitive to MTH1 inhibitors in comparison to parental NTUB1 cells. As Pol κ is involved in the incorporation of 8-oxodGTP into DNA, we hypothesized that high expression level of Pol κ in cisplatin resistant cells make them more sensitive to MTH1 inhibitors as more 8-oxodGTP would be incorporated into DNA, resulting in more DNA damage and cell death in comparison to parental cells. Indeed, we observe higher induction of cleaved-PARP, γH2AX, cleaved-Caspase 3 and more annexin v positive cells in cisplatin resistant NTUB1/P cells in comparison to parental NTUB1 cells upon treatment with MTH1 inhibitors. MTH1 inhibitor also significantly delayed the NTUB1/P xenograft tumor growth in comparison to vehicle treatment in immunosuppressive mice. Depletion of Pol κ in cisplatin resistant NTUB1/P cells by siRNA resulted in decreased incorporation of 8-oxodGTP and sensitivity to MTH1 inhibitors compared to non-target control cells. Overexpression of Pol κ in NTUB1 and NTUB1/P cells results in further sensitization to MTH1 inhibitors. In conclusion, elevated levels of Pol κ in cisplatin resistance cells determine increased sensitivity towards MTH1 inhibitors and MTH1 inhibitors can be a potential promising therapy for the treatment of cisplatin resistant tumors in patients.

Poster 8

Project:

Treatment strategy for severe human hantavirus infection, H-G Ljunggren

Endothelial damage and B-cell response in hantavirus infection - potential approaches for treatment

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Objective:

Hantaviruses are a rodent-borne viruses that are transmitted to humans via inhalation causing potentially lethal infections. The Puumala hantavirus (PUUV) is highly endemic in northern Sweden, and we have collected a large number of longitudinal patient samples at the Dept of Infectious Diseases at Umeå University Hospital. These samples are now used to better understand the pathogenesis of the infection, and for the development of novel treatment methods to combat the disease.

The endothelium of blood vessels have the potential to regulate inflammation and vascular permeability, and is therefore believed to play a central role for the pathogenesis of the infection. *The main objective of the Ahlm group is to decipher mechanisms behind endothelial damage and function of platelets and to identify potential targets of disease-intervention.*

The elicitation of neutralizing antibody responses are critical for protection against virus infection. Consistently, low levels of circulating anti-hantavirus antibodies has been correlated with a poor prognosis of hantavirus-induced disease. *The main objective of the Forsell group is to single-cell clone broadly neutralizing anti-hantavirus antibodies from patient samples, and to characterize these for their potential as a future antibody-based therapy against hantavirus-mediated disease.*

Methods:

Endothelial glycocalyx degradation markers (soluble TM, heparan sulphate and syndecan-1) were determined in blood collected from PUUV patients. Pulmonary biopsies were obtained through bronchoscopy in hospitalized PUUV patients and in age, sex and smoking-matched healthy volunteers and stained for thrombomodulin (TM) and EN-4. To identify patients with circulating anti-PUUV antibodies that can cross-neutralize the Hantaan and Andes strains of hantavirus, a Vesicular Stomatatis Virus-based pseudotype assay was used. Flow cytometry was used both to analyze hantavirus-specific B cells in patient samples, and to sort these on a single cell level.

Results:

PUUV causes systemic endothelial glycocalyx degradation. Levels of syndecan-1, heparan sulphate and soluble TM were all significantly higher during acute disease compared to follow-up. The ratio of TM-stained pulmonary blood vessels was significantly lower in PUUV-patients compared to controls.

We have identified that a subset of patients that had mounted an anti-PUUV response with significant neutralizing potential also against hantaan and the andes strains of hantaviruses. In addition, we have verifed that PUUV-infected patients have a distinct and expanded population of circulating B cells from where antibodies can be single cell cloned.

Conclusions:

Systemic and pulmonary endothelial glycocalyx degradation during hantaviral may result in plasma leakage and lethal pulmonary complications during hantavirus infection. Consequently, the endothelium warrants further notice when investigating pathogenesis and designing future therapeutic interventions. Moreover, a subset of the patient samples that are kept at Umeå University can be used to develop a potential antibody-based therapy against PUUV and likely also other hantaviruses including the deadly American hantaviruses. The clinical efficacy of cloned monoclonal antibodies will subsequently be assessed in an animal model.

Poster 9

Human hantavirus infection elicits marked redistribution of mononuclear phagocytes from peripheral blood to the airways

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Hantaviruses infect humans via inhalation of virus-contaminated rodent excreta. Infection can cause severe disease with up to 40% mortality depending on the viral strain. Exaggerated immune responses may inadvertently contribute to disease development. Since hantaviruses are transmitted via inhalation, studying immunological events in the airways is of importance to understand the processes leading to immunopathogenesis. Here, we sampled bronchial biopsies and longitudinal blood draws from 17 patients infected by the Puumala hantavirus. During the acute stage of disease, a significant influx of HLA-DR$^+$, CD11c$^+$ and CD123$^+$ MNPs was detected in the patients' bronchial tissue. In parallel, absolute numbers of MNPs were dramatically reduced in peripheral blood, coinciding with viremia. Expression of CCR7 on remaining MNPs in blood suggested migration to peripheral and/or lymphoid tissues. Numbers of MNPs in blood subsequently normalized during the convalescent phase of the disease when viral RNA was no longer detectable in plasma. Mechanistic analysis revealed that hantavirus infected blood MNPs *in vitro*, and that infection lead to induced CCR7 expression. In conclusion, the present study demonstrates marked redistribution of blood MNPs to the lung during acute hantavirus infection, a process that may underlie local immune induction and contributing to immunopathogenesis in human hantavirus infection.

SSF Rambidrag SB12-0003

Poster 10

Human hantavirus infection elicits marked CD8+ and NK cells response due to induction of apoptosis resistance in infected cells

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Following an outbreak of a Puumala hantavirus infection in the human population, we longitudinally analyzed the primary CD8 T cell response in infected individuals from the first onset of clinical symptoms until viral clearance. A vigorous CD8 T cell response was observed early following the onset of clinical symptoms, determined by the presence of high numbers of Ki67(+)CD38(+)HLA-DR(+) effector CD8 T cells. This response encompassed up to 50% of total blood CD8 T cells, and it subsequently contracted in parallel with a decrease in viral load. We subsequently demonstrated that NK cells can rapidly expand and persist at highly elevated levels for >60 d after human hantavirus infection. A large part of the expanding NK cells expressed the activating receptor NKG2C and were functional in terms of expressing a licensing inhibitory killer cell immunoglobulin-like receptor (KIR) and ability to respond to target cell stimulation. In time, this response extends far beyond what is considered normal for an innate immune response. Expanding NK cells expressed markedly increased levels of activating NK cell receptors and cytotoxic effector molecules. In search for possible mechanisms behind this NK cell activation, we observed virus-induced IL-15 and IL-15Rα on infected endothelial and epithelial cells. Hantavirus-infected cells were shown to strongly activate NK cells in a cell-cell contact-dependent way, and this response was blocked with anti-IL-15 antibodies. Surprisingly, the strength of the IL-15-dependent NK cell response was such that it led to killing of uninfected endothelial cells despite expression of normal levels of HLA class I. An intriguing observation in patients with HFRS and HCPS is that on one hand the virus infection leads to strong activation of CD8 T cells and NK cells, on the other hand no obvious destruction of infected endothelial cells is observed. We recently provided an explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from cytotoxic lymphocyte-mediated induction of apoptosis. When dissecting potential mechanisms behind this phenomenon, we discovered that the hantavirus nucleocapsid protein inhibits the enzymatic activity of both granzyme B and caspase 3. This provides a tentative explanation for the hantavirus-mediated block of cytotoxic granule-mediated apoptosis-induction, and hence the protection of infected cells from cytotoxic lymphocytes. These findings may explain why infected endothelial cells in hantavirus-infected patients are not destroyed by the strong cytotoxic lymphocyte response.

SSF Rambidrag SB12-0003

Poster 11

Structure and function of surface-associated and released virulence proteins during pneumococcal colonization

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Streptococcus pneumoniae (pneumococcus) is a major human pathogen and the leading cause of pneumonia, bacteremia and meningitis worldwide. Pneumococcus is commonly carried in the human nasopharynx and can spread to other body sites to cause disease. Surface-associated or released virulence-related pneumococcal proteins, such as the pneumococcal serine-rich repeat protein (PsrP) and peptido-glycan-degrading autolysin LytA, respectively, are key factors during pneumococcal adhesion, colonization, biofilm formation and immune evasion, thus representing promising future drug targets.

We have recently determined the ***crystal structure of the keratin-binding domain*** $\boldsymbol{BR_{187\text{-}385}}$ of PsrP, that is present in 60% of pneumococcal capsular serotypes causing pneumonia in humans. Furthermore we demonstrated that $\boldsymbol{BR_{187\text{-}385}}$ does not only bind to the acidic helical rod structure of lung associated keratin, but also to biofilm-associated extracellular DNA, in order to promote adhesion and bacterial aggregation, respectively (Schulte et al. Open Biol. (2014), Schulte et al. Sci. Rep. (2016)).

Crystal structures of the highly conserved LytA amidase alone and in complex with a large peptidoglycan (PG) fragment revealed a large Y-shaped binding crevice and conserved key residues, that are required for efficient binding and cleavage of a complex PG substrate. These structures and detailed biochemical studies of site-directed LytA-mutants demonstrated the unprecedented importance of multivalent binding to PG saccharides for LytA activity (Mellroth et al. *mBio* (2014), Sandalova et al. *Mol. Microbiol.* (2016)).

Thus, our ***multidisciplinary approach*** comprising X-ray crystallography, small angle X-ray scattering as well as a large array of biophysical techniques and functional *in vitro* and *in vivo* assays allowed us to provide a ***molecular understanding for PsrP-mediated pneumococcal colonization and LytA-mediated autolytis.***

Poster 12

Identification, Synthesis and Biological Evaluation of Tetrahydrocarbazole Analogues that Trigger LytA-mediated Lysis of *Streptococcus pneumoniae*

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A high-throughput screening procedure was developed for identifying bactericidal agents that target the respiratory tract pathogen *Streptococcus pneumoniae*. Active compounds triggered autolysin (LytA)-mediated lysis, which was used as a read out. A total of 17, 500 compounds was screened and 71 hits distributed over 11 compound classes was afforded. The most active compound class was based on the tetrahydrocarbazole skeleton. A structural-activity relationship (SAR) study identified a diamino moiety as essential for the lysis-inducing activity and a series of analogues could be synthesised and the biological properties were evaluated. Compounds with equal activity but with lower cytotoxic activity compared to the best screening hit was found. Analogues from this compound class was also tested against multi-antibiotic resistant pneumococcal isolates and it was pleasing to find that the compounds were equally active as towards the wild type TIGR4 strain.

Poster 13

Streptococcus pneumoniae RrgA and PspC interact with two blood-brain barrier endothelial receptors, mediating bacterial invasion of the brain

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Streptococcus pneumoniae (the pneumococcus) is the main cause of bacterial meningitis. Despite access to antibiotics mortality ranges between 8% to 37% worldwide depending on geographical region. Neurological sequelae, such as hearing loss, focal deficits, motor and cognitive impairments, significantly affect the quality of life of survivors. Bacteria reach the brain through the bloodstream. To invade the brain from the blood, bacteria first encounter the blood-brain barrier (BBB) endothelium and develop strategies to pass this barrier. Receptor-mediated transcytosis was originally proposed as a mechanism employed by pneumococci to cross the BBB. Using high-resolution microscopy of brain biopsies from patients that died due to pneumococcal meningitis, we observe that pneumococci on the BBB endothelium co-localize with two endothelial receptors. Furthermore, we show that the major adhesin of the pneumococcal pilus-1, RrgA, binds both receptors, while the choline binding protein PspC binds, but to a lower extent, only one of the receptors. Using a bacteremia-derived meningitis model and mutant mice, and antibodies against the two receptors, we prevent pneumococcal entry into the brain and meningitis development. By adding antibodies to antibiotic (ceftriaxone) treated mice we further reduce the bacterial burden in the brain. Our data suggest that blockade of the two endothelial receptors has the potential to become a new therapeutic strategy to prevent meningitis development.

Poster 14

Targeting *Pseudomonas aeruginosa* ADP-ribosylating Exoenzyme S

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Pseudomonas aeruginosa is a gram-negative bacterium and one of the leading pathogens behind hospital-acquired infections including blood, pneumonia, and wound infections. Due to the remarkable antibiotic resistance of *P. aeruginosa* there is a need to develop novel anti-pseudomonal agents that can be used in combination therapy to ensure treatment of resistant strains.

In this project, we target the type III secretion system (T3SS) as an essential virulence factor and a valid therapeutic target in many gram-negative bacteria including *P. aeruginosa*. The T3SS is a syringe-like apparatus spanning the bacterial membranes and responsible for transporting effector proteins into eukaryotic cells. Exoenzyme S and T (ExoS and ExoT) are two toxins that are secreted by the *P. aeruginosa* T3SS. They are ADP-ribosyltransferase (ADPRT) enzymes that modify various eukaryotic proteins, such as small GTPases, and lead to signal-transduction malfunction and eventually cell death. Mutation of *exoS* at the ADPRT domain attenuates virulence and ExoS-ADPRT is thus a valid therapeutic target.

In order to identify organic molecules that target ExoS-ADPRT, we developed an *in vitro* enzymatic assay and performed high-throughput screens of chemical libraries of > 30,000 compounds including NAD-mimicking molecules, diverse small molecules, natural products and natural-product inspired library. As a result, we identified a small-molecule competitive-inhibitor of ExoS-ADPRT (STO1101) with an IC_{50} of 20 μM[1]. An extended hit validation and structure activity relationship (SAR) revealed important structural features and allowed us to improve the activity of the hit compounds.

Eventually, all promising inhibitors will be investigated for their *in vivo* activity, and a follow up pharmacokinetics study in a mouse model will allow us to support the scientific community with a novel chemical probe(s) to study bacterial virulence and to develop novel anti-pesudomonal therapy.

References:

1) Pinto, A.F., et al., *J. Biomol. Screen*. 2016, 21(6):590-5

Poster 15

High-throughput screening for inhibitors of *Pseudomonas aeruginosa* twin arginine translocation

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Antibiotics are becoming less effective in the treatment of infections caused by multidrug-resistant *Pseudomonas aeruginosa*. An alternative approach to antimicrobial therapies, based on the inhibition of specific virulence-related traits, offers the advantage of reducing the bacterial adaptability to the host environment, without creating the selective pressure normally associated with conventional antibiotics. The twin-arginine translocation (Tat) pathway, responsible for the export of folded proteins across the cytoplasmic membrane of bacteria, is known to play an important role in the pathogenesis of *P. aeruginosa*. A functional Tat system is required for pyoverdine production, secretion of phospholipase C (PLC) and full virulence in a pulmonary mouse model, and therefore constitutes a promising target for the development of an anti-pseudomonal therapy. The first aim of this work was to develop high-throughput screening (HTS) assays able to detect compounds inhibiting the Tat activity. Tat mediates export of hemolytic and non-hemolytic PLC (PlcH and PlcN, respectively) and PvdN, an essential component in pyoverdine biogenesis into the periplasm. A compound able to inhibit both pyoverdine and PLC is therefore likely to target Tat function. An assay for pyoverdine inhibitors, based on the intrinsic fluorescence of pyoverdine, was developed and employed in the screen of a library consisting of ca. 28000 compounds. The screening resulted in the identification of 49 potential hits. However, these molecules also exhibited a significant effect on bacterial growth. Subsequently, an assay based on the direct measurement of PLC activity in the culture medium was developed and optimized for HTS. Following Tat-mediated export into the periplasm, PLC is further translocated across the outer membrane by the type II secretion system (T2SS). Our assay is thus able to identify both Tat and T2SS inhibitors, which can be distinguished in secondary assays for products relying on one of these mechanisms for their secretion or activity, such as pyoverdine (Tat-dependent) and LasB elastase (T2SS-dependent). For its demonstrated role in the pathogenesis of *P. aeruginosa*, the T2SS is also considered a potential anti-virulence target. Our novel PLC assay was employed in the screen of two chemical libraries and a small collection of novel compounds from natural products, for a total of ca. 40000 molecules. Out of 59 primary hits identified, 11 were confirmed by dose-response analysis and are currently being characterized with regard to their target(s) and effect on virulence-related phenotypes. The most promising hits will eventually serve as lead compounds for drug development. The identification and development of virulence inhibitors active on different mechanisms and targets will broaden the spectrum of treatment options available against *P. aeruginosa* infections.

Poster 16

Chemical probes to study and block bacterial virulence applied to *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen that can cause severe infections in patients suffering from burn wound, cystic fibrosis or cancer. The bacterium is also a common cause of pneumonia, severe eye infection and urinary tract infection. The intrinsic and acquired resistance to antibiotics that gives *P. aeruginosa* a selective advantage and promotes its colonization is a major clinical problem. *P. aeruginosa* has a wide range of different virulence factors, for example the Type III secretion system (T3SS) that translocates virulence effectors into the cytosol of the host cell to evade immune defense and facilitate colonization.

The T3SS consists of a protein complex spanning the two membranes with a needle protruding from the bacterial surface. The PcrV protein is localized at the tip of the needle and this protein is required for translocation of toxins into the host cells. Antibodies against PcrV block T3SS function and promote passive protection in animal infection models, which makes it a good target for development of novel antimicrobials. The natural ligand for PcrV has not been identified. The aim is to discover and develop chemical probes that target PcrV.

A library of small molecules (7600 compounds) has been screened for binding to the PcrV protein using surface plasmon resonance (SPR). The screen resulted in 395 potential binders to PcrV that were screened for biological activity in cell infection experiments. Out of those, 53 compounds could inhibit *P. aeruginosa* from infecting cells. These compounds were further investigated and the binding of the compounds to PcrV was confirmed by NMR experiments. Two interesting compound classes have been identified among the compounds that proved to bind to PcrV by SPR and inhibit the infection in the cell infection experiments in a dose-dependent manner. Analogs to these compounds are currently synthesized and biologically evaluated to improve efficacy.

Poster 17

Biomarker profiles in CVD – The ABC risk score project

ABC scores in atrial fibrillation (AF)

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Background: Atrial fibrillation (AF) is associated with an increased risk of stroke. The benefit of oral anticoagulation is therefore based on a balance between reduction in ischemic stroke and increase in major bleeding. To improve the prediction of stroke and major bleeding, respectively, we developed and externally validated novel risk scores based on clinical variables and novel biomarkers associated with cardiovascular outcomes in AF.

Methods: Novel biomarker-based risk scores for stroke and major bleeding, respectively, in anticoagulated patients with AF were developed and internally validated in 14,537 AF patients randomized to either warfarin or apixaban in the ARISTOTLE trial, and externally validated in 8,468 AF patients randomized to either warfarin or dabigatran in the RE-LY trial. Plasma samples for determination of candidate biomarker levels were obtained at randomisation. All outcomes were centrally adjudicated. The predictive value of biomarkers and clinical variables were assessed in Coxregression models. The most important variables were included in the score with weights proportional to the model coefficients.

Results: The most important predictors for stroke were the variables age, prior stroke, and the cardiac biomarkers high-sensitivity cardiac troponin (cTn-hs) and NT-proBNP. For major bleeding, the most important predictors were the levels of the biomarkers growth differentiation factor-15 (GDF-15), cTnhs, and haemoglobin, and information of age, and prior bleeding. The scores were named the ABCstroke (Age, Biomarkers [cTn-hs and NT-proBNP), and Clinical history of prior stroke] and the ABCbleeding (Age, Biomarkers [GDF-15, cTn-hs, and haemoglobin], and Clinical history of prior bleeding) scores, respectively. The ABC scores were well calibrated. The ABC scores yielded higher c-indices than the widely used conventional stroke (CHA_2DS_2-VASc) and bleeding (HAS-BLED) scores in both the derivation cohort (0.67 vs. 0.62 and 0.68 vs. 0.61, respectively) and the external validation cohort (0.65 vs. 0.60 and 0.71 vs. 0.62, respectively).

Conclusions: The ABC risk scores, using age, biomarkers, and clinical history of stroke/bleeding, were well calibrated and provided better discrimination for stroke and bleeding, respectively, in large cohorts of patients with AF. Thus, the ABC risk scores should be useful as decision support regarding oral anticoagulation treatment in patients with AF.

Poster 18

Biomarker profiles in CVD – The ABC risk score project

Exploration of novel biomarkers in cardiovascular disease (CVD) and atrial fibrillation (AF) using new techniques

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Cardiovascular diseases (CVDs) are among the most common causes of death, and for deterioration of health worldwide. Although treatment options for CVD have improved during the last decades, and despite guidelines for secondary prevention are followed, progression and recurrence of disease is a large problem. Hence new tools with higher precision are needed for both risk prediction and for monitoring treatment.

Proximity Extension Assay (PEA)
Proseek® Multiplex is an analysis method based on the DNA-assisted molecular technique proximity extension assay (PEA) where 92 different proteins can be detected in 90 samples simultaneously from only 1 µl of biological material. This technology is particularly suitable for multiplex analysis of proteins and provides a high-throughput assay with high specificity and sensitivity without the issue of crossreactivity, a major limiting factor in conventional methods. With the use of this technique biomarkers have been analyzed in large cohorts of patients with stable coronary artery disease (STABILITY study, 4300 patients), acute coronary artery disease (PLATO study, 400 patients), in patients with atrial fibrillation (AF) (ARISTOTLE study, 5500 patients), and in population-based samples (ULSAM and PIVUS studies, 1700 patients). The interpretation of all these data is currently ongoing, and the goal is to identify novel diagnostic and/or prognostic biomarkers, and patterns of biomarkers, for distinguishing between different types of CVDs and to find the underlying pathophysiological mechanisms.

Extracellular vesicles
Elevated levels of sub-populations of cell-derived extracellular vesicles (EVs) are found in patients with CVD, and in groups with risk factors for CVD, and are promising biomarkers. However, most techniques used to date for detecting EVs have limitations in detection due to their small size and low protein levels, and are not suitable for clinical use. We have developed an assay that is suitable for clinical use that can capture both small and large EVs with high specificity and sensitivity using proximity ligation assay (PLA), and real-time PCR quantification. Using these novel techniques we are currently analyzing samples from a sub-set of AF patients from the ARISTOTLE study (cases and controls, in total 840 samples) to evaluate the prognostic value of EVs from endothelial cells, platelets, leukocytes and erythrocytes in the development of stroke during AF. The aim is to discover novel EV biomarkers of AF that may add to the ABC scores and that may be suitable for clinical use.

MicroRNAs
MicroRNAs (miRNAs) are a class of short non-coding RNA molecules that are expressed just like protein-coding genes. MiRNAs are present in cells, tissues and as stable entities in various body fluids, such as plasma. Cells can release miRNAs that act as paracrine cell-cell signaling molecules. In various diseases including CVD the expression pattern of miRNAs changes making miRNAs ideal biomarkers. In collaboration with Exiqon A/S (Denmark) we have developed a novel high-throughput expression profiling method for multiplexing miRNAs using their Locked Nucleic Acid (LNA)™-probes on the Biomark HD system. This method offers a robust detection-system and provides a unique platform for screening large cohorts by qRT-PCR in a short time for miRNAs. Currently the first clinical study using this method is ongoing in the same sub-population of AF patients (cases and controls, in total 840) from the ARISTOTLE study presently being analyzed for EVs. The goal is to find patterns of miRNAs that may represent novel biomarkers of AF and that will further the understanding of the underlying mechanism of prethrombotic status.

Poster 19

Biomarker profiles in CVD – The ABC risk score project

Biomarker-based prediction of ischemic events and major bleeding in acute coronary syndromes – Development of the ABC-ACS prediction models

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Introduction
Management of acute coronary syndromes (ACS) involves treatment with potent antiplatelet and anticoagulant treatment. Patients with ACS are, however heterogeneous in terms of risk of ischemic events and risk of major bleeding. Biomarkers and angiographic extent of coronary artery disease provide additional prognostic information. We aimed to develop novel biomarker-based clinical prediction models for cardiovascular death/spontaneous myocardial infarction(MI)/Stroke, and for non-CABG-related major bleeding.

Methods
The models were developed and internally validated in 9,448 (ischemic model) and 9,351 patients (bleeding model) included in the Platelet inhibition and patient outcomes (PLATO) study and undergoing revascularization. All patients received dual antiplatelet therapy with either ticagrelor or clopidogrel. For each outcome, we fitted rich Cox models containing a large number of clinical variables and candidate biomarkers. Based on variable importance and fast backward selection, we created smaller models to approximate the large models. Discrimination was assessed with Harrell's C index and calibration was visually assessed with calibration plots. For the ischemic model, we compared our novel model with the established TIMI and GRACE risk scores.

Results
The final ischemic model included age, gender, body mass index, ECG findings, extent of CAD, NT-proBNP, GDF-15, prior stroke/TIA, prior MI, and diabetes mellitus. It had a c index of 0.68 for CVD/Spon MI/Stroke, which was higher than for TIMI and GRACE scores (0.60 for each), and was well calibrated. The final bleeding model included age, gender, hemoglobin and GDF-15. It discriminated well, with a c index of 0.71 for non-CABG-related major bleeding, and was well calibrated. Higher predicted ischemic risk seemed to predict benefit of ticagrelor over clopidogrel, and similarly, higher predicted bleeding risk seemed to identify patients at elevated risk of bleeding on ticagrelor compared with clopidogrel.

Conclusions
In patients with acute coronary syndromes undergoing revascularization, our novel biomarker-based ABC-CHD risk scores were well calibrated, and discriminated well for both ischemic and bleeding events. After external validation, these models might be used as decision support regarding antithrombotic treatment.

Poster 20

Poster communication from the project "**Non-Invasive, Point-Of-Care, Biomarkers of Asthma"** within the SSF-action **"Biomarkers 2013"**

Affinity proteomics in blood from BIOAIR and U-BIOPRED

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Asthma and chronic obstructive pulmonary disease (COPD) are major respiratory diseases where there is a need for biomarkers that can identify disease subgroups as well as predict response to treatment. We have here applied an affinity proteomics approach, performing protein profiling of 675 individuals and in total 895 samples from patients with asthma of different severities, COPD and healthy controls.

To analyse relative levels of proteins, samples were analysed using antibody suspension bead arrays combined with a direct labelling single-binder assay. Here, biotinylated plasma samples were incubated with a multiplex mixture of antibody-coupled beads. Bound proteins were detected with a streptavidin-conjugated fluorophore and measured on Luminex instruments. Proteins interesting in the context of asthma, COPD, lung and inflammation in general formed the basis of the target selection, resulting in 180 unique proteins targeted by 377 antibodies.

The bead array was used to screen a subset of patient samples from the BIOAIR study (Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airways Disease). This sample set consisted of patients with mild to moderate asthma, severe asthma or COPD. The patients were part of a double-blind placebo-controlled parallel steroid intervention trial, with before and after samples available. In addition, the same bead array was used to profile baseline samples from the U-BIOPRED study (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes): mild to moderate asthma, severe asthma, severe asthma and current or ex-smoker, and healthy controls.

In the intervention study, the majority of protein levels decreased after steroid treatment in all groups. The number of significantly changing proteins was larger in the mild asthma group followed by severe asthma and COPD. The U-BIOPRED study revealed more significant differences between severe asthma or severe asthma smokers and mild asthma or healthy controls. Further analysis will focus on connecting the results to clinical information with the aim to reveal new subgroups for increased understanding and better management of disease.

Poster 21

Poster communication from the project "**Non-Invasive, Point-Of-Care, Biomarkers of Asthma"** within the SSF-action **"Biomarkers 2013**

Rambidrag [RB13-0196]

How to define sub-phenotypes of asthma by measurement of eicosanoid metabolites in urine

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Introduction: Eicosanoids are important inflammatory mediators in respiratory disease. We hypothesised that sub-phenotypes of severe asthma (SA) could be identified by unbiased clustering using data from eicosanoid profiles in human urine. We therefore quantified the urinary excretion of the main metabolites of cysteinyl-leukotrienes (CysLTs), prostaglandins, (PGs) and isoprostanes (IPs) in the U-BIOPRED cohort (IMI project) for phenotyping of severe asthma.

Methods: 597 baseline subjects were included: non-smoking SA (SAn; *n*=302), smoking SA (SAs/ex; *n*=109), mild-to-moderate asthma (MMA; *n*=86), and healthy controls (HC; *n*=101). Eicosanoid were measured in spot urine samples by UPLC-MS/MS. Patient clustering was evaluated by means of unbiased principle component analysis (PCA) and consensus clustering using a bootstrap methodology (1000 times) as previously done for other outcomes (Lefaudeux et al. JACI, 2016). The obtained patient clusters were characterized by clinical and chemical endpoints to evaluate clinical validity.

Results: Quality assessment of the long-term LC-MS/MS measurement qualified 11 of 13 urinary eicosanoids having QC CV´s between 8-35%. Principle component analysis (PCA) did not provide significant separation between the study groups. Instead, consensus clustering, using partitioning around the medoids(PAM) and the Euclidean distance measure, generated a stable five-cluster model (U_1-U_5). Molecular derived clusters demonstrated clinical characteristics suggesting a Type II asthma, Female-high BMI, and an Obstructive phenotype. In addition, two new and distinctly different sub-phenotypes could be defined.

Conclusion: Molecular based consensus clustering using urinary eicosanoids identified sub-phenotypes of asthma with clinically distinct traits. Small molecular profiling of key inflammatory eicosanoid metabolites in human urine from asthma patients therefore provide a promising novel and non-invasive strategy for characterisation of asthma disease heterogeneity.

Poster 22

Poster communication from the project "**Non-Invasive, Point-Of-Care, Biomarkers of Asthma"** within the SSF-action **"Biomarkers 2013".** Rambidrag [RB13-0196]

Pattern of phospholipids and Surfactant Protein A in Particles in Exhaled Air (PExA) in European Respiratory Health Survey- III.

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Introduction: The lining fluid from small airways mainly consist of surfactant, important for airway patency and hostdefence. Both lipid and protein composition of surfactant has been shown to be altered in respiratory disease in broncho-alveolar samples. A method for the collection of exhaled particles non-invasively have been developed, the PExA method. The exhaled particles are mainly composed of surfactant, and the most abundant pulmonary surfactant phospholipid is di-palmitoylphosphocholine (DPPC) and the most abundant lung-specific protein is surfactant protein A (SP-A).

Methods:200 participants (mean age 53 years, range 40-66) in the Gothenburg part of the European Respiratory Community Health Survey (ERCHS)- III was were examined with the PExA method in addition to a clinical examination including spirometry and FENO, a biomarker for eosinophilic inflammation. The PExA samples were analyzed for SP-A and albumin using validated ELISA method as well as the pattern of the major phospholipids using GC-MS-MS allowing quantification of DPPC whereas other phosphatidylcholine (PC) species are reported as percent of the signal-intensity of DPPC (%SI).

Results: 36 subjects reported physician diagnosed asthma, and the majority had mild disease and had no alterations of SP-A, albumin or phospholipid pattern in PExA samples. Subjects reporting an asthma attack the last year (n=17) had increased levels of PC 16:0/18:2 (11.3 vs 10.2 %SI, p=0.01) and decreased levels of albumin (6.8 vs 5.6 wt%, p=0.03) in PExA samples compared to healthy subjects. In the whole group, the levels of DPPC were correlated with FENO (r_s=0.3, p=0.03), and weakly with the FEV1/FVC ratio (r_s=0.17, p=0.02). Smokers had increased levels of DPPC (9.9 vs 11.4 wt%, p= 0.02) and PC16:0/18:2 (28.3 vs 25.1 %SI, p=0.05) compared to non-smokers.

Conclusions: In subjects with mild asthma and few symptoms the composition of SP-A and the major phospholipids in PExA samples are not altered. In subjects with more recent asthma symptoms, the levels of PC16:0/18:2 was increased and may be a result of increased leakage of plasma, where the levels of this PC are much higher. Why albumin is decreased in asthma is not known, but this has also been observed in an earlier study. One may speculate that the increase in DPPC in smokers may have a protective function against the inhaled toxic cigarette fume.

Poster 23

Detection and isolation of antigen reactive B cells in patients with rheumatoid arthritis

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Background: The majority of autoimmune diseases, e.g. rheumatoid arthritis (RA), are characterized by autoantibodies that are produced by B cells. We have identified several novel post-translationally modified epitopes in collagen type II (CII), a major cartilage constituent that are autoreactive. In mouse models, autoantibodies that bind to these epitopes can either induce or protect against inflammatory arthritis. However, very little is known about the frequencies or phenotype the B cells that produce these autoantibodies in humans.

Aim: The aim of this project is to study the B cells that produce autoantibodies reactive to CII-epitopes in RA patients.

Material and methods: Clinical data and peripheral blood were collected from patients with RA (n=100). Titres of CII-reactive serum-autoantibodies were determined by Luminex. The different subsets of B cells that expressed a CII-reactive B cell receptor (BCR) were analysed and isolated in patients with positive (n=10) or negative (n=5) titres for autoantibodies recognizing the CII-epitopes cyc48 (CII-F4-R-Cit) and cyc49 (CII-F4-Cit-R) as well as in healthy controls (n=3) using flow cytometry.

Results: In patients with detectable autoantibodies to cyc48 and cyc49, their titres were 13761±3815 and 5585±1992 (mean ± SEM), respectively. The corresponding titres in the patients negative for these autoantibodies were 122±55 and 234±75. In autoantibody-positive patients, the frequencies of cells expressing a BCR reactive for cyc48 and cyc49 in different subsets were: transitional cells 0.88±0.18 % and 1.33±0.33 %; naïve cells 0.09±0.0008% and 0.13±0.06 and memory cells 0.31±0.25 and 0.30±0.12%. All frequencies were well above those detected in patients without cyc48/49 titres and in healthy controls. A higher proportion (p=0.03) of the cyc48/49 positive patients were treated with methotrexate compared to cyc48/49 negative patients, no other clinical differences were recorded. CII-reactive single B cells were isolated in order to clone the BCR.

Conclusions: In patients with manifest RA there is a relatively high and detectable frequency of transitional B cells that express a joint-specific BCR, which suggests that the deletion of autoreactive B cells in the bone marrow in RA patients is defective. As there is a decrease in the proportion of naïve CII-specific B cells, a substantial amount of the autoreactive cells are deleted in the periphery. However, peripheral B-cell tolerance is incomplete, as CII-specific B cells are enriched in the pool of memory B cells.

Poster 24

Synthesis of triple helical peptides from collagen for multiplex analysis

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Rheumatoid arthritis (RA) is an autoimmune inflammatory disease where joint inflammation and cartilage destruction are mediated by autoantibodies directed to various self antigens. Type II collagen (CII) is a major cartilage-specific protein and anti CII autoantibodies have been detected in early-RA patients. With the aim to develop a predictive and sensitive diagnostic test against RA before disease onset, antibodies against CII and all its modified epitopes constitute interesting opportunities for new biomarkers in RA.
Type II collagen is composed of three identical α chains of primarily repeating Gly-Xxx-Yyy triplets, which induce each α chain to adopt a left-handed polyPro II helix. Three left handed chains then intertwine to form a right-handed superhelix. The triple helical conformation of CII has been shown to be crucial for autoantibody recognition. Post-translational modifications of certain positions of CII, such as citrullination of arginine residues, have also been suggested to contribute to the induction of joint infection in RA.
Here we report on the solid-phase synthesis and biophysical characterization of triple-helical peptides (THPs), including native and modified sequences from CII. The homo trimeric THPs, which consist of around 170 amino acids, are synthesized starting from a pre-synthesized trimeric branch. After HPLC purification the yield ranges between 15-25 % and results in more than 50 mg of each THP. Characterization is done by CD spectroscopy to analyze both the triple helicity and thermal stability, and also by mass spectrometry to identify the THPs and proteolytic fragments thereof. More than 40 THPs have been successfully synthesized, which now are used in multiplex analysis of RA.
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Poster 25

Luminex-based diagnostic kit for Rheumatoid Arthritis

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Background
Rheumatoid Arthritis (RA) is a chronic inflammatory disease affecting up to 1% of the population. One of the major diagnostic criteria for RA is the presence of anti-citrullinated protein antibodies (ACPA) that can be detected in serum of RA patients with a very high specificity, yet a large group of patients still lack biomarkers for an early diagnosis.

Aim
To develop a diagnostic kit for RA with:

- Higher sensitivity and specificity than current test used in clinics (CCP2).
- Prediction of treatment outcome & disease severity to help clinicians for better diagnosis.

Method
Using a Luminex-multiplex protocol we have screened over 400 new joint peptides in patient cohorts with inflammatory joint diseases and healthy controls.

Results
Multivariable analysis has identified a set of new biomarkers for a subset of patients (CCP2 negative) that currently lack biomarkers. New biomarkers correlating with clinical data on future disease activity (DAS28), pain (VAS) & joint destruction (Larsen score) have also been identified.

Poster 26

Pseudomonas aeruginosa elastase cleaves a C-terminal peptide from human thrombin that inhibits host inflammatory responses*

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Pseudomonas aeruginosa **is a Gram-negative opportunistic pathogen notoriously persistent in chronic infective conditions such as non-healing leg ulcers. This species is known for its** immune evasive abilities amongst others by degradation of a large variety of host proteins. However, it has never been investigated whether protein degradation by *P. aeruginosa* enzymes may lead to the direct formation of bioactive peptides exerting novel functions. Therefore, the aim of this study was to investigate whether *P. aeruginosa* can generate peptides that modulate host responses.

To study this concept we investigated thrombin degradation. Using a broad range of methods we found that *P. aeruginosa* elastase cleaves a C-terminal derived peptide from thrombin, which inhibits pro-inflammatory responses to several pathogen-associated molecular patterns *in vitro* and *in vivo*. FACS, slot blot assays and electron microscopy showed that the peptide prevents receptor dimerization and subsequent activation of down-stream signalling pathways. Finally, the peptide was found in chronic wound fluids and observed on leukocytes derived therefrom.

Taken together, *P. aeruginosa* elastase cleaves thrombin, resulting in the formation of a peptide that dampens inflammation. These findings constitute a novel concept of pathogen-host interactions, where bacteria mimic an endogenous anti-inflammatory mechanism that can aid in circumvention of host responses.

*This work was supported by a grant from The Swedish Foundation for Strategic Research (*Synergy Grant in Infection Biology*)

Poster 27

DNA-fragmentation is a source of bactericidal activity against *Pseudomonas aeruginosa**

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Pseudomonas aeruginosa airway infection is common in cystic fibrosis (CF), a disease also characterized by abundant extracellular DNA (eDNA) in the airways. The eDNA is mainly derived from neutrophils accumulating in the airways and contributes to a high sputum viscosity. The altered environment in the lower airways also paves the way for chronic *P. aeruginosa infection*. Here, we show that mice with *P. aeruginosa* airway infection have increased survival and decreased bacterial load after topical treatment with DNase. Furthermore, DNA from sputum of CF patients showed increased bactericidal activity after treatment with DNase *ex vivo*. Both degraded DNA of neutrophil extracellular traps (NETs) and genomic DNA degraded by serum, acquired bactericidal activity against *P. aeruginosa In vitro*, small synthetic DNA-fragments (<100 base pairs) but not large fragments nor intact genomic DNA, were bactericidal against Gram-negative but not Gram-positive bacteria. Addition of divalent cations reduced bacterial killing, suggesting that chelation of divalent cations by DNA results in destabilization of the LPS-envelope. This is a novel antibacterial strategy where fragmentation of eDNA and DNA-fragments can be used to treat *P. aeruginosa* airway infection.

*This work was supported by a grant from The Swedish Foundation for Strategic Research (*Synergy Grant in Infection Biology*)

Poster 28

Innate host defense properties of collagen VI and its potential as a therapeutic agent*

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Collagen VI is a ubiquitous extracellular matrix component that forms extensive microfibrillar networks in most connective tissues. Collagen VI contains several domains homologous to the A-type domain found in von Willebrand factor. Interestingly, von Willebrand factor harbors cationic sequence motifs associated with heparin affinity that may confer antimicrobial properties. Here, we describe for the first time that the collagen VI von Willebrand factor type A-like (VWA) domains exhibit a broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria in skin infections *ex vivo. In silico* sequence and structural analysis of VWA domains revealed that they contain cationic and amphipathic peptide sequence motifs, which might explain the antimicrobial nature of collagen VI. *In vitro and ex vivo* studies show that these peptides exhibited significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa through membrane disruption*. In addition, some of the peptides also exhibited wound healing and anti-endotoxic properties *in vitro*. Our findings shed new light on the role of collagen VI-derived peptides in innate host defense and provide templates for development of peptide-based antibacterial therapies.

*This work was supported by a grant from The Swedish Foundation for Strategic Research (*Synergy Grant in Infection Biology*)

Poster 29

Characterization of leukocyte chemotactic activity of triggered by streptococcal M1 protein *

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Streptococcal skin infections range from superficial to life-threatening deep tissue infections. The M1 serotype is associated with causing invasive infections compared to other M serotypes. The M1 surface protein is an important virulence factor for the bacteria of which can be shed from the bacterial surface by both host- and bacterial proteases. Thus, it remains to be investigated whether this bacterial protein is important in order to cause deeper tissue infections.
We recently published that keratinocytes recognize the streptococcal surface protein M1 through pathogen-associated molecular pattern signaling. Keratinocytes responds to the bacterial protein by expressing pro-inflammatory cytokines, particularly IL-8. Here, we investigated keratinocytes chemotactic activity of white blood cells (WBCs) when encountering M1 protein and studied the subsequent effects of the bacterial protein in relation to streptococcal skin infection pathology.

*This work was supported by a grant from The Swedish Foundation for Strategic Research (*Synergy Grant in Infection Biology*)

Poster 30

Molecular Interplay between man and *Plasmodium falciparum (Wahlgren, Nilsson)*

Giant Unilamellar *Glycocalyx*-Coated Vesicles as Artificial Red-Blood Cells for Investigating the Molecular Basis of Malaria Parasite Invasion

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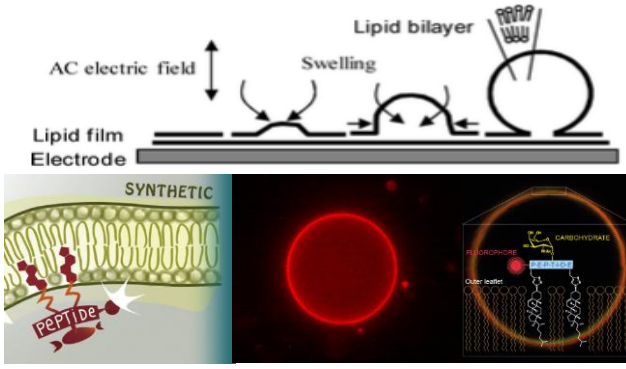
Plasmodium falciparum antigenically varies proteins at the surfaces of the infected red blood cell (iRBC) and the merozoite to evade immunity and facilitate entry. These are fundamental elements of parasitism and are to large extent facilitated by the complex RBC surface glycocalyx. As part of the Molecular Interplay between man and *Plasmodium falciparum* Programe, we aim to systematically investigate these adhesive-invasive mechanisms via a cell-sized model membrane system, so called Giant Unilamellar Vehicles (GUVs) displaying relevant carbohydrate ligands in a controlled manner. Thus, GUVs will be holding single synthetic glycocalyx components such as blood group, sialylated and sulfated glycans and complexity will be created to match the optimal host receptor interaction by the parasite.



In preliminary experiments we have used novel cholesterylated glyco-modules and demonstrated carbohydrate specific rosetting of iRBCs which will be presented during the conference.

Poster 31

Framework: Infection biology

Poster title: Molecular interplay of man and *Plasmodium falciparum*

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Malaria still takes the life of nearly half a million people a year. *Plasmodium falciparum* is by far the most virulent of the human malaria parasites and responsible for nearly all morbidity and mortality associated to the disease. Much of the virulence is attributed to this particular parasite's remarkably efficient invasion of host erythrocytes and sequestration in the microvasculature. Understanding the molecular interplay between parasite and host in regards to these virulence features presents viable approaches to target severe malaria with therapeutic interventions. In the work presented here we have uncovered new clues to which proteins partake in development of severe malaria and show that we can target these with a small compound to interfere with the viability of the parasite. First, we reveal the structure of IgM and PfEMP1, a known virulence factor of the parasite, separately and in complex. This work uncovers that IgM participate in the clustering of PfEMP1 on parasitized red blood cells to mediate robust host-parasite interactions and suggest that the IgM-PfEMP1 interaction prevents IgM-mediated complement activation. Second, we characterize a new surface exposed virulence factor that preferentially binds to blood group A and thereby forms clusters of infected and uninfected erythrocytes known as rosettes. We suggest that this family of adhesins has a fundamental role in the development of severe malaria and thereby contribute to the varying global distribution of ABO blood groups in the human population. Finally, we present results from two clinical studies with a novel drug against severe malaria developed in the lab. This drug, sevuparin, is a depolymerized glucose amino glycan that interferes with the parasite invasion and sequestration. Sevuparin proved to be safe and well tolerated in both trial groups and showed promising results as a future treatment for severe malaria.

Poster 32

Luminescent conjugated oligothiophenes – multimodal tools for molecular imaging in bacterial infections

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Recent advancement of non-invasive imaging techniques offers the possibility to visualize the dynamics and biochemical activity of pathological or biological processes in real-time. Magnetic resonance imaging (MRI and positron emission tomography (PET) can be used in organ to the full body scale, whereas resolution at the cellular and molecular level is obtained using optical imaging, such as two-photon microscopy. In this regard, we have developed a class of optoelectronic tools, denoted luminescent conjugated oligothiophenes (LCOs), which can be utilized for real-time optotracing of disease-associated molecular targets. In the present project, we develop LCOs that produces specific signals for bacterial infection-associated targets, as exemplified by constituents in the extracellular matrix that defines bacterial biofilms.

From a chemical perspective, the LCOs consist of a repetitive flexible thiophene backbone having an exact number of thiophene units and upon interaction with different biomolecular targets, a conformational restriction of the thiophene rings leads to distinct optical finger prints from the LCOs. Thus, specific biomolecular targets can be traced due to unique spectral signatures from the LCOs. By addition of particular side-chain functionalities along the thiophene backbone, the LCOs can be tailored to interact with distinct biological targets, such as protein aggregates, carbohydrates, certain cells or bacterial biofilms. Furthermore, the color of the LCOs can be tuned by varying the length of the thiophene backbone or by adding other heterocyclic chemical moieties than thiophene to the backbone of the LCO. These chemical modifications are also essential for creating LCOs suitable for different detection schemes such as two-photon imaging. A variety of options also exists for synthesizing radio-labelled derivatives of the LCOs that can be utilized for PET imaging of distinct biomolecular targets and we have successfully attached the common radioisotopes ^{11}C and ^{18}F to the LCOs. Overall, LCOs that can be utilized for multimodal imaging of a variety of biological targets have been developed.

Poster 33

Real-time optotracing of curli and cellulose in live bacterial biofilms using luminescent oligothiophenes

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Biofilm is a special bacterial lifestyle defined as an aggregation of bacteria enclosed by an endogenously produced extracellular matrix (ECM). The ECM consists of proteins and polysaccharides that act as a defensive barrier towards physical and chemical challenges. Biofilm can form on surfaces of both abiotic and biotic origin, and poses as such a challenge in clinical, environmental and as well as industrial settings. Although biofilm has been suggested by us and others to form *in vivo* by bacteria infecting an organ, no proof that this is the case exists. This is because currently used dyes for staining of biofilms are toxic as well as non-specific. Thus, study of the dynamics of biofilms and their formation in their native environments has been hindered by a lack of research tools. We have developed a method for simultaneous, real-time, *in situ* detection and differentiation of the biofilm ECM components curli and cellulose, using non-toxic, luminescent conjugated oligothiophenes (LCOs). These flexible conjugated polymers emit a conformation-dependent fluorescence spectrum, which we use to kinetically define extracellular appearance of curli fibers and cellulose polysaccharides during bacterial growth. Applicability of this technique is demonstrated by defining biofilm morphotypes of *Salmonella enterica* serovars Enteritidis and Typhimurium, and their isogenic mutants in liquid culture and on solid media, and by visualizing the ECM components in native biofilms. Reporting the use of LCOs across a number of platforms, including intracellular cellulose production in eukaryotic cells and in infected tissues, we demonstrate the versatility of this opto-tracing technology, and its ability to redefine biofilm research.

Poster 34

The reality show of infection – studying UPEC infection of the kidney using intravital imaging

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INTRODUCTION: We have previously developed a model of renal infection where bacteria are microinfused directly into the tubules of rat kidneys and we could visualize, with single cell resolution, the progress of infection in the living tissue. Over the first hours of infection bacteria multiply within the lumen, activate a rapid coagulatory response in local small vessels and leukocytes are recruited to the infection site. The Phillipson group has shown that leukocytes are directed towards a site of inflammation by a mechanism termed ”intra-vascular crawling”, via a chemokine gradient, and initial vascular adhesion to optimal trans-migration sites at endothelial junctions. In this project, we combine our expertise to study renal host responses to bacterial infection, focusing on the epithelial-endothelial crosstalk, and effects on infection progress and outcome.

METHODS AND RESULTS: To investigate the fundamentals of cell-cell signaling between infected epithelium and nearby endothelium, we first developed an *in vitro* system, using primary like human cells. Using a proteome array screen we have identified two novel candidates excreted by infected epithelium, CD147 and CD26, that may influence the pro-coagulatory response in endothelia. Further, we have shown that the excretion of CD147 and CD26 is dependent on the bacterial virulence factor alpha-haemolysin.

In parallel we have re-established the micropuncture infection model in rats and translated the procedure into mice. Using transgenic mice, we have been able to visualize the presence of a previously un-described population of sentinel immune cells in the kidney cortex *in vivo*. We have found that a large population of resident macrophages are present in the interstitium and in the glomeruli of naïve mouse kidneys, where they show a scanning behavior and appear to probe the environment. Following both infection and laser induced injury, a rapid recruitment of neutrophils occurs. The recruitment of neutrophils during infection seem to correlate with the initial attachment of bacteria, and it appears that neutrophils can cross into the infected tubules and clear the infection. Quantification of neutrophil recruitment is underway.

We have seen a similar distribution of sentinel leukocytes, including resident macrophages ($CD163^+$), leukocytes ($CD18^+$) and dendritic cells ($CD80^+/86^+$), in rat kidney using ex-vivo immunohistochemistry. We have also been able to visualize a change in number and location of these cells in both a 4 day ascending urinary tract infection as well as within 4 hours of the micropuncture kidney infection.

CONCLUSIONS: We have identified signaling candidates for the epithelial-endothelial coagulatory signaling and mapped the sentinel leukocyte populations in the kidneys of both mice and rats. We are currently investigating the roles for the signaling candidates and leukocytes during infection in the *in vivo* setting.

Poster 35

Anaplastic lymphoma kinase addictive neuroblastoma cell lines are associated with growth upon treatment with MEK inhibitor trametinib.

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Background

MEK inhibitor, such as trametinib, suppress signalling through the mitogen-activated protein kinase (MAPK) cascade has anti-cancer activity and are approved as a single agent for treatment of BRAF positive melanoma. Recently, trametinib has been suggested to be included with RTK inhibitors, such as different ALK inhibitors, in a rational polytherapy strategy for treating both ELM4-ALK and K-RAS-mutant lung cancer and of both naïve and relapse neuroblastoma containing ALK-RAS-MAPK mutations to alter the incomplete and temporary responses obtained with ALK inhibitors so far.

Materials and Methods

To test this polytherapy hypothesis we employed trametinib and ALK inhibitors which were investigated in a set of various neuroblastoma and lung cancer cell lines. Resazurin assay was performed to evaluate cell viability. Protein levels were determined using western blotting.

Results and Conclusion

We show that pharmacological inhibition of MEK-ERK pathway using trametinib results in increased levels of activation of AKT and ERK5. Further, we are able to show that ``superactivation`` of AKT is via Sin1 T86 phosphorylation. We investigated the molecular mechanisms upon treatment with the MEK inhibitor trametinib and found that targeting MEK kinase is favourable in ELM4-ALK mutated lung cancer cells and RAS-MAPK mutated neuroblastoma but not beneficial in ALK addictive neuroblastoma cells, although these cell lines activate the RAS-MAPK pathway.

Poster 36

PAN cancer analysis of S-phase-enriched lncRNAs identifies oncogenic drivers and novel biomarkers

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Abstract

Recent evidence suggests that lncRNAs take part in cancer progression and metastasis through influencing several oncogenic pathways. However, our efforts to find clinically relevant cancer associated functional lncRNAs on global scale are still lacking. By employing a nascent RNA capture sequencing we identified 1145 S-phase-associated lncRNAs. Out of 1145, 571 lncRNAs showed significant differential expression at least in one tumor type across pan cancer TCGA datasets. Interestingly, 73% of these transcripts exhibit higher expression in tumors. Using an integrated multistep statistical pipeline on 14 pan-cancer datasets, we identified 520 independent prognostic markers. Based on our high-throughput transcriptomic analysis of S-phase enriched lncRNA across pan-cancer TCGA datasets followed by large-scale functional and clinical investigations, we provide a comprehensive list of lncRNA-based oncogenic drivers with potential prognostic value.

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Exome- and whole genome sequencing for clinical evaluation and precision medicine in neuroblastoma

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Background

Despite major progress in treatment of pediatric cancer, aggressive neuroblastoma (NB) still constitutes a major clinical problem. Currently, the event free survival of high-risk NB is about 40% implicating that 60% either don't go into full remission or falls into relapse. Most relapses have an acquired drug resistance meaning that conventional treatment options may not work. Therefore, further studies of this group are of great importance. Massive parallel sequencing has now become a valuable tool in both cancer research and in clinical evaluation as it provides valuable information regarding patient specific alterations that might be used in targeted therapy.

Material and Methods

DNA from 54 patients in total have been subjects for massive parallel sequencing; 16 tumor/normal pairs and 27 single tumors have been analyzed using exome sequencing, 8 tumor/normal pairs with whole genome sequencing and three using both exome and whole genome sequencing. Exome sequences were mapped using BWA with GATK realignment followed by variant calling through SNPeff and copy number analysis trough ControlFreec. Whole genome data were analyzed using the CLC Genomic Workbench with annotation and filtering done in Ingenuity variant analysis software.

Results

On average, 14 somatic protein changing single nucleotide variants were detected per patient (range 2-71) with recurrent alterations mainly detected mainly in *ALK*. Predicted deleterious germ line alterations in well-established cancer genes were detected in three patients. Structural variants included genes such as *ATRX* and *TERT*, as described by others previously, but also novel homozygous focal deletions at chromosome 19. Shortest regions of overlapping deletions of this chr19 region include seven gens whereof *CIC* (Capicua) might be the most interesting in a cancer context. Copy number analysis of both exome- and whole genome sequencing gives genomic profiles that are comparable to the genome profiles generated from the SNP-microarrays that are in current clinical use. Specific breakpoints for structural variants could be detected in all sample subjects for whole genome sequencing as well as for all for all *MYCN* amplified neuroblastomas that subjects for exome sequencing.

Conclusions

Exome- and whole genome sequencing can be used for clinical evaluation of NB providing information regarding both constitutional and tumor specific alterations. The information can be used for clinical decision of patient specific therapeutic options such as ALK- or CDK4/6 inhibitors, but can also in rare cases give information regarding which treatments not to use. Furthermore, whole genome sequencing also gives the possibility to identify tumor specific structural alterations such as translocation breakpoints that can be used to monitor tumor burden in liquid biopsies.

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Targeting amyloid fibrillar binding sites and alpha 7 nicotinic receptors - in silico to imaging binding studies

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Background: The presence amyloid beta fibrils in brain tissue is one of the key features of Alzheimer´s disease (AD).. The amyloid oligomer are probably the most toxic entities in AD pathogenesis and therefore potential targets for early imaging biomarkers. Likewise, α7 nicotinic acetylcholine receptors (α7-nAChR), which show opposite aberrant expression levels in neurons and astrocytes in AD patients, also serve as attractive targets. The aim of this project is to design novel PET (Positron Emission Tomography) tracers which can specifically bind to these targets and to be used for improved and selective diagnosis of AD as well as new drug targets. Detailed understanding of the nature of the target binding sites is needed using both competition binding experiments and computational modeling studies.

Method: We capitalize on modern *in silico* methods to gain insight into the basic mechanisms and properties of biostructures at the molecular and nanoscale levels that are connected to early stages of AD Our approaches are generic and rational, i.e. based on the principles of quantum and classical physics where each electron or atom can make a difference allowing for accurate characterization of binding and spectroscopic properties of molecules bound at the target biostructures. We are employing supercomputers to carry out the sequence of computational approaches - molecular docking, molecular dynamics, metadynamics, free-energy calculations. The modeling furthermore addresses the structural dynamics of oligomerization and fibrilization processes and the misfolding of proteins with relevance for AD. All modeling is carried out with feedback and validation, where critical experimental data, from *in vitro* as well as *in vivo binding* studies, are used to secure the applicability or for further refinement of the models.

Results: Several core and surface sites of amyloid beta fibrils have been explored. The modeling studies could reproduce experimental obtained affinity data for most of the existing tracers and amyloid binding molecules, suggesting that the available computational models can be reliably used for further improving the specificity of the tracers by optimizing their interaction in specific core sites. Competition binding studies in AD autopsy brain tissue show that tracers like PIB, BTA-1, Me-BTA1, florbetaben, AZD2184, bind to multiple sites in amyloid fibrils targets with varying binding affinity. The computational studies could reveal the binding sites with larger binding affinity. Recent experiment studies also showed difference in nature of the tracers binding for the sporadic and autosomal variants of AD suggesting that conformational nature of amyloid beta aggregates can alter the binding profiles of tracers. The modeling studies are in progress to address the difference in tracers binding to mutants of amyloid fibril. In the case of α7-nAChR, we have designed novel tracer molecules with improved binding affinity based on virtual screening and free energy calculations (FEP+). These are currently used for experimental validation.

Conclusion: Based on our scientific multidisciplinary approach including a large-scale *in-silico* molecular profiling protocol we demonstrate the possibility to unravel the nature of binding sites, modes and binding dependent spectroscopic properties of small molecules in amyloid fibril targets. Specific PET tracers have from theoretical modelling been designed for ligands of alpha7 nicotinic receptors and awaiting for experimental biological validation.

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Targeting tau pathology in Alzheimer brain-translational characterization of binding properties of new PET tracers.

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The aggregation of abnormally hyperphosphorylated tau protein into paired helical filaments represents a major pathological hallmark of Alzheimer disease (AD) as well as other tauopathies Molecular imaging and, in particular, the recent development of tau-specific tracers in Positron Emission Tomography (PET) allowed for *in vivo* visualization of tau aggregates. Tau aggregates are present in different forms and it is important to compare the binding properties of different tau PET tracers in AD as well as other tauopathies. In this study we compared two tau PET tracers belonging to different chemical families[^{11}C]THK5351 and [^{11}C]PBB3, both *in vitro* using binding assay as well as autoradiography techniques and *in vivo* using PET imaging.

Methods: The two PET tracers have been labeled with ^{11}C via direct alkylation with [^{11}C]Methyl iodide. *In vitro* binding assays using [^{3}H]THK5351 in competition with T807 (tau PET tracer) were performed on hippocampal brain homogenate from AD (n=3). *In vitro* autoradiography on adjacent large frozen brain hemisphere sections from three AD cases was performed using both [^{11}C]THK5351 and [^{11}C]PBB3. Competition autoradiography studies were done using unlabeled THK5351, PBB3 as well as T807. A different group of patients with clinical diagnoses of mild cognitive impairment or AD was recruited for the *in vivo* PET investigations. Each patient underwent [^{11}C]THK5351 as well as [^{11}C]PBB3 PET scans at close interval (< 1 month).

Results: The regional cortical distributions observed *in vitro* were comparable between [^{11}C]THK5351 and [^{11}C]PBB3 although more binding in subcortical regions was observed in case of [^{11}C]THK5351. The quantitative analysis showed that the specific binding was much lower for [^{11}C]PBB3 compared to [^{11}C]THK5351. The competition study both in vitro binding assay as well as in autoradiography demonstrate that the different tau PET tracers have different binding sites. *In vivo,* [^{11}C]THK5351 compared to [^{11}C]PBB3 showed greater grey matter binding and greater binding in subcortical regions, in agreement with the *in vitro* observations. Both tracers showed binding in the temporal lobes and other isocortical areas, however with a distinct regional distribution for every tracer.

Conclusions: To date, this is the first translational study to investigate the comparability of two different tau-specific ^{11}C-labeled PET tracers in the same patients with AD. This study will shed new light into the binding properties of the tracers as well as the underlying interrelationship between the tau tracers' binding with other markers of AD.

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MRI and PET approaches to define AD subtypes

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Background: Alzheimer's disease (AD) is a neurodegenerative disease, characterized by abnormal aggregation of amyloid-beta (Aβ) peptides into and hyperphosphorylated tau protein into tangles in the brain. Subtypes of AD have been defined based on the distribution of tangles (typical AD, limbic predominant and hippocampal sparing). These findings have been confirmed using magnetic resonance imaging (MRI) and positron emission tomography (PET) imaging, with atrophy patterns and regional hypometabolism matching the postmortem distribution of tangles. Both structural MRI and PET are now playing a great role in clinical settings, increasing the specificity of differential diagnosis.

Aim: The first aim of this study is to confirm these findings as well as translate them into clinical practice by defining subtypes using visual assessment of MRI (used to support the clinical diagnosis today). The second aim is to characterize the subtypes (cross-sectionally and longitudinally), using a combination of clinical variables as well as more advanced MRI based methods, and PET glucose metabolism and amyloid plaque deposition.

Method: Validated visual assessment scales for temporal, frontal and posterior atrophy were used to define subtypes in patients with mild cognitive impairment (MCI), possibly on the way to develop AD, and patients with a diagnosis of AD. The clinical progression was characterized as well as structural and functional changes using MRI in combination with cluster analysis and techniques to model complex brain networks (graph theory). Finally, we characterized the subtypes using PET, a molecular imaging technique that allows for the assessment of functional (metabolism and neuroinflammation) and pathophysiological (Aβ and tau deposition) features in the brain of living patients.

Results: Clinical progression and biomarker patterns have been defined in different subtypes based on patterns of atrophy. Work is currently on going to translate these findings to the early symptomatic (prodromal) stages of AD, and to explore the possibility of defining additional subtypes via the application of MRI based methods and PET biomarker data.

Conclusions: In addition to an improved further understanding of the clinical heterogeneity of AD, our findings will likely help to further refine clinical management and clinical trial enrichment strategies for evaluation of new drug in AD therapies.

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Establishment of a murine norovirus model system for evaluating novel mucosal vaccines

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Norovirus (NoV) is a leading cause of gastroenteritis and winter vomiting disease. Research on human NoV has been hampered by the inability to infect and propagate the virus in cell cultures or in animal models. However, for murine norovirus (MNV), both efficient cell culture systems and mouse models have been documented. In this project we have established a MNV-3 infection model. The aim was to use this model to acquire a detailed understanding of the interaction between NoV and host cells and to allow for the identification of molecular targets for a mucosal vaccine. A TaqMan RT-qPCR system was used to detect MNV-3 RNA in faeces and tissues samples, including Peyer´s patches, mesenteric lymph nodes, small intestine and caecum of orally infected BALB/c mice. MNV-3 induced a chronic infection in the mouse and viral RNA was detected in faeces at least up to 30 days post infection. At that time the mice had developed neutralizing antibodies against the virus. We developed a unique FISH probe for targeting viral RNA which allowed us to monitor infection in vitro and in vivo. We observed MNV-3 infection in dendritic cell and macrophages, but not in B cells, questioning the results from earlier published reports. Interestingly MNV-3 infection of primary murine macrophages was found to induce a significant release of TNF-alpha ($p<0.001$) and IL-6 ($p<0.001$) after 24 hours of infection. To assess protection following mucosal vaccination we established criteria for reduced MNV-3 viral load in different target tissues as we evaluated our unique vaccine candidates based on the CTA1-noro-DD fusion protein .

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The next generation of mucosal vaccines based on combinations of targeted fusion proteins and lipid nanoparticles

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Immune protection against infectious diseases is most effective if located at the portal of entry of the pathogen. Hence, there is an increasing demand for vaccine formulations that can induce strong protective immunity at mucosal surfaces. We aimed at developing the next generation of effective mucosal vaccines based on the combination of targeted fusion proteins and lipid nanoparticles. Recent technological advancements and a better understanding of the principles that govern priming of mucosal immune responses have greatly facilitated this development and contributed to a more optimistic view on mucosal vaccines. We used the adjuvant platform CTA1-DD with incorporated infection-specific peptides to immunize against influenza or norovirus infections. This proof-of-concept study revealed that strong protection was achieved against a lethal challenge-infection with virus, The liposome technology proved ideal for combining protein antigen and adjuvant into an effective mucosal vaccine. More specifically, a liposome vaccine against pandemic flu, based on the conserved M2e peptide from influenza virus and adjuvanted with the CTA1-enzyme from cholera toxin, effectively stimulated M2e-specific antibody and CD4 T cell immunity of life-long duration. We developed unique models allowing for detailed analysis of vaccine-targeting to dendritic cells. These model systems were then used for optimizing the lipid composition and protein loading of the liposome to generate strong protective immunity. Dendritic cell targeting was greatly augmented by employing a single chain antibody against CD103, which replaced the DD-element in the fusion protein. We believe the combination of liposome technology and targeted fusion proteins with adjuvant function based on CTA1 holds much promise for the next generation of highly effective mucosal vaccines.

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Subcomponent vaccine development against human norovirus infections

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Norovirus (NoV) is the leading cause of outbreaks of viral gastroenteritis across all settings and age groups in the world and, thus, a major challenge to global health care systems, driving high societal costs and resources.The goal of our project is to find novel ways to block or prevent NoV infections. We are using different approaches, which target antiviral drugs to inhibit binding to host cell molecules crucial for entry of NoV or are focused on developing effective mucosal vaccines against NoV. Since NoV strains of the GII.4 genotype are causing the majority of worldwide outbreaks, we choose to work with this genotype using three distinct but complementary avenues. 1) The first employs the “classical way” of immunization with recombinantly expressed virus-like particles (VLP) of the GII.4 Dijon or Ast6139 strains to generate monoclonal antibodies (MAbs). The VLPs were used as immunogens and we established a panel of 15 unique monoclonal antibodies (MAbs) of different isotypes, which showed different binding patterns to the two VLPs. By total internal reflection fluorescent microscopy (TIRFM) we found distinctly different inhibition characteristics when assaying for their pseudo-neutralizing capacity of target VLPs to various histo-blood group antigens (HBGAs) of glycosphingolipids in membranes . 2) The second avenue was based on the CTA1-DD fusion protein platform carrying linear peptides for stimulation of NoV specific CD4 T cells and B cells, with peptides representing P1-2 and S-domains of GII.4 NoV. 3) In the third avenue we took advantage of our recently established anti-Dijon MAbs, and well characterized human polyclonal plasma samples with varying antibody titers against NoV, to screen by automated peptide arrays binding activities against linear sequences deduced from the Dijon-strain capsid protein. Several interesting sequences were identified both from the protruding P1-P2 domains as well as from the shell (S) domain. For an original and unique vaccine approach we have selected the best combinations of peptide candidates for a novel mucosal fusion-protein vaccine construct with genotype or strain specificity.

Our experiments will greatly contribute to the development of a second generation of mucosal vaccines against NoV. Our studies also provide important conceptual advancements on how to prevent NoV infections in general by blocking.

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SSF Framework Project for Research on Novel Biomarkers of Clinical Relevance

Project title: BIO IBD – A multi-modal national study to identify BIOmarkers for diagnosis, therapy response and disease progression in IBD

Cultured blood T cell responses predict anti-TNF therapy response in patients with ulcerative colitis

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Background
Anti-tumour necrosis factor (TNF) therapy is used for treatment of ulcerative colitis (UC). Since approximately 30% of patients with UC do not benefit from the treatment, it is of clinical interest to identify biomarkers of response before therapy is initiated.

Aim
To identify prognostic biomarkers of anti-TNF therapy response in anti-TNF therapy-naïve patients with UC.

Methods
Peripheral blood cells were obtained from 56 patients with UC before therapy started. Thirty-four **patients were included in an exploratory cohort and 22 patients in a validation cohort.** Blood cells **were stimulated *in vitro* with influenza vaccine with and without anti-TNF. T cell surface receptor expression and** cytokine release were determined (in total 17 variables). Treatment response was evaluated using the Mayo score 12–14 weeks after the first infusion.

Results
In the **exploratory cohort, b**lood cells from the patients showed stronger anti-TNF dependent suppression of T cell surface receptor expression and cytokine secretion among therapy responders than non-responders. In particular, anti-TNF suppressed the expression of CD25 on T cells and secretion of interleukin 5, to a higher degree in responders than in non-responders. These variables were used to a create model to predict therapy outcome, which was confirmed in the validation cohort. Correct classification of future therapy response was achieved in 91% of the cases in the validation cohort.

Conclusion
The effect of anti-TNF on cultured blood T cells, obtained before therapy started, predict treatment outcome in patients with UC.

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SSF Framework Project for Research on Novel Biomarkers of Clinical Relevance

Project title: BIO IBD – A multi-modal national study to identify BIOmarkers for diagnosis, therapy response and disease progression in IBD

SUBPHENOTYPES OF INFLAMMATORY BOWEL DISEASE ARE CHARACTERIZED BY SPECIFIC SERUM PROTEIN PROFILES

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Objective
The inflammatory bowel disease (IBD) serum proteome is still to be defined. We aimed to identify inflammatory serum proteins that discriminate between Crohn's disease (CD), ulcerative colitis (UC), and healthy controls (HCs).

Methods
A panel of 91 inflammatory proteins were quantified in a discovery cohort of CD (n=54), UC patients (n=54), and HCs (n=54) using a proximity extension assay. Candidate proteins were identified by univariate analyses using t-test, with false discovery rate correction. A sparse partial least-squares (sPLS) approach was used to identify additional discriminative proteins by creating a prediction model optimized for its parameters using a double cross-validation leave one out method. A replication cohort was used for validation were mean protein levels were compared to 95% confidence intervals of the discovery cohort. The performance of the prediction models was evaluated by application on the replication cohort and by resampling of the discovery cohort with the disease class permuted, to assess statistical significance level.

Results
By univariate analysis, 17 proteins were identified with significantly different abundances in CD and HCs, and 12 when comparing UC and HCs. Additionally, 64 and 45 discriminant candidate proteins, respectively, were identified with the multivariate approach. Correspondingly, significant cross-validation error rates of 0.11 and 0.19 were observed in the discovery cohort. Only FGF-19 was identified from univariate comparisons of CD and UC, but 37 additional discriminant candidates were identified using the multivariate approach. Using univariate comparisons, 16 of 17 CD-associated proteins and 8 of 12 UC-associated proteins were validated in the replication cohort. The area under the curve for CD and UC was 0.92 and 0.94, respectively, when the sPLS model from the discovery cohort was applied to the replication cohort.

Conclusions
By investigating a panel of inflammatory proteins, we identified several candidate markers that seem to be discriminant for subphenotypes of IBD. Our data highlight the potential of serum proteins in future biomarker identification.

Poster 46

Framework grant - Host pathogen interactions: ETEC and *V.cholerae*

Host genetic factors influence susceptibility to Enterotoxigenic *Escherichia coli*: an association between the Lewis blood group system, *FUT2* and infection.

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There is strong evidence to suggest that changes in the host expression of ABH and Lewis antigens could be major determinants of altered susceptibility to infection. The likely reason for this being blood group antigens can serve as receptors or co-receptors for microorganisms, parasites or viruses. For instance, in humans a homozygous inactivating mutation of the α1,2-fucosyltransferase 2 (*FUT2*) gene leads to the absence of ABH blood group antigens and changes in Lewis antigen expression (commonly known as non-secretor status) in the intestinal mucosa. Non-secretor status is extremely heterogeneous and ethnic specific and is found in approximately 20% of the worldwide population.

However, in Bangladesh published studies have interestingly shown that as many as 40% of all Bangladeshis could have non-secretor status. Moreover, children under the age of two who are living in Dhaka have been found to be more likely suffering with symptomatic enterotoxigenic *E. coli* (ETEC) infection if they are non-secretors. A plausible explanation for this association being that ETEC expressing the colonisation factor antigen I (CFA/I) fimbriae are thought to bind to the Lewis non-secretor antigen known as Le^a.

To evaluate if *FUT2* non-secretor status can play a direct role in altering susceptibility to symptomatic ETEC CFA/I infection, we are performing an association study in over 200 healthy and ETEC infected Bangladeshi children to determine if a *FUT2* single nucleotide polymorphism (SNP) is directly associated with altered susceptibility to ETEC CFA/I infection. Results so far indicate a homozygous *FUT2* missense mutation known as rs200157007-TT is highly prevalent in the Bangladeshi population and is associated with non-secretor status ($P<0.0006$).

To support to this work, we have also constructed genetically engineered CHO-K1 cells with different glycosyltransferase cDNAs involved in human Lewis antigen biosynthesis (Le^a, Le^b and H). Using quantifiable immunofluorescence techniques and qRT-PCR, we are using these CHO-K1 lines as a model system to study the binding of ETEC CFA/I fimbrae and closely related ETEC colonization factors to different Lewis antigen determinants found in the human small intestine. So far we show that the major subunit CfaB of the CFA/I colonization factor fimbrae binds stronger to CHO-K1 cells expressing Le^a compared to those CHO-K1 cells expressing Le^b and H.

Poster 47

Host pathogen interactions: ETEC and *V. cholera*

Defining virulence: plasmid gene content and conservation within a clonal lineage of enterotoxigenic *Escherichia coli* (ETEC)

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Enterotoxigenic *Escherichia coli* (ETEC) remains a major cause of child mortality in low-income endemic countries. We have recently found that this pathogen consists of several major clonal lineages that are isolated from patients globally. One of these lineages, L1, expresses the ETEC toxins LT and STh and the colonization factors CS1, CS3 and CS21. L1 is associated with diarrheal disease in children and all strains that express this virulence profile appear to be clonal and derived from one single ancestral isolate that emerged approximately 60 years ago and then managed to spread globally. Thus, L1 isolates must harbor inherent genetic traits that confer virulence and ability to spread in the environment and we hypothesize that the acquisition of specific virulence plasmids in an ancestral *E. coli* created L1. However, if plasmid content in pathogens like L1 change or evolve over time is not known.

To study this we determined the genetic content of L1 chromosomes and plasmids and their evolution over time. We used PacBio sequencing and two libraries of 10 kb and 2 kb to assemble the full genome and plasmid contents of 15 L1 ETEC isolated from children with diarrhea in Latin America, Africa and Asia between 1980-2008. The sequencing revealed that all isolates had a chromosome of 4.8 million bp encoding an average of 4550 genes. The chromosome phylogeny divided the strains into two major groups but no temporal or geographical distribution patterns could be found. In addition, all isolates contained 3-5 plasmids. The biggest plasmid p110 (110 kb) encoded LT, STh and CS3. The p70 plasmid (54-76 kb) contained CS1. Most of the clinical isolates also had an additional plasmid; p56 (73-51 kb), and a p43 plasmid (39-45 kb) that harbored the gene for EatA.

Antibiotic resistance profiles and metabolic analyses showed low prevalence of antibiotic resistance that did not increase over time. Only three isolates from Latin America isolated 1994, and 2007-2008 carried small plasmids with resistance genes against streptomycin, beta-lactamase (TEM) and trimetroprim. Resistance against ampicillin and amoxycillin was confirmed using E-tests in the three isolates. One of the 2008 isolates was also resistant to tetracycline. All other isolates were susceptible to the antibiotics tested. Metabolic analysis of 48 carbon sources showed that the L1 isolates were able to ferment deoxyribose but generally unable to ferment fucose. Both carbon sources have been suggested to facilitate pathogenic intestinal colonization.

Our results show that L1 harbors a generally stable chromosome and plasmid content over time and the success of this pathotype is not due to acquisition of antibiotic resistance.

Poster 48

Rambidrag: Host pathogen interactions: ETEC and *V. cholera*

Poster title**: A retrospective analysis of serotype switching of *Vibrio cholerae* O1 in cholera endemic region shows it to be a non-random process: Further findings**

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Vibrio cholerae of the O1 serogroup cause epidemic cholera. The serogroup consists of two serotypes called Ogawa and Inaba that differ only in the methylation of the terminal sugar of the O-antigen. The methylation is dependent upon the *wbeT* gene product (an S-adenosylmethionine-dependent methyltransferase) and mutations in this gene that abrogate methylation result in a change from the methylated Ogawa to the non-methylated Inaba serotype. Currently, since both cause a similar disease, there is little perceived difference between the two serotypes. In the effort to develop a novel simplified oral cholera vaccine formulation (now undergoing clinical trials), we have manipulated the *wbeT* gene so as to generate a gene product with reduced methylation activity so that both Ogawa and Inaba antigens are expressed on the same cell (the so-called Hikojima serotype). In the course of this work we found that naturally occurring Inaba isolates are of two general types: One (type a) in which the *wbeT* gene is disrupted by mutations resulting in a truncated gene product and one (type b) in which a single base change results in an amino acid change that abrogates the methylation activity of a full length protein.
A detailed epidemiological study of *V. cholerae* isolates in an endemic area in Kolkata, India indicated that the two serotypes emerge in response to selective pressure and it has become evident that there are significant differences between them. Overall, selection is driven by the structure of the O-antigen such that Ogawa strains normally predominate, but Inaba isolates emerge during periods when Ogawa isolates are suppressed. However, in periods of Inaba predominance there also appears to be a difference between the different Inaba isolates such that type (a) mutants have limited ability to spread and only cause sporadic cases whereas type (b) mutants are able to expand and cause significant outbreaks. The reasons for this are unclear, but independent emergence of similar mutations in different settings shows that the phenomenon is widespread. Since the two types of mutant have the same Inaba serotype we were interested in finding underlying differences between them.
We have therefore generated isogenic Ogawa and Inaba strains with Inaba strains of both the (a) and (b) types and shown significant differences in the transcriptome between the different mutant strains compared with the Ogawa wild type grown under similar conditions, with significant differences in the expression of more than 150 genes. Significantly, there are also important differences between the *wbeT* (a) and (b) type mutants that may contribute to the epidemiological observations.
Further work aims to investigate the role of these genes by generating specific mutants and testing them in the infant mouse cholera model. The role of the virulence genes directly involved in pathogenesis are well studied, but it is clear from these studies that the impact of more subtle changes in the genome can have a direct impact on the ability of emerging strains to cause cholera outbreaks.

Karlsson, S. L., N. Thomson, A. Mutreja, T. Connor, D. Sur, M. Ali, J. Clemens, G. Dougan, J. Holmgren and M. Lebens (2016). "Retrospective Analysis of Serotype Switching of Vibrio cholerae O1 in a Cholera Endemic Region Shows It Is a Non-random Process." PLoS Negl Trop Dis 10(10): e0005044.

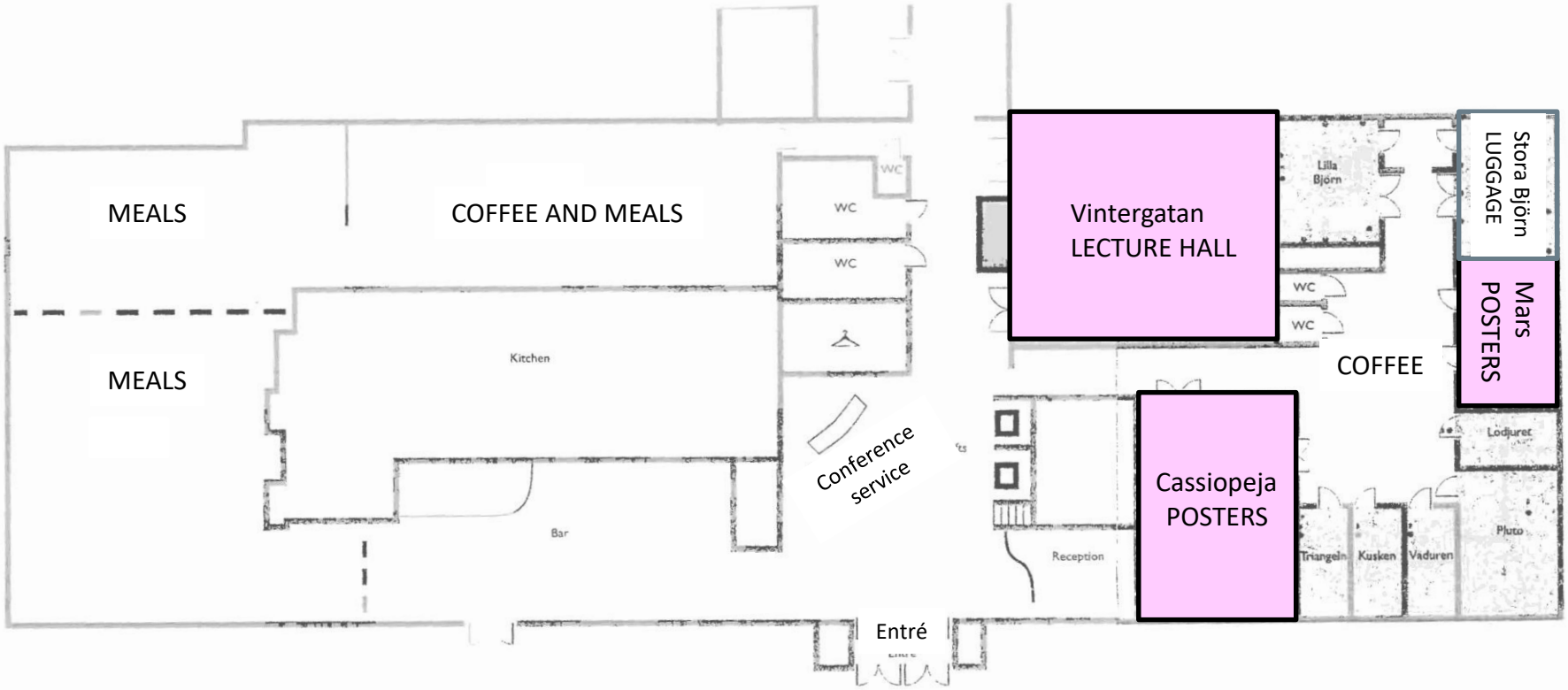
Poster 49

Methodologies and tools for generating international press coverage for a research article

Ben Libberton, Agneta Richter-Dahlfors

Swedish Medical Nanoscience Center, Karolinska Institutet

The research at the Swedish Medical Nanoscience Center is constantly being promoted to the public as well as the international press. A specific workflow has been designed in order to incorporate outreach in to the day-to-day running of the center. We use different social media platforms to both solicit engagement from the public, but also to help us develop a culture where collecting media friendly content such as photos, videos and popular-science summaries becomes second nature. In this poster, I will share the strategy that we use as well as how to incorporate it into your own group workflow. The strategy revolves around the social media platform Twitter which has been used to gain the attention of many authoritative journalists from places such as the BBC, CNN, the Washington Post, New Scientist and Nature News.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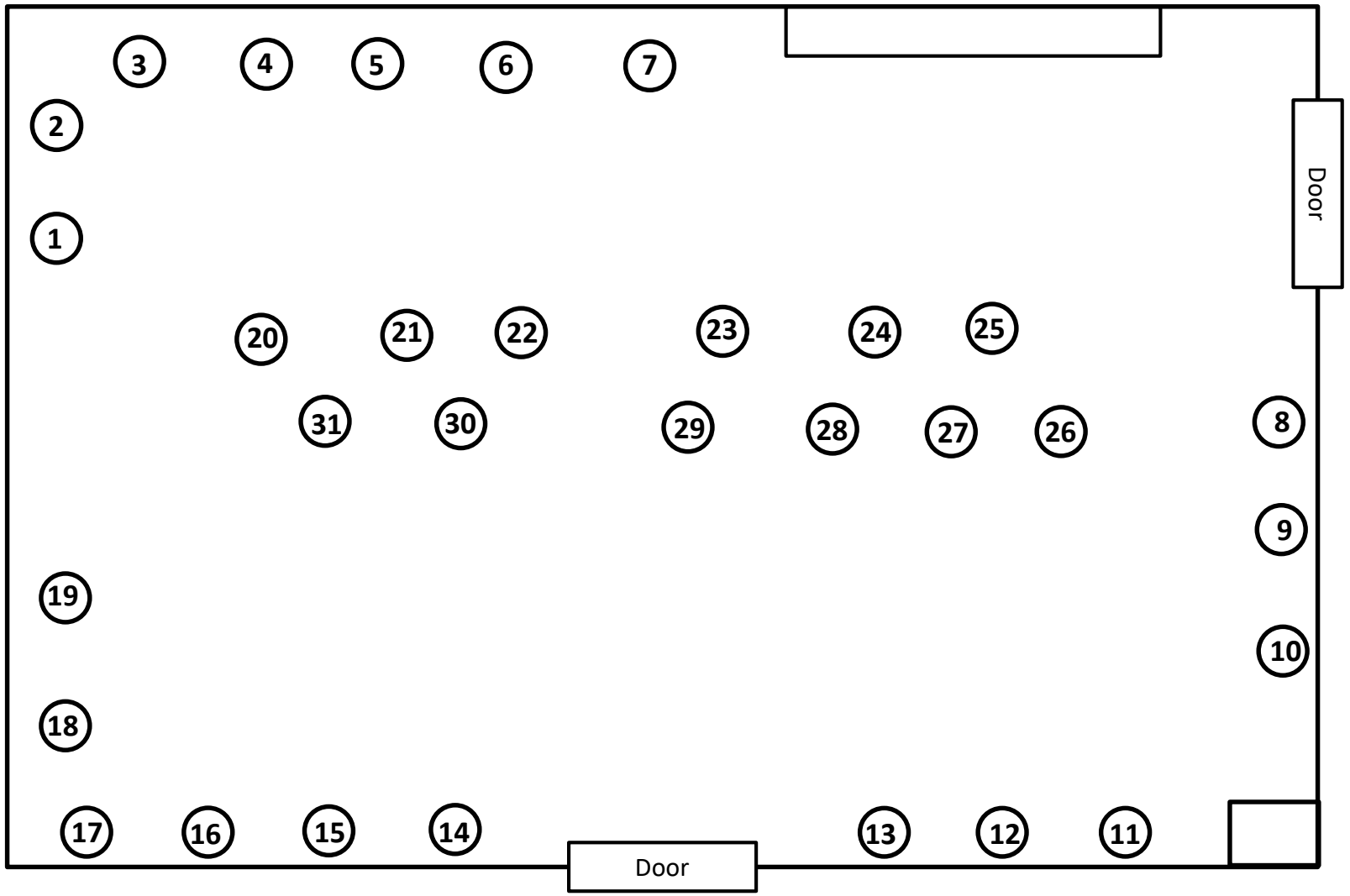
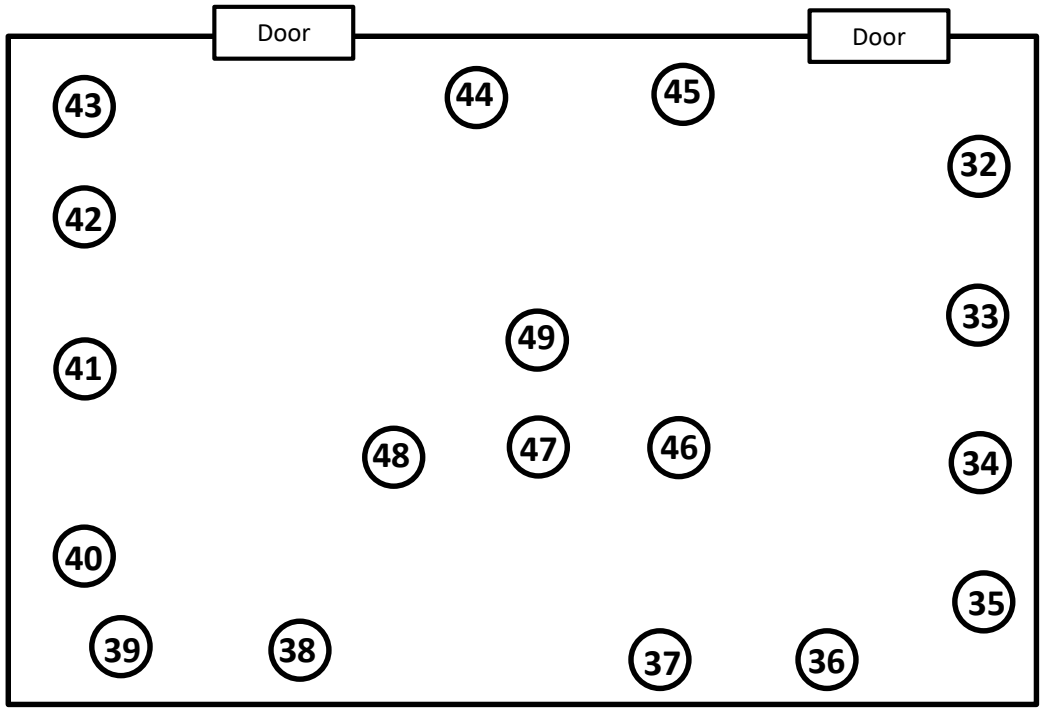


Room Mars – display of posters



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