Nordic Flow Cytometry Meeting

August 30 – September 1, 2017

CPH Conference at DGI Byen

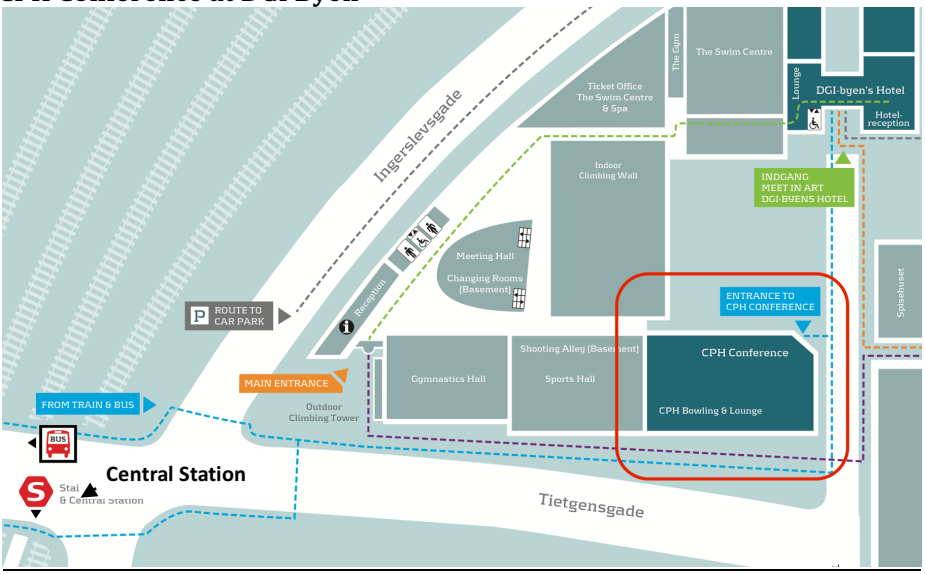
Copenhagen, Denmark

DSFCM Dansk Selskab for Flowcytometri

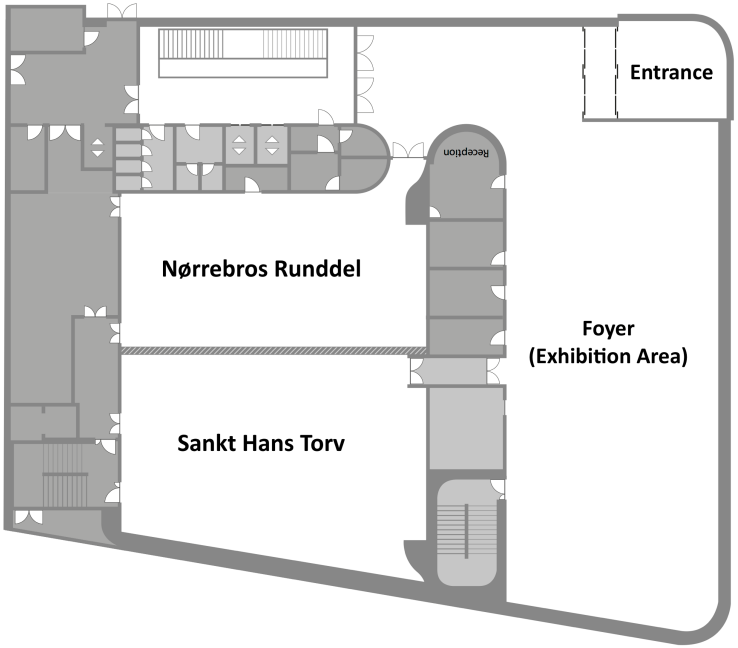
NFCF Norsk FlowCytometriForening

SVENSK FÖRENING FÖR FLÖDESCYTOMETRI

CPH Conference at DGI Byen



CPH Conference Ground Floor



WELCOME

Dear Colleagues,

Welcome to the Nordic Flow Cytometry Meeting – NFCM2017 – in Copenhagen.

With a vision of establishing a joint Nordic flow cytometry meeting of high quality and international appeal, this conference is the end product of a fruitful collaboration between the Norwegian Society for Flow Cytometry, the Swedish Society for Flow Cytometry, and the Danish Society for Flow Cytometry.

We have put together a program packed with excellent speakers from around the world - the common denominator being that they are all outstanding flow cytometrists. With this line-up, we look forward to lively and engaging discussions in and out of the sessions.

In addition to networking with fellow flow cytometrists during the coffee and lunch breaks in the foyer, there will also be unique opportunities to interact with companies providing flow cytometry related technology, software and reagents. The company profiles for all exhibitors can be found in this booklet.

We hope you enjoy the conference and we hope to see you again in future meetings in the Nordics and around the world!

On behalf of the organizing committee,

Line Nederby
Jan Pravsgaard Christensen
Charlotte Christie Petersen
Anna Fossum
Jacob Larsen
Gelo de la Cruz

For more information about the Nordic flow cytometry societies:

Denmark: www.flowcytometri.dk
Norway: www.flowcytometri.no
Sweden: www.sfff.se

PROGRAM

Day 1 Wednesday, August 30

08:00 - 13:00	**Registration**	*Foyer*
09:00 - 12:00	**Summer school** (Mandatory for PhD students)	*Sankt Hans Torv*
09:00 – 09:10	**Welcome** Jan Pravsgaard Christensen (DSFCM Board member)	
09:10 – 09:40	**Time to Flex: multiple applications of flow cytometry** Karen Hogg, *University of York (UK)*	
09:40 – 10:15	**Applications of intracellular staining** Karen Hogg, *University of York (UK)*	
10:15 – 10:30	**Coffee break**	
10:30 – 11:00	**RNA analysis using flow cytometry** Rui Gardner, *Memorial Sloan Kettering Cancer Center (USA)*	
11:15 – 11:40	**Tips and tricks for panel design and panel optimization** Andrew Filby, *Newcastle University (UK)*	
11:40 – 12:00	**Best practice for publishing flow cytometric data** Andrew Filby*, Newcastle University (UK)*	
12:00 - 13:00	**Lunch**	
13:00 - 13:10	**Welcome by all three Scandinavian societies**	*Sankt Hans Torv*
13:10 - 13:50	Session 1 **Measurement of DNA using flow cytometry** Trond Stokke, *Oslo University Hospital (NO)*	*Sankt Hans Torv*
13:50 - 14:30	Session 2 **Cutting edge tools for cell sorting** Rui Gardner, *Memorial Sloan Kettering Cancer Center (USA)*	*Sankt Hans Torv*
Session 1 & 2 Chair:	Bo Baldetorp, Lund University (SE)	
14:30 - 15:00	**Coffee**	*Foyer*
15:00 - 15:45	Session 3 **NK cells and treatment options for human cancer** Hans-Gustaf Ljunggren*, Karolinska Institute (SE)*	*Sankt Hans Torv*

15:45 - 16:15 Session 4 *Sankt Hans Torv*
Standardizing Antibody Panel Design for Multicolor Flow Cytometry
Michael Kapinsky, *Beckman Coulter (FR)*

Session 3 & 4 Chair: Bjarne Kuno Møller, Aarhus University Hospital (DK)

16:30 - 17:30 Session 5 - Keynote and ISAC Lecture *Sankt Hans Torv*
Precision Immunology Through Deeper Single Cell Profiling
Pratip Chattopadhyay*, New York University, (USA)*

Session 5 Chair: Gelo de la Cruz, The Danish Stem Cell Center (DK)

17:30 - 18:30 **Poster viewing and happy hour** *Foyer*

18:30 **Departure for Canal tour** *Entrance*

19:00 - 20:00 **Copenhagen Canal tour** (advanced registration required)

Day 2 Thursday, August 31

08:00 - 10:00 **Registration**

09:00 - 09:45 Session 6 *Sankt Hans Torv*
Transcriptional mechanisms that promote differentiation while preserving potency in embryonic stem cells - how stem cells can become heterogeneous
Joshua Brickman, *NNF Center for Stem Cell Biology - DanStem (DK)*

09:45 - 10:30 Session 7 *Sankt Hans Torv*
Development of high throughput flow cytometry assays
Johannes Landskron, *University of Oslo (NO)*

Session 6 & 7 Chair: Hans Christian Aass, Oslo University Hospital (NO)

10:30 - 11:00 **Coffee** *Foyer*

11:00 - 12:30 **Parallel Session 1**

Clinical *Sankt Hans Torv*

Co-Chairs: Line Nederby, Vejle Hospital (DK) and Jacob Larsen, Roskilde Hospital (DK)

(1) 11:00 - 11:15 **The formation of activated platelet-complexed leukocytes is augmented in cirrhosis and may be enhanced by platelet transfusion**. Sidsel Støy, *Aarhus University Hospital (DK)*

(2) 11:15 - 11:30 **Generation of reference material for flow cytometry cross match and Luminex alloantibody single antigen assays**. Anna Nowocin, *National Institute for Biological Standards and Control (UK)*

(3) 11:30 - 11:45 **Peptide-MHC-directed expansion of multifunctional antigen-responsive T cells**. Vibeke Mindahl Rasmussen, *National Veterinary Institute of the Technical University of Denmark (DK)*

(4) 11:45 - 12:00 **MRD assessment by flow cytometry in the NMSG/EMN02-MRD study – Status and preliminary results from an ongoing study.** Alexander Schmitz, *Aalborg University Hospital (DK)*

(5) 12:00 - 12:15 **Cellular reference materials for flow cytometry**. Luisa De Jesus Saraiva, *National Institute for Biological Standards and Control (UK)*

(6) 12:15 - 12:30 **T cell recognition of large T and small T antigen in Merkel cell polyomavirus-associated cancer**. Ulla Kring Hansen, *National Veterinary Institute of the Technical University of Denmark (DK)*

Basic Research A *Nørrebros Runddel*

Co-Chairs: Jan Pravsgaard Christensen, University of Copenhagen (DK) and Charlotte Christie Petersen, Aarhus University (DK)

(7) 11:00 - 11:15 **Delineating distinct stages of early erythropoiesis in human bone marrow employing imaging flow cytometry.** Carina Agerbo Rosenberg, *Aarhus University Hospital (DK)*

(8) 11:15 - 11:30 **Zika virus infection: a new in vivo model to define protective immunity.** Loulieta Nazerai*, University of Copenhagen (DK)*

(9) 11:30 - 11:45 **Could natural killer cells play a role in type 1 narcolepsy?** Matilda Degn*, Rigshospitalet (DK)*

(10) 11:45 - 12:00 **The impact of GSK-3 inhibitor and IL-2, IL-7 and IL-21 on memory profile after in vitro culture of T cells.** Ditte Elisabeth Jæhger*, The Technical University of Denmark (DK)*

(11) 12:00 - 12:15 **Automated analysis of flow cytometry data to reduce inter-lab variation in the detection of MHC multimer binding T cells.** Natasja Wulff Pedersen, *The Technical University of Denmark (DK)*

(12) 12:15 - 12:30 **B-cell development is dependent on the transcription factor ERG**. Elisabeth Søndergaard, *University of Copenhagen (DK)*

12:30 - 13:30 **Lunch**

13:30 - 14:30 **Parallel Session 2**

Basic Research B *Sankt Hans Torv*

Co-Chairs: Jan Pravsgaard Christensen, University of Copenhagen (DK) and Charlotte Christie Petersen, Aarhus University (DK)

(13) 13:30 - 13:45 **Assessment of opsonisation potential of chicken serum by flow cytometric based phagocytosis assay**. Frederik Larsen, *Aarhus University (DK)*

(14) 13:45 - 14:00 **Small-particle flow cytometry: a new era in the characterization of extracellular vesicles in liquid biopsies.** Jaco Botha, *Aalborg University Hospital (DK)*

(15) 14:00 - 14:15 **Immunological characterization of the oncopig model and detection of cell-mediated immune responses to cancer.** Nana Overgaard, *The Technical University of Denmark (DK)*

(16) 14:15 - 14:30 **Flow cytometry as analytical technique to evaluate the influence of shear stress on the interaction of a novel drug delivery vehicle with different cell lines.** María José York-Durán, *The Technical University of Denmark (DK)*

Cell Sorting and Flow Applications *Nørrebros Runddel*

Co-Chairs: Anna Fossum, Biotech Research & Innovation Centre (DK) and Gelo de la Cruz, The Danish Stem Cell Center (DK)

(17) 13:30 - 13:45 **Fluorescence reporter gene platform applicable for the detection and quantification of horizontal gene transfer in anoxic environments.** Rafael Pinilla*, University of Copenhagen (DK)*

(18) 13:45 - 14:00 **Molecular heterogeneity of mantle cell lymphoma**. Oriane Cédile, *Odense University Hospital (DK)*

(19) 14:00 - 14:15 **Functional and regulatory characterization of a novel tumor promoting factor in acute myeloid leukemia**. Linea Laursen*, University of Copenhagen (DK)*

(20) 14:15 - 14:30 **Large-scale detection of antigen-responsive t cells using Peptide-MHC multimers labelled with DNA barcodes**. Amalie Kai Bentzen*, The Technical University of Denmark (DK)*

14:30 - 15:00 Coffee *Foyer*

15:00 - 15:45 Session 8 *Sankt Hans Torv*
Analysis and sorting of extracellular vesicles by flow cytometry; a detailed description of sample and machine requirements
Ger Arkesteijn, *University of Utrecht (NL)*

15:45 - 16:30 Session 9 *Sankt Hans Torv*
Drug traffickers, genetic engineers and germ warfare
Karen Hogg, *University of York (UK)*

Session 8 & 9 Chair: Jens Bæk Simonsen, The Technical University of Denmark (DK)

16:30 - 17:30 **Poster session and happy hour**

18:30 - 23:00 **Conference dinner** *Østerbro*
3rd floor of CPH Conference

Day 3 Friday, September 1

09:00 - 09:45 Session 10 *Sankt Hans Torv*
DNA ploidy and S-phase fraction analysis in endometrial cancer- clinical application and comments
Rasmus Green, *Lund University (SE)*

09:45 - 10:30 Session 11 *Sankt Hans Torv*
The role of flow cytometry in the personalized medicine approach to diagnostics and therapeutics of immunodeficiencies.
Bjarne Kuno Møller, *Aarhus University Hospital (DK)*

Session 10 & 11 Chair: Idun Dale Rein, Oslo University Hospital (NO)

10:30 - 11:00 **Coffee** *Foyer*

11:00 - 11:45 Session 12 *Sankt Hans Torv*
Man versus machine: Deep learning for Imaging Flow Cytometry data analysis
Andrew Filby, *Newcastle University (UK)*

11:45 - 12:30 Session 13 *Sankt Hans Torv*
Mass cytometry, beyond the hype - New and powerful discovery tool for biology and medicine
Vinko Tosevski, *University of Zürich (CH)*

Session 12 & 13 Chair: Charlotte Christie Petersen, Aarhus University (DK)

12:30 - 12:45 Closing remarks *Sankt Hans Torv*

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SVENSK FÖRENING FÖR FLÖDESCYTOMETRI

Speaker Abstracts

Session 1: **Measurement of DNA using flow cytometry**

Trond Stokke and Paula DeAngelis
Oslu University Hospital, The Norwegian Radium Hospital, Oslo, Norway

We will in this review briefly go through the history of (DNA) flow cytometry. We will further discuss limitations present when assessing DNA content, with weight on resolution and noise. Practical obstacles will also be discussed, e.g. how the presence of dead cells and aggregates may affect the results, and how to remove such unwanted events. The measurement of "DNA content" by flow cytometry intrinsically relies on the assumption that the binding and fluorescence of the fluorescent DNA stain is stoichiometric, i.e. is proportional to DNA content. This may not always be the case, and, rather than considering this a nuisance, it may be taken advantage of for e.g. chromosome analysis/sorting and distinction of mitotic cells from G2 cells with the same DNA content. We will further discuss the prognostic value of ploidy and S phase fraction in different tumors. At last, some new instruments will be discussed, including the imaging flow cytometer (Imagestream) and mass spec single cell cytometry (CyTOF).

Session 2: **Cutting edge tools in cell sorting**

Rui Gardner
Memorial Sloan-Kettering Cancer Center, New York, NY, USA

From centrifugation to high-speed droplet cell sorting, the capability to separate cells individually or in bulk based on different physical and chemical properties of the cells has revolutionized science in the last decades. In particular, Immunology as well as cell and cancer biology, have benefited from the development of droplet cell sorters, which can separate cells with remarkable purity, relatively low loss, and at incredible speeds.

However, we have become so accustomed to this technology that we have learned to take it for granted. Yet the process itself of charging and sorting single droplets has not evolved that much since its early development. Indeed, there are some limitations in droplet cell sorting that have mostly been overlooked and not appropriately addressed.

In this brief talk I will address some of the limitations of droplet high-speed sorting, but also discuss some of the current great uses of this technology and present a few different emerging tools in cell sorting that will address some of the limitations of the current technology.

Session 3: **Phenotypic and functional characterization as well as therapeutic implications of human natural killer cells**

Hans-Gustaf Ljunggren
Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Natural killer (NK) cells represent a unique subset of lymphocytes, distinct from classical T and B cells and more recently identified innate lymphoid cells (ILCs). NK cells were originally identified on a functional basis, because of their ability to lyse certain tumor cells in vitro without the requirement for prior immune sensitization of the host. We now know that the cells, in an integrative manner with other immune cells, contribute to efficient host anti-microbial and anti-tumor immunity. Furthermore, they are well characterized with respect to origin, differentiation, receptor repertoire, and effector functions. Current insights into NK cell molecular specificity and function have suggested that it might be possible to treat human cancer with NK cells. To approach this goal, we have carefully addressed questions of importance for the development of NK-cell-

based immunotherapies. Based on the questions addressed, we have designed and initiated clinical trials with NK cells in patients with treatment refractory hematological malignancies.

Session 4: **Standardizing antibody panel design for multicolor flow cytometry**

Michael Kapinsky
Beckman Coulter, Marseille, France

When it comes to comparability of flow cytometry data “experimental noise” may impose a major challenge. Standardization obviously is an important counter strategy that can mitigate risk of assay failure and minimize human error sources. Less obvious, also the question of appropriate, hence sensitive panel design can be resolved by standardized approaches. Antibody panels can be constructed towards optimal sensitivity according to objective measures, mainly depending on the antigen expression patterns in the interrogated biological system and the instrument performance characteristics using a defined set of dyes. Novel computational models can help in managing these complex interdependencies to preserve sensitivity in multicolor cytometry.

Session 5: KEYNOTE AND ISAC LECTURE

Precision Immunology Through Deeper Single Cell Profiling

Pratip Chattopadhyay
New York University, New York, NY, USA

Three trends have dominated biomedical research over the last decade. The first, the NIH Roadmap's Single Cell Analysis Program, was founded on the principle that cells are extremely heterogenous, and that this heterogeneity is important in health and disease. For this reason, cells must be characterized individually, rather than by insensitive and misleading analysis of bulk cell populations. This trend renewed appreciation for cellular heterogeneity, and incited a revolution of new technologies that could comprehensively analyze single cells (the second trend, deep profiling). Finally, a third biomedical research trend was sparked by President Obama's Precision Medicine Initiative, which aims to define genomic and proteomic differences between patient groups, and use this information to inform treatment decisions. In this talk, I will discuss my work at the intersection of these three trends, and demonstrate the value of new technologies for comprehensive and complete cellular analysis. I will provide examples of how deep knowledge about immune responses can be attained, using examples drawn from our recent work in immunotherapy and fundamental immunology. This talk will highlight our work developing 30 parameter flow cytometry, single cell RNA sequencing, CITE-Seq (for simultaneous measurement of protein and transcripts), and new bioinformatic tools.

Session 6: **Transcriptional mechanisms that promote differentiation while preserving potency in embryonic stem cells - how stem cells can become heterogeneous**

William Hamilton, Jurriaan J. Hölzenspies, Rob Illingworth, Wendy Bickmore and Joshua Brickman
Novo Nordisk Foundation for Stem Cell Biology (DanStem), Copenhagen, DK

Embryonic stem cells (ESCs) are immortal cell lines derived from the peri-implantation mammalian embryo. Both ESCs and the embryos from which they are derived are remarkable, in that individual cells retrain the capacity to begin developmental anew, despite having undergone a degree of patterning or differentiation. Understanding how single cells harness this plasticity and use it to generate heterogeneity in vivo and in vitro has been a major focus of our group. What is the basis for this plasticity at a transcriptional level? We have identified two novel mechanisms regulating this process. The first involves a protective activity of Polycomb co-repressor complexes, that enables transcriptional stimulation in the absence of commitment. The second relies on the reversible manipulation of transcription by Erk signalling. Erk stimulates the induction of differentiation at a transcriptional level, while the stability of specific transcription factors regulates

an Erk independent transition into commitment. The differential regulation stabilities of these factors combined with the reversible activity of Erk on transcription leads to evolving heterogeneities that could represent transition states in development and differentiation.

Session 7: **Development of high-throughput flow cytometry assays**

Johannes Landskron, PR Berg, E Solberg, D McClymont, M Enger, AJ Stokka, K Taskén
University of Oslo, Centre for Molecular Medicine Norway, Nordic EMBL Partnership, Oslo, Norway

Over the years, the technical progress in flow cytometers increased the number of parameters analyzed and the sample throughput significantly. Today, state of the art instruments are equipped with up to five lasers and offer options to sample from 96- or 384-well plates, transforming flow cytometers from single-tube to high-throughput instruments. These developments offer two possibilities to increase the throughput. i) Some channels can be used for sample multiplexing utilizing a method called fluorescent cell barcoding (FCB). There, different samples are stained with concentration series of fluorescent dyes which are coupled to amine-reactive esters. Samples are then combined and run as a single sample on the flow cytometer. Up to 48 samples can easily combined into one. ii) Acquiring directly from 384-well plates. Here, sample preparation is the limiting factor. Since manual handling is not further applicable, assays have to be automated using robotics, which can be a challenge for “difficult protocols” like intracellular staining. In our lab, we developed several automated assays using a Hamilton microlab STAR pipetting robot that contains heat elements for incubations and is connected to a plate centrifuge via a robotic arm. Very small volumes are dispensed using an acoustic liquid handler.

Session 8: **Analysis and sorting of extracellular vesicles by flow cytometry; a detailed description of sample and machine requirements**

Ger Arkesteijn
Utrecht University, Faculty of Veterinay Medicine, Department of Biochemistry and Cellular Biology, Utrecht, The Netherlands

Flow cytometry has become an indispensable tool in biological research. Besides multi-color analysis of biological samples, flow cytometry is widely used to sort and purify populations of cells at high speed based on single cell characteristics. Flow cytometers, as they are currently designed, fulfill the demands to process cells or cell sized particles. During the last decade however, there is an increasing demand to process smaller particles, not least driven by the rapidly expanding research field of extra cellular vesicles (EVs). EVs are in the size range of 50 to 500 nm and it has become evident that these EVs play crucial roles in biological processes. For characterization of EVs, flow cytometry seems to be the method of choice due to the fact that particles can be measured quantitatively and qualitatively with high speed on a single particle basis. However, to be able to analyze particles in the range of EVs, some requirements have to be met before this can be achieved on a flow cytometer. In this presentation a closer look is taken at every step in the process of flow cytometric analysis of EVs. This includes the choice of trigger signal, specific hardware adaptations, the influence of sheath pressure, isolation procedure, concentration of sample and the occurrence of swarm, sorting requirements and the reduction of background signal. This presentations thereby aims to create awareness with respect to the possibilities and impossibilities to measure EVs by flow cytometry.

Session 9: **Drug traffickers, genetic engineers and germ warfare**

Karen Hogg
University of York, Department of Biology, York, UK

Drug traffic by protein transporters has a significant impact on the behaviour and efficacy of a number of drugs. Flow cytometry for the quantitation of fluorescence and proven to correlate the

fluorescence intensity to the number of protein on cells surface. Drug discovery and development groups routinely use active drug trafficking studies in human cell lines and hepatocytes: the interpretation of these results can be limited by the inability to quantify the number of transporters present in the test samples. Here we provide a flow cytometric method for quantification of transporter levels on the cell surface and within the cell and demonstrate that flow cytometry is an important tool for future protein analysis as it may not only quantify the number of proteins that a cell express but also identify the number of proteins on the surface and it is easy to apply for routine assays.

Quantification of DNA by flow cytometry is a well-established method; there are many dyes that bind stochiometrically to DNA. Applications using bacteria, plant and mammalian samples to quantify or study changes in the genome will be described.
Controlling the germ load, with or without drugs, we all have to do. We have used flow cytometry to study models of anti-biotic resistance in E. coli; plasma treatment of Salmonella and the host symbiont interaction between Paramecium and Chlorella.
The applications described all use the quantitation of fluorescence using the powerful tool of flow cytometry to provide absolute numbers.

Session 10: **DNA ploidy and S-phase fraction analysis in endometrial cancer- clinical application and comments**

Rasmus Green, A Hahn, M Bjurberg, L Hartman, A Måsbäck, B Baldetorp
Lund University, Department of Clinical Sciences, Lund, Sweden

Introduction: DNA flow cytometry (FCM) has for many years routinely been used in the Nordic countries as part of the pre-operative risk stratification in endometrial cancer (EC). However, the usefulness of DNA ploidy analysis and the proliferative marker S-phase fraction (SPF) has varied considerably over the years and between different studies. In my presentation I will discuss the improvement of DNA-FCM analyses, the variances between studies and provide a hypothesis as to why the differences in results are observed. I will conclude by presenting our own findings and make some remarks for the future.
Method: Using a retrospectively collected material with 1140 women with stage I EC we performed multivariate Cox regression analysis with data on age, stage, grade, DNA ploidy status, SPF and adjuvant treatment on death in EC, overall survival and time-to-progression.
Results: DNA aneuploidy was not a prognostically adverse factor when adjusted for other included factors on any outcome. SPF retained prognostic significance on all outcomes.
Conclusion: SPF is a significant prognostic factor in early stage EC. In our study DNA aneuploidy did not show any significant prognostic value. We published our findings 2015 and since then the Swedish national clinical guidelines for EC (in which our study is referenced) have been updated to no longer include DNA aneuploidy as a clinically guiding factor, while still being reported in national registries. The optimal cut-off value for SPF is still to be sought, which our study group is currently working on. Preliminary findings may be presented at the conference.

Session 11: **The role of flow cytometry in the personalized medicine approach to diagnostics and therapeutics of immunodeficiencies**

Bjarne Kuno Møller, *on behalf of ICID at Aarhus University Hospital*
Aarhus University Hospital, Department of Clinical Immunology, Aarhus, Denmark

During recent years it has emerged that an increasing number of primary immune-deficiencies are caused by monogenic defects. In particular, the development in NGS technologies has revolutionized this field and allowed identification of the genetic basis of a rapidly growing list of primary immunodeficiencies within and in addition to the classical diagnostic groups.
In the framework of International Center of Immunodeficiency Diseases (ICID), our research program has aimed at developing genetic diagnosis of known immunodeficiencies and, notably,

identification of novel genetic defects predisposing to infectious diseases. This has been achieved employing whole exome sequencing (WES), and through the establishment of different gene panels for specific clinical infectious phenotypes. However, the identified genetic defects are often personal and their significance needs to be verified by functional immunological studies.
Flow cytometry is initially utilized for phenotypical characterization of lymphocyte subsets and thus creating immunological profiles of patients referred for diagnostics to aid in the selection of gene panels for WES. A set of unique cases is presented to illustrate our approach. We furthermore actively employ flow cytometry to establish evidence that identified mutations are causal for the immunodeficiency observed in individual patients.

Session 12: **"Man versus machine": machine/deep learning for imaging flow cytometry**

Andrew Filby
Director of the Cytometry Platform (FCCF), Newcastle University, Newcastle-upon-Tyne, UK

Cellular and molecular heterogeneity pervades all biological systems creating a complex set of challenges for those who wish to understand how individual cells within communities interact with one another in order to influence the phenotype and function of higher organisms. "Cytometry" is the study of cellular systems at the single cell level and can include any technique capable of making multiple, informative measurements at the single cell level. While cytometric technologies such as fluorescence and mass based flow cytometry can currently measure 30-40 parameters per cell, the parameter output from Image-based cytometry systems can be almost infinite and are often continuous (non-discrete) in nature. One such cytometric technology is Imaging flow Cytometry (IFC) that combines the high-throughput capabilities of conventional flow cytometry with the ability to capture spatially registered multi-spectral imagery for each cell as it passes through the system. These include fluorescence-based images as well as transmitted bight-field and laser side scatter (dark-field) imagery that are not dependent on introduced fluorescence (label-free). While these approaches are extremely powerful it is a significant challenge to derive meaningful, objective data from such high parameter output. Here we demonstrate label-free prediction of DNA content and quantification of the mitotic cell cycle phases by applying supervised machine learning and deep learning approaches to morphological features extracted from bright-field and the often overlooked dark-field images of cells using IFC. This method facilitates non-destructive monitoring of cells avoiding potentially confounding effects of fluorescent stains while maximizing available fluorescence channels. The method is effective in cell cycle analysis for mammalian cells, both fixed and live, and can accurately assess the impact of an agent that perturbs the cell cycle at mitosis. As the same method is also effective in predicting the DNA content of fission yeast, it is likely to have a broad application to other cell types and other cellular systems.

Session 13: **Mass cytometry, beyond the hype - new and powerful discovery tool for biology and medicine**

Vinko Tosevski
University of Zürich, Head of Mass Cytometry Facility, Zürich, Switzerland

Today's modern biomedical science critically depends on advanced analytical instrumentation to support the new discoveries. Usually, the development of innovative technologies allows researchers to address hypotheses in previously unattainable ways and quickly leads to novel discoveries and greater insight into the complexity of biological systems. One such very recent development is mass cytometry.

Mass cytometry is an analytical platform that combines the sample preparation workflow typical of flow cytometry and detection capacity of atomic mass spectroscopy. This fusion allows for the measurement of up to 50 different parameters off a single cell at rates between 500 and 1000 cells per second. So far, the technology has been applied with great success in applications like

immunophenotyping, functional cell profiling, quantification of protein levels and their modifications, simultaneous measurement of mRNA transcripts and corresponding proteins, DNA synthesis, cell cycle and more. Owing to its currently unparalleled multiplexing capacity, mass cytometry found wide acceptance and is rapidly transitioning from emerging technology to well established and developed method for high-dimensional single cell analysis.

Parallel Session Abstracts

Clinical Flow Cytometry

(1) **The formation of activated platelet-complexed leukocytes is augmented in cirrhosis and may be enhanced by platelet transfusion** (Poster # 42)

Sidsel Støy[1,2], VC Patel[2], JP Sturgeon[2], GKM Vijay[2], T Lisman[4], W Bernal[3], D Shawcross[2]
[1] Aarhus University Hospital, Department of Hepatology and Gastroenterology, Aarhus, Denmark
[2] King's College London School of Medicine at King's College Hospital, Institute of Liver Studies and Transplantation, London, UK
[3] King's College London School of Medicine at King's College Hospital, Liver Intensive Care Unit, London, UK
[4] University Medical Center Groningen, Surgical Research Laboratory, Groningen, The Netherlands

Background: Dysfunctional immune cells and altered platelet numbers and activation are characteristics of patients with cirrhosis. Activated platelets may form complexes with leukocytes. We have recently reported increased frequencies of platelet-complexed neutrophils (PCNs) in cirrhosis with evidence of neutrophil activation upon contact with healthy platelets in vitro. Whether this occurs in vivo following platelet transfusion and contributes to systemic inflammation and endothelial activation is unknown. We therefore characterised platelet-complexed leukocytes (PCLs) in cirrhosis and investigated the effects of elective platelet transfusion.
Methods: Utilizing flow cytometry, we measured PCLs, activation and function as well as plasma markers of systemic inflammation and endothelial activation in patients with cirrhosis before and after platelet transfusion. Coagulation status was assessed by thromboelastometry and plasma levels of haemostatic proteins.
Results: Patients with cirrhosis had more PCNs than healthy subjects and more platelets attached per individual monocyte and lymphocyte. All PCLs expressed higher levels of activation markers and PCNs had higher resting oxidative burst and phagocytic capacity ($p<0.001$) than their non-platelet-complexed counterparts. Paradoxically, PCL frequency decreased with increasing MELD score. Platelet transfusion reduced time to clot development, accelerated the onset of coagulation and led to greater clot firmness. Furthermore, it augmented platelet activation as measured by sCD40L ($p=0.01$) and increased the percentage of PCMs ($p<0.05$).
Conclusions: Patients with cirrhosis have circulating activated PCLs and following elective platelet transfusion demonstrate evidence of platelet activation and rapid and augmented clot formation.. Furthermore, as platelet leukocyte aggregation accompanies leukocyte activation, this suggests that elective platelet transfusion might exacerbate immune dysfunction in cirrhosis.

(2) **Generation of reference material for flow cytometry cross match and Luminex alloantibody single antigen assays** (Poster # 30)

Anna Nowocin, D Eastwood, L Saraiva, S Diebold
National Institute for Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, UK

Introduction: Flow Cytometry Cross Match (FCXM) is a sensitive and predictive assay for hyper-acute organ rejection. It is a deciding tool in testing HLA sensitisation of matched transplant recipients, used by Histocompatibility and Immunogenetics (H&I) labs. Immunoassays have been found to be particularly problematical from the standardization view point. Use of reference

materials in diagnostic immunoassays helps to reduce variability and provides reliable results regardless of location, time or measurement methods. Most labs use NIBSC reference material for FCXM standardisation in addition to, or instead of in-house controls, since the latter lack uniformity across transplant centres. Here we present a way of replenishing a source of negative diagnostic FCXM reference materials that can be used as a uniform diagnostic control across the H&I labs. In addition, we show the challenges of developing a new positive reference material for crossmatching.

Methods: To generate uniform FCXM reference material human sera or plasma were purified of aggregates and screened using sets of different PBMC donors in FCXM and Luminex alloantibody single antigen (LSA) assays to identify diverse levels of HLA sensitisation. The sera that showed similar levels of reactivity were then pooled, re-tested for changes in allospecificity and freeze-dried to preserve biological activity. The resulting fluorescence shift was calculated as Relative Fluorescence Intensity (RFI) separately for T and B cells populations in FCXM. The potency of the reference material has been assessed against the negative and positive NIBSC standards, which have well-established via previous collaborative studies RFI values. LSA readouts allowed the adjustment for single-antigen specificities in final pool.

Results: As the pooling can affect sera reactivity different pools of human sera were prepared based on their alloantibody titters and pan-reactivity (PRA). High %PRA (≥50%) or high titter (≥10,000MFI) of single alloantibody specificity did not always give the positive crossmatch (RFI ≥2.0) when pooled with negative (MFI ≤ 2,000) or low titter positive (MFI = 2,000-10,000) sera. At the same time single anti-HLA binding by sera pool was achieved when individual low-positive or negative sera were mixed together. It has presented a challenge for precisely planned inclusion/exclusion criteria for positive and negative FCXM reference material that could also be used in LSA assays. However, we succeeded in identifying sera for a negative pool based on the lack of single-antigen cumulative specificities in LSA assay and a negative crossmatch. This pooled trial material was proved to be acting as a good negative control giving RFI between 1 and 1.4 on both T and B cells. The preparation of positive reference material proved to be more challenging with lack of minimal volumes of adequate candidate sera to prepare a pan-reactive FCXM and LSA freeze-dried control.

Conclusions: The in-house controls can give variable levels of background from lab-to-lab and can affect the final decision on levels of HLA sensitisation of patients. The developments of reference materials for FCXM needs to be based on well planned initial preparation and collaborative commutability studies that cover a wide range of operating procedures. NIBSC role is to provide a continuous and long-lasting source of reliably performing reference materials for transfusion and transplantation medicine that can be used by a number of diagnostic labs. The screen of healthy donor sera for FCXM allowed us to generate a pool of negative but not positive sera with HLA class I and II pan-reactivity. The search for a source of allosensitised human material within Europe continues to be hugely challenging.

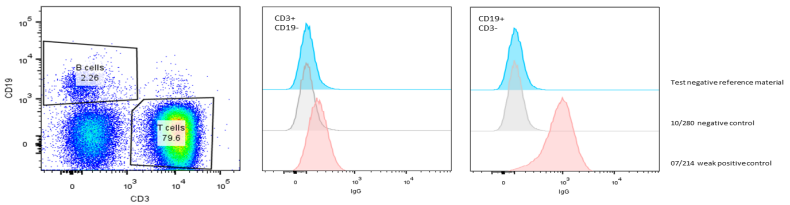

Figure 1. MFI shifts (RFI) of test pool destined to become negative control against 10/280 negative control and 07/214 weak positive control on T and B cells. As the reference material is a negative class I and II control it has to have RFI close to 1 when compared with 10/280 and give RFI of ≥2.0 on both T and B cells when used as negative control with 07/214.

(3) **Peptide-MHC-directed expansion of multifunctional antigen-responsive T cells** (Poster # 1)

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Introduction: The immunotherapeutic approach, adoptive cell transfer (ACT) where tumor infiltrating lymphocytes (TILs) are extracted from the patient's tumor, activated and expanded ex vivo, and subsequently given back to the patient, have in malignant melanoma studies showed clinical durable responses in more than 50% of patients. However, the expansion of TILs requires extensive ex vivo culturing often at the cost of T cell differentiation and functional capacity. Most current strategies involve non-specific expansion of bulk TILs, often providing growth preference to co-infiltrated virus specific T cells and driving an exhausted phenotype of the expanded T cell product. A potential way to resolve this challenge, is the use of artificial antigen-presenting scaffolds providing both an antigen specific stimulation through peptide-Major histocompatibility complex (MHC) interaction and additional the required co-stimulatory and growth signals through associated stimulatory molecules and cytokines.
Method: It is aimed to develop a new technology to expand tumor reactive T cells, through use of MHC-loaded artificial antigen-presenting scaffolds to provide the cells with specific functional stimulation to obtain phenotypic and functional properties to mediate tumor regression. These scaffolds will be build using a dextran-based polysaccharide backbone associated with streptavidins where biotinylated peptide-MHC class I molecules are attached to govern the specific interaction with a specific T cell, and a combination of biotinylated cytokines and co-stimulatory molecules are co-attached to provide stimulation to the T cell to achieve increased functional properties. The artificial antigen-presenting scaffolds interacts specifically with T cells based on recognition of the peptide-MHC molecule and effectively expand and functionally stimulate specific T cells in a peptide-MHC-directed fashion, while leaving all other T cell specificities untouched in a heterogeneous mixture of cells. The specific expansion of the T cells will be traced by tetramers staining and analyzed using flow cytometry.
Results: Results from in vitro experiments have showed that antigen specific CD8 T cells stimulated with these artificial antigen-presenting scaffolds, express a less differentiated phenotype, with high CD28 expression and low PD-1 expression, associated with high proliferation potential and enhanced antitumor effect in vivo. Furthermore, this expansion strategy provides a high frequency of multifunctional antigen specific CD8 T cells expressing IFN-γ, TNF-α, and CD107a upon target recognition. Furthermore the current strategy should allow for simultaneous expansion of numerous (>20) different T cell populations, required to generate T cell products with broad recognition profiles based on the personal cancer-antigen and mutational profile.
Conclusion: This expansion technology could with great advantage be used in ACT, to increase the anti-tumor effect of the transferred T cell product, as all of the achieved T cell characteristics are of significant importance for in vivo tumor cell recognition following ACT of expanded T cell products. Thus, the present strategy is ideal for expansion of cancer-restricted T cells for adoptive cell therapy.

(4) **MRD assessment by flow cytometry in the NMSG/EMN02-MRD study – Status and preliminary results from an ongoing study** (Poster # 37)

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Introduction: Treatment outcome in multiple myeloma (MM) has resulted in a major improvement in the overall response depth due to high dose melphalan and introduction of novel drugs. This resulted in a demand on highly sensitive methods to detect minimal residual disease (MRD) beyond

the response assessment criteria such as complete remission (CR) and stringent CR (sCR), such as MRD assessment by multicolor flow cytometry (MFC). Here we describe our flowMRD method and present current results based on patient data received in a NMSG study as a part of the EMN-02/HOVON-95 MM trial framework.
Aim/Goal: The goals of our study are 1) to generate prospective evidence for the MFC based definition of sCR and 2) to document the predictive potential of ongoing MRD assessment in patients in sCR, following consolidation and during maintenance therapy.
Methods: 138 patients from >20 different nordic centers have been enrolled in the study, and from 48 patients assumed to have reached sCR during/after treatment, we received at least 1 (and up to 15 sequential) follow up sample(s). Using MFC, we analysed 1-2ml of fresh bone marrow sample (optimally within 48h after sending), utilizing the 2 tube diagnostic Euroflow PCD-Panel. Corresponding clinical date were received from the EMN02/Hovon trial database.
Results/Conclusion: Based on the current dataset in this ongoing study, we show preliminary evidence that (1) the definition of sCR by flow cytometry is a number of mPC below 1:10.000, (2) MRD assessment and sCR (k/l) correlates well in the majority of analysed cases, and (3) for individual cases, malignant plasma cell frequencies change with time, even before substantial changes can be measured by the plasma level of free k/l.

(5) **Cellular reference materials for flow cytometry** (Poster # 16)

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Flow cytometry is the work horse of the clinical laboratory. Its major advantages are the quick turnaround time and its relative simplicity. However, its simplicity can be misleading, especially when attempting to quantify rare cells (<1% of WBC). To help establish robust, reliable quantifications there is a real need for physical materials to serve as reference samples. We have generated reference materials for CD4 T cell and CD34 cell counting validated through international collaborative studies. We are currently developing a positive cellular control for Mesenchymal Stromal Cell identity check by flow cytometry and are looking to further support clinical diagnostic needs through the provision of physical reference materials.

(6) **T cell recognition of large T and small T antigen in Merkel cell polyomavirus-associated cancer** (Poster # 21)

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Introduction: Merkel Cell Carcinoma (MCC) is a rare but aggressive human skin cancer induced by Merkel Cell Polyomavirus (MCPyV). The virus is commonly found in human, but the oncogenic transformation primarily takes place in immunosuppressed and elderly people. Two mutations, often inflicted by UV light, allow the clonal integration of the viral genome into the host genome and the translation of the two viral genes large T (LTA) and small T antigen (STA). Standard treatment with chemotherapy shows poor clinical outcome instead immunotherapy could be a new potential strategy given the viral origin of the cancer. The use of PD-1 checkpoint inhibitors in MCC has shown promising results (>50% response rates, RECIST). However, not all patients are able to mount an immune response. Instead adoptive transfer of MCPyV-reactive T cells is an attractive strategy for this patient cohort.

Aim: We have previously identified several T cell epitopes from the MCPyV-derived proteins LTA, STA and viral capside protein1 (VP1), restricted to HLA-A01, -A02, -A03, -A11, -A24 and B07. Here we aim to expand the knowledge about T cell epitopes by including a broader range of HLA restrictions (HLA-B08, -B35 and -B44).
Method: We analyzed 31 patients' peripheral blood mononuclear cells (PBMC) by first using a peptide-MHC-based enrichment, which allowed detection of low frequency T cell clones. T cell reactivity against any of the MCPyV-derived epitopes was revealed using combinatorial color-encoding of pMHC multimers and flow cytometry analysis. 3 patients' tumor infiltrating lymphocytes (TIL) were also analyzed by direct ex vivo detection of MCPyV-reactive T cells using combinatorial color-encoding of pMHC multimers and flow cytometry analysis. Detected responses against MCPyV-derived epitopes were further investigated in terms of T cell functionality where specific lysis of target cells carrying the epitope could be monitored with flow cytometry.
Results: In total 28 T cell responses against 18 different peptides were detected in the PBMC samples. So far functional testing has confirmed functional T cell reactivity against a single of these epitopes. 5 T cell responses against 5 different MCPyV-derived epitopes were detected in the TIL samples.
Conclusion: Investigating the functional T cell response towards the newly detected MCPyV-derived epitopes is still in progress and will use MCC tumor cell lines as target cells. This will also reveal whether the epitope is actually presented on MCC tumor cells. Defining MCPyV-derived epitopes which are both presented on tumor cells and cable of eliciting an immune response could potentially lead to a new personalized T cell based therapy based on the patient's HLA type.

Basic Research A

(7) **Delineating distinct stages of early erythropoiesis in human bone marrow employing imaging flow cytometry** (Poster # 33)

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Introduction: Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal diseases characterized by dysplasia in one or more of the myeloid cell lines in the bone marrow (BM). Consequently, MDS patients presents with various degrees of anaemia, infections and bleeding as a consequence of dyserythropoiesis, dysgranulopoiesis and/or dysmegakaryopoiesis. While morphological assessment of BM samples combined with cytogenetic analyses are key parameters in diagnosing and prognosticating theses patients identifying and estimating the extent of dysplasia is difficult, especially in cases where the dysplastic changes are sparse. To obviate this, multicolor flowcytometry (FCM) is gaining acceptance as a diagnostic tool in MDS by identifying antigenic expression aberrancies in the granulocytic and monocytic lineages together with a robust enumeration of immature myeloid cells. By contrast, the erythroid and megakaryocytic cell lineages are less well characterized by FCM. Imaging flow cytometry (IFC) provides a novel methodology, which allows for simultaneous assessment of immunophenotypic and morphometric parameters in a high number of cells. We hypothesized that this approach could delineate early erythropoiesis in BM from healthy donors, providing a prerequisite for studying dysplastic features in BM samples from patients with MDS.
Methods: Thawed human BM samples were DNA stained with 0.5 µg/mL Hoechst 33342 (Chemometec) in complete growth medium 15 min/37°C prior to antibody staining. The cells were washed and stained with the following monoclonal antibodies: CD235a-PE, DAKO; CD45-ECD, Beckman Coulter; CD71-PerCP-Cy5.5, BioLegend; CD117-PE-Cy7 and CD105-APC, BD Bioscience. For RNA staining 0.5 ug/mL Thiazole Orange (TO, Sigma-Aldrich/Merck) was included during antibody staining. All antibodies and stains were titrated to secure the optimal concentration for use. Single

stained samples either cells (TO/Hoechst), or capture beads (antibodies) were used as compensation controls. Cells were acquired on an ImageStreamX imaging flow cytometer (Amnis/Merck) using the INSPIRE software with lasers adjusted to maximum values without saturating the brightest stains/cells. Data analysis was performed with the IDEAS software package including its compensation wizard, appropriate masks and features.

Results: Employing the ImageStream we were able to delineate erythropoietic intermediates in BM from a healthy donor by integrating immunophenotypic changes of relevant surface markers, loss of RNA, and enucleation. Erythroblasts were initially gated as CD45neg/lowSSClowHoechstposCD71pos (transferrin receptor) single cells in focus. Expression of CD117 (c-kit) defined the most immature erythroid precursor, the proerythroblast (ProE), and distinguished it from the more mature basophilic erythroblast (BasoE), with both developmental stages expressing CD105 (endoglin) (Fig. 1a). CD105 was gradually lost while expression of CD235a (glycophorin A) was increased during differentiation towards polychromatic- and orthochromatic erythroblasts (PolyE and OrthoE) (Fig. 1b). CD235a persisted throughout the final differentiation to reticulocytes (Retic) and mature erythrocytes (RBC) (Fig. 1c). Immunophenotypically defined developmental stages were confirmed by the image gallery, DNA condensation, enucleation and loss of RNA (Fig. 1c).

Conclusion: Using IFC we were able to identify the seven distinct erythroid developmental stages previously described for healty erythropoiesis in human BM. Together with a refined morphometric analysis, these preliminary data aims at applicability for the identification and characterization of dyserythropoiesis in the BM of MDS patients.

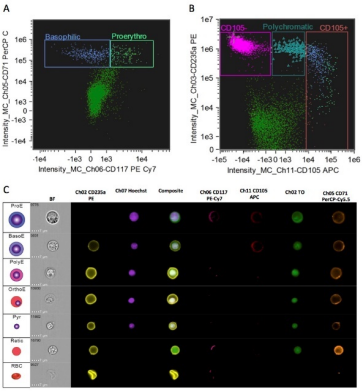

Figure 1. Gating for (A) proerythroblast (light green) and basophilic erythroblasts (blue) based on their CD117 expression in the CD45neg/lowSSC/ lowHoechst/posCD71pos/CD105pos population, and (B) polychromatic and orthochromatic (CD105neg) erythroblasts on the basis of decreased CD105 expression and increased CD235a expression in CD45neg/lowSSClow/Hoechstpos/ CD71pos cells. The lymphocyte population (dark green) served as an internal negative control. (C) Image galleries of representative cells from the various developmental stages.

(8) **Zika virus infection: a new in vivo model to define protective immunity** (Poster # 10)

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Zika virus (ZIKV) is a mosquito-borne flavivirus that has drawn worldwide attention due to its association to neurologic complications, particularly severe congenital malformations. While ZIKV can replicate efficiently and cause disease in human hosts, it fails to replicate to substantial titers in mice except when these lack IFN-I immunity (i.e IFNAR1-/- or STAT1-/- mice). In this study, having in mind that immunocompromised mice are not ideal for studying the complex interplay of ZIKV infection with the host's immune defenses, we developed a murine model where lethal infection is induced in WT mice by introducing the virus directly in the brain via intracerebral (i.c) inoculation. In this way, the antigen is precisely placed at the site of interest, evading the first line of defense, and thus rendering the mice susceptible to infection. We found that, while intravenous (i.v) inoculation of two different strains of WT mice with low doses of ZIKV does not result in viremia, it

is nevertheless able to induce both cell-mediated and humoral immunity as well as clinical protection against subsequent i.c challenge with lethal doses of the virus. In order to determine the contribution of key components of the immune system to the observed protection we employed several KO strains as well as in vivo T-cell depletion and adoptive transfer assays. So far, our results point to a key role for antiviral Abs in clinical protection; however, in the absence of pre-existing Abs a minor impact of cell mediated immunity is revealed.

(9) **Could natural killer cells play a role in type 1 narcolepsy?** (Poster # 17)

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Type 1 Narcolepsy (NT1) is an autoimmune disease targeting the hypocretin-producing neurons leading to sleep/wake disturbances and cataplexy. Genetic studies have found a strong association to the HLA DQB1*0602 and T-cell receptor polymorphisms pointing towards immune cell implication in the pathogenesis. However, an effector cell type has not been characterized in NT1. Pathogenic and regulatory effects of Natural Killer (NK) cells are implicated in several autoimmune diseases, but have not previously been investigated in NT1.
Peripheral Blood Mononuclear Cells from 23 patients and 23 gender and HLA-matched healthy controls (HC) were isolated by Lymphoprep. We analyzed the frequency of CD4, CD8 T-cells, B-cells, NKT cells and NK cells (CD56+CD3-). We investigated the expression of CD57, NKG2D and Natural Cytotoxicity triggering Receptor1 (NKp46) a key receptor initiating NK-mediated cell lysis on NK subsets by flow cytometry.
Patients with NT1 had normal frequencies of T-cells and B-cells, but had higher frequency of NK cells (p=0.0033). To determine the potential role of NK cells in NT1 we investigated the frequency of surface markers of activation and found that patients had higher frequency NK cells in the CD56highNKp46highNKG2Dhigh population (HC 59.4 ± 22.8 (mean ±STD) and NT1 78.6 ±11.6 % of CD56highCD3-) and a lower frequency of the CD56highNKp46lowNKG2Ddim population. The expression levels of Nkp46 and NKG2D, measured as mean fluorescent intensity (MFI), were comparable between controls and NC.
The CD56high NK cells are of a more immature phenotype and can modulate T-cells and the CD56intermediate cells. NKp46high NK cells are highly cytotoxic secreting Interferon gamma (IFN-γ). Mouse studies have found that NKG2D+ NK cells can migrate into the CNS and kill neuronal progenitor cells. Your findings suggest that the NKp46highNKG2Dhigh NK population, more frequent in NT1, could play a role in the loss of hypocretinergic neurons.

(10) **The impact of GSK-3 inhibitor and IL-2, IL-7 and IL-21 on memory profile after in vitro culture of T cells** (Poster # 23)

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Introduction: Adoptive T cell transfer (ACT) is the principle of expanding tumor-reactive T cells from patient samples in vitro before re-administration into the patient for therapeutic effect. The current standard expansion protocol includes the addition of high dose IL-2 to T cell cultures in vitro. The possible limitations with this protocol includes the induction of regulatory T cells and T cell activation-induced cell death. Furthermore, culture in IL-2 enriched media leads to the progressive loss of CD44, driving the T cells towards an effector phenotype. This outcome contrasts with the goal of creating prolonged T cell engraftment after transfer, due to the shorter lifespan of fully differentiated T cells. In contrast, IL-21 in combination with IL-7 or IL-15 has been shown to increase the activity of effector T cells as well as favoring a stem cell like or central memory phenotype, both believed to have a longer lifespan and higher capacity to eradicate tumors. In addition, ex vivo

culture with a small molecule GSK3β inhibitor (TWS119) results in a population of less differentiated T cells, with superior anti-cancer properties. A protocol consisting of pre-enrichment for naïve T cells and culture in the presence of GSK3β inhibitor plus IL-21 and IL-7 can be used to generate a population of 90% stem cell like CD19-specific CAR-T cells. In the following experiments, we assessed the memory phenotype of T cells isolated from pmel-1 transgenic mice after culture with IL-2, IL-21, IL-7 and/or the GSK3β inhibitor (TWS119).
The aims were i) to evaluate a multi-color flow panel for distinguishing memory phenotype and ii) to identify optimal culture conditions for future ACT in vivo experiments.
Methods: Spleens were collected from pmel-1 transgenic mice. T cells were purified from splenic single cell suspensions using a Pan T Cell Isolation Kit. Pmel-1 naive CD8+ T cells were primed in vitro with anti-CD3 and anti-CD28 specific antibodies in conjunction with cytokines and/or TWS119. Four days following T cell activation, phenotype was addressed using flow cytometric analysis using a BD Fortessa X-20 and cytolytic capacity was addressed with a Pierce LDH assay.
Results: We found that addition of TWS119 to the culture media yielded a less differentiated phenotype, as evidenced by a higher preservation of CD44 expression in conjunction with a decrease in in T cell–specific cytotoxicity towards target B16.F10 cells. The effect of TWS119 was markedly decreased when combined with IL-2, whereas a combination of IL-21 and/or IL-7 independently and in combination with TWS119 maintained a subset of less differentiated cells. More studies are required to identify the optimal dosing and combination of cytokines and inhibitor in terms of preserving high cell viability and numbers, while limiting progressive CD44 loss during in vitro culture.
Conclusion: The combination of IL-21, IL-7 and TWS in culture media can be used to expand less differentiated T cells, with a potential impact for ACT clinical application.

(11) **Automated analysis of flow cytometry data to reduce inter-lab variation in the detection of MHC multimer binding T cells** (Poster # 35)

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Introduction: Manual analysis of flow cytometry data and subjective gate-border decisions taken by individuals continue to be a source of variation in the assessment of antigen-specific T cells when comparing data across laboratories, and also over time in individual labs. Therefore, strategies to provide automated analysis of MHC multimer-binding T cells represent an attractive solution to decrease subjectivity and technical variation. The challenge of using an automated analysis approach is that MHC multimer-binding T cell populations are often rare and therefore difficult to detect.
Methods: We used a highly heterogeneous dataset from a recent MHC multimer proficiency panel to assess if MHC multimer-binding CD8+ T cells could be analyzed with computational solutions currently available, and if such analyses would reduce the technical variation across different laboratories. We used three different methods, FLOCK, SWIFT, and ReFlow to analyze flow cytometry data files from 28 laboratories. Each laboratory screened for antigen-responsive T cell

populations with frequency ranging from 0.01-1.5% of lymphocytes within samples from two donors.

Results: Experience from our analysis shows that all three programs can be used for the identification of high to intermediate frequency of MHC multimer-binding T cell populations, with results very similar to that of manual gating. For the less frequent populations (<0.1% of live, single lymphocytes), SWIFT outperformed the other tools.

Conclusion: In this study, we demonstrate the feasibility of using automated analysis pipelines for assessing and identifying even rare populations of antigen responsive T cells and discuss the main properties, differences and advantages of the different methods tested.

(12) **B-cell development is dependent on the transcription factor ERG** (Poster # 38)

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Introduction: B-cells constitute an indispensible part of the adaptive immune system, and several transcription factors have been shown to be essential for their formation.

The transcription factor ERG has previously been shown to have an influence on hematopoietic stem cells and various cancers, including B-cell acute lymphoblastic leukemia.

However, the role of ERG in normal B-cell development is largely uncharacterized. The aim of this project is, thus, to characterize the role of the transcription factor ERG in B-lymphocyte development with particular emphasis on elucidating the underlying transcriptional and translational mechanisms.

Method: We have created an Ergfl/fl:CD2iCre mouse model, in which Erg is deleted exclusively in the lymphoid lineage.

The main method for characterization of the mouse model is flow cytometry. Other methods include in vitro differentiation assays and several high-throughput methods such as proteomics, gene expression and ATAC-sequencing (performed on FACS sorted cells).

Results: The lymphoid-specific knock-out of Erg leads to a dramatic loss of B-cells, which can be detected already at an early progenitor stage.

This cell loss can partly be ascribed to changes in apoptosis and proliferation profiles of the progenitors. Accordingly, gene expression and proteomics analyses show an increase in apoptosis-associated proteins and changes in cell cycle-related genes. Furthermore, we observe a down-regulation of ribosomal proteins followed by a decrease in protein synthesis.

Signaling through the pre-B-cell receptor (pre-BCR) is essential for progenitor B-cell differentiation and proliferation, and several proteins in this pathway are de-regulated. In particular, we see a large decrease in the pre-BCR component immunoglobulin M, which could explain the great loss of B-cells.

Conclusion: We have, for the first time, identified ERG to be of central importance for the development of B-cells. Loss of ERG in the lymphoid cell compartment leads to a severe reduction in B-cells. The deficiency of ERG in the B-cell progenitors leads to changes in key cell properties such as proliferation, apoptosis and translation, which is possibly due to a decreased pre-BCR signaling.

Basic Research B

(13) **Assessment of opsonisation potential of chicken serum by flow cytometric based phagocytosis assay** (Poster # 25)

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Introduction: Antibiotic resistant pathogens pose a serious threat to human health and cause problems in poultry meat production. Antibiotic resistant pathogens, together with the consumers' increased demand for animal welfare and food with better quality and health benefit, have led to a rapidly developing organic poultry production. Sustainable strategies to prevent diseases and improve bird performance, such as selective breeding of robust and immunocompetent chickens, are therefore needed. Innate immunity proteins in serum, such as the opsonins C3 of the complement system and mannose-binding lectin (MBL), play a pivotal role in the first line of defence in chickens against common pathogens. Assessing opsonisation potential in serum of widely used organic chicken lines can thus be an important selection parameter in organic poultry production. Here we report a flow cytometric assay to characterize serum opsonisation potential to assess the robustness of different chicken breeds.
Method: Sera from chickens with different genetic background were used to opsonise fluorescent carboxylate-modified polystyrene latex beads. Latex beads were subsequently added to the culture medium of HD11 chicken macrophages and incubated at 41°C or 4°C. Opsonisation potential was determined using flow cytometry.
Results: Latex beads of size 0.9 – 1.1 µm were selected for the phagocytosis assay. Beads were incubated with serum from two inbred White Cornish chicken lines selected for high MBL serum concentration, L10H (33.4 µg/mL, n=3), and low MBL serum concentration, L10L (7.6 µg/mL, n=3). Opsonisation potential of L10H serum appeared greater than opsonisation potential of L10L serum. An animal experiment was performed to compare the immunocompetence of the local Danish chicken breed Hellevad with two chicken lines widely used in organic production, Bovans Brown (Bovans) and Hisex White (Hisex). Opsonisation with serum from the Hisex chicken line (n=12) resulted in less phagocytosis with a high variation compared to opsonisation with serum from the Bovans chicken line (n=12) and Hellevad chicken line (n=12), which resulted in greater phagocytosis with less variation. The results, hence, suggest differential opsonisation potential of serum from the different chicken lines used.
Conclusion: We show that flow cytometric analysis can be employed to assess immune robustness of organic chickens in respect to serum opsonisation potential, which might be of great value in future selective breeding of immunocompetent organic chicken lines.

(14) **Small-particle flow cytometry: a new era in the characterization of extracellular vesicles in liquid biopsies** (Poster # 13)

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Extracellular vesicles (EVs) are a heterogeneous group of membrane-encapsulated particles ranging from 50-1000nm containing cellular components that are released by cells in latent, activated, and apoptotic states. Therefore, EVs hold great potential as biomarkers in liquid biopsies. Flow cytometry has been a widely used method for quantitation and characterization of EVs. However, the applicability of flow cytometry has been somewhat limited due to the inability of conventional flow cytometers (FCM) to detect smaller EVs (< 500nm). To overcome this issue, recent advances in flow cytometry have led to the development of FCM dedicated to the analysis of small particles (sp-FCM). Here, we report on the performance of an Apogee A60 Micro-PLUS sp-FCM with regards to EV characterization.

In order to assess the sensitivity of sp-FCM, we analysed 100nm silica nanospheres with a refractive index value close to that of EVs. We found that sp-FCM could sufficiently discriminate 100nm nanospheres from background on light scatter alone, thus demonstrating that sp-FCM is significantly more sensitive than conventional FCM. Next, we evaluated the resolution of sp-FCM with a sample consisting of a mixture of six distinct populations of silica nanospheres ranging from 180nm to 1300nm. sp-FCM could effectively discriminate the six nanosphere populations from each other on both small-angle and large-angle light scatter, and the discrimination was consistent for both the small and large nanospheres. When overlaying the silica nanospheres onto a plasma sample labelled with lactadherin-FITC, we demonstrated that sp-FCM is capable of measuring EVs down to 100nm in size, and that the bulk of EVs measured with sp-FCM is within the 100nm to 300nm range, which is consistent with previous studies. Finally, we analysed the reproducibility of EV measurements by analysing 21 aliquots of a single platelet-free plasma (PFP) sample on a single day. PFP aliquots were labelled with a combination of lactadherin-FITC for detecting phosphatidylserine on the surface of EVs, anti-CD41-APC to detect EVs of platelet origin, and anti-CD36-PE to determine the expression of the fatty acid scavenger CD36 on EVs. Little variability was observed in concentration determination of various EV phenotypes (CV=3,68-7,32%) and median fluorescence intensities (MFI) of the EV phenotypes (CV=1,44-6,63%) between aliquots of the PFP sample.

Thus, we conclude that sp-FCM is a powerful tool for EV characterization with sufficient sensitivity, resolution, and reproducibility to accurately characterize the bulk of EVs present in bio-fluids. We believe that sp-FCM holds great potential for increasing our understanding of EVs in pathological conditions, which could lead to discovery of highly sensitive, disease-specific biomarkers in liquid biopsies.

(15) **Immunological characterization of the oncopig model and detection of cell-mediated immune responses to cancer** (Poster # 31)

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In recent years, immunotherapy has shown great breakthroughs; however, the majority of preclinical studies has been based solely on rodent models and tends to experience a troublesome translation to human patients often related to the differences between mice and humans. Instead, we have developed a large animal model, the Oncopig, with inducible tumor formation resulting from KRASG12D and TP53R167H expression upon exposure to an adenoviral vector Cre-recombinase. Due to the high degree of similarity in the immunome, metabolism, and size between humans and pigs, we believe that the Oncopig can serve as a valuable large animal model for translational cancer research. With the establishment of the Immunoscore as a new approach for staging cancer patients, it has become evident that the immune status of the tumor microenvironment has a crucial impact on the patient's outcome and response to therapies. For this reason, we performed an immunological characterization of Oncopig-derived tumors to elucidate the potential in using this model for testing cancer immune therapeutic approaches. We firstly confirmed the ability to induce transgene expression and tumor formation in pigs, and observed substantial T cell infiltration to the tumors independent of the site of induction. We subsequently demonstrated an intratumoral enrichment of the CD8β+ cytotoxic subset, which was found to significantly differ in their expression of perforin, when compared to the circulating T cell pool. Additionally, granzyme B-production was widely distributed within the tumors, further suggesting a cytotoxic tumor microenvironment. Various FoxP3-expressing T cell subsets were also

readily detectable, thus indicative of either an intratumoral regulatory compartment and/or newly activated T cells. To investigate the immunogenicity of the tumor cells themselves, we developed a fluorescence-based in vitro porcine cytotoxicity assay with co-culturing of immune effector cells and tumor target isolates. Here, we demonstrated pronounced killing of autologous tumor cells in an effector-target dependent manner. Hence, our results revealed immunological recognition and tumor-specific cytotoxicity; supporting the potential of using the fully immunocompetent Oncopig as a large animal model for future preclinical testing of immunotherapies against human cancer.

(16) **Flow cytometry as analytical technique to evaluate the influence of shear stress on the interaction of a novel drug delivery vehicle with different cell lines** (Poster # 7)
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Introduction: Although several platforms acting as drug delivery carriers have been developed over the years, only few of them have successfully transitioned from pre-clinical to clinical studies. Even after promising performance in in vivo studies, most of the platforms fail in the clinical trials. A better transition from in vitro studies to in vivo that could help to reducing and optimize the expensive animal studies could be achieved by employing in vitro set ups which mimic better the dynamic human physiology.
Upon intravenous injection, the carriers are exposed to mechanical forces, including shear stress, due to the blood flow which highly affects the cell-carrier interaction. Recent studies have started to give insights on the impact of shear stress in the development of drug delivery carriers in several parameters such as the carriers' cytotoxicity, targeting efficiency or transfection efficiency, among others. Therefore, in vitro studies which improve the traditional well-plate experiments towards more realistic physiological conditions are needed. Aiming to assess the impact of shear stress on the cell-carrier association/uptake, we have exposed two cell lines that are directly exposed to the blood flow (namely vascular endothelial cells and macrophages) to a novel drug delivery system under different shear stress conditions.
Method: The novel carrier will be functionalized with different surface coatings to decrease the uptake by the cells which will, in turn, result in an increased circulation time in vivo. The endothelial and macrophage cells were exposed to zero, static conditions, low or high shear stress mimicking the physiological conditions in the vasculatory system. Finally, the association/uptake of the carrier with the different cells, by means of fluorescence intensity due to the fluorescently labelled drug delivery vehicle, was investigated by flow cytometry by analysing at least 2000 cells.
Results: The exposure to low or high shear stress, corresponding to veins and capillaries, respectively, in all tested cell lines resulted in a different association/uptake depending on the coating of the drug delivery vehicle as compared to static conditions. Confocal laser scanning microscopy was also employed to evaluate the degree of cell internalization of the novel drug delivery vehicle depending on the surface coating and the shear stress conditions employed.
Conclusion: Our results demonstrated that cell internalization and cell association of drug delivery vehicles with different surface coatings, is highly dependent on the presence and the intensity of shear stress used. Thus, we have demonstrated that the effect of shear stress on the interaction of the carrier with different cells that are exposed to the blood flow is an important parameter that needs to be taken into consideration when performing in vitro experiments.

Cell Sorting and Flow Applications

(17) **Fluorescence reporter gene platform applicable for the detection and quantification of horizontal gene transfer in anoxic environments** (Poster # 2)
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Background: The study of horizontal gene transfer (HGT) in microbial communities has been revolutionised by advances in cultivation-independent methods based on fluorescence reporter-gene technologies. However, the use of fluorescent markers like green fluorescent protein (GFP) and mCherry is limited by environmental constraints that affect the correct maturation of their fluorophores, such as oxygen availability and pH levels. Few studies have focused on elucidating their impact, and the sheer amount of distinct protein variants requires each system to be examined in an individual fashion. The wealth of ecologically and clinically relevant oxygen-deprived micro-habitats in which bacteria thrive, calls for the urgent development of suitable tools that permit their study.
Objectives: Development of an aerobic fluorescence recovery method for mCherry and GFPmut3, as well as characterisation of the impact the pH has on their fluorescence intensities. Validation of the dual-labelling system for the study of HGT in anoxic milieus.
Methods: The time-course fluorescent recovery of mCherry and GFPmut3 in vivo, as well as the effect of the extracellular pH on fluorescence was monitored through flow cytometry. The applicability of the findings was validated in anaerobic filter mating experiments.
Conclusions: The findings present a solution to an intrinsic problem that has long hampered the utilisation of this system to study HGT in environments devoid of oxygen, highlight its pH limitations, and provide the experimental tools that will help broaden its horizon of application to other fields.
Keywords: Horizontal Gene Transfer, Aerobic Fluorescence Recovery, Green Fluorescent Protein, mCherry, pH

(18) **Molecular heterogeneity of mantle cell lymphoma** (Poster # 14)

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Introduction: Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma characterized by the translocation t(11;14)(q13;q32), which transposes the cell cycle regulator cyclin D1 (CCND1) (11q13) under control of the immunoglobulin heavy chain (IGH) locus (14q32). Cyclin D1 forms a complex with cyclin-dependent kinases 4 and 6 (CDK4/6) which promotes cell cycle progression. In MCL, the translocation leads to the constitutive overexpression of CCND1 and cell cycle deregulation. The survival of MCL patients is still poor, especially for patients resistant to frontline drugs. Although patients are brought in remission, relapses often occur with disseminated lymphoma, which is more difficult to treat. There is a need for a better understanding of the clonal heterogeneity of this disease and to identify new signaling pathways with genes which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.
Aims: To address the genetic heterogeneity in MCL in paired patient samples at diagnosis and relapse.
Methods: Four patients diagnosed with MCL were analyzed. Frozen peripheral blood mononuclear cells from bone marrow or blood at diagnosis and relapse were used. 7AAD-CD3-CD19+light chain+CD5+CD20+FMC7+ cells were sorted using a FACS ARIA III. 7AAD-CD3+ T cells were sorted from the same patients as paired non-malignant control samples. DNA and RNA were extracted from sorted cells and both exome and transcriptome sequencing were performed. Mutations were detected by exome sequencing and evaluated against the Catalogue of Somatic Mutations in Cancer (COSMIC; Wellcome Trust Sanger Institute), dbSNP and PubMed database. The expression of somatic variants were confirmed with transcriptome analysis. Exemption from informed consent was approved by the National Ethical Committee.

Results: Intersecting somatic variants between whole exome sequencing and variants in the transcriptome showed preserved mutations between diagnosis and relapse. Some of the somatic gene mutations observed were already well-known in B-cell malignancies (e.g. TP53, NOTCH1, ATM and MYD88). We identified new aberrations not previously described in the COSMIC database at diagnosis and at relapse (e.g. SPEN (associated to NOTCH pathway regulation) or TRAF3 (associated to B-cell malignancies)). No somatic mutation was observed to be shared by all four patients. However, aberrations in the same signaling pathways were identified across individuals, such as the NOTCH-pathway. All together, these data support the heterogeneity of the MCL clones.
At relapse we identified new gene mutations (e.g. MYD88 or ATM) as well as loss of previous one (e.g TRAF3), suggesting that a modified malignant clone had evolved and progressed. This was supported by the allele frequency distributions which detected discrete clonal or competing subclonal involvement: A patient harbored one major discrete clone at diagnosis while at relapse two clearly separated groups, with respect to allele frequency, were present.
Conclusion: Our work shows examples of the molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the malignant transformation of MCL and identify new ones involved in the B-cell signaling pathways. This adds valuable knowledge to the biological understanding of MCL which is pivotal in the era of precision medicine.

(19) **Functional and regulatory characterization of a novel tumor promoting factor in acute myeloid leukemia** (Poster # 28)

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Introduction: C/EBPa is a central transcription factor in normal hematopoiesis, as well as a target of key cancer driver mutations. A frequent mutation gives rise to a truncated version of C/EBPa-p30. Using a mouse AML model precisely recapitulating this human molecular driver event, we have used a bioinformatics approach to identify specific C/EBPa-p30 target genes, whose expression affect tumor progression in AML. The aim is to validate and determine the mechanisms by which a specific C/EBPa-p30 target gene supports AML progression and to assess, to which extent its expression is dependent on an active enhancer bound by C/EBPa-p30.
Methods: The studies are based on the C/EBPa-p30 mouse model. Methods include shRNA and CRISPRi mediated knock-down, applied to in vivo experiments as competitive bone marrow transplantation assays as well as survival studies. Cell transductions are evaluated using fluorescent labeled plasmids, and fluorescent activated cell sorting to sort for transduced cells. Hematopoietic differentiation populations are evaluated by flow cytometry by antibody stainings.
Results: Among the top, conserved hits was a gene associated with a prominent, 40 kb upstream, p30-specific, putative enhancer. shRNA mediated knock-down of this gene resulted in delayed tumor progression assessed by both competitive bone marrow transplantation and survival analysis, thus confirming a tumor promoting role of the protein in vivo. The identified region 40 kb upstream of the transcriptional start site, was functionally validated as a gene-specific enhancer using 3C-qPCR and CRISPRi.
Conclusion: The top hit gene was identified as a putative and potentially drugable tumor promoting target, which is up-regulated as a direct consequence of the altered target specificity of the mutated C/EBPa allele. The target is highly subtype specific, and it's knock-down results in a strong survival advantage.

(20) **Large-scale detection of antigen-responsive t cells using Peptide-MHC multimers labelled with DNA barcodes** (Poster # 36)

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Introduction: Identification of antigenic peptides recognized by T cells is important for understanding disease development and for treating immune-related diseases. Current cytometry-based approaches are limited to simultaneous screening of T cell reactivity towards 10-100 distinct peptide specificities in a single sample, which poorly match the large diversity of T cell recognition in humans. Consequently it has been impossible to comprehensively analyze T cell responsiveness in most immune-related diseases.
Method: We have developed a technology that enables parallel detection of numerous different peptide-MHC responsive T cells in a single sample, using >1000 different peptide-MHC multimers labeled with individual DNA barcodes. After isolation of MHC multimer binding T cells, using cell sorting, their recognition are revealed by sequencing the MHC multimer-associated DNA barcodes.
Results: We have demonstrated the feasibility of using large panels of >1000 DNA-barcoded MHC multimers for single-tube detection of rare T cell populations. When T cell reactivity was tested in a cohort of 10 healthy donors and 11 melanoma patients, the large-scale approach performed comparable to fluorescently-labeled MHC multimers in terms of sensitivity (0.005% of CD8 T cells) and frequency of the detected T cell populations (r2=0.99). Additionally, using the DNA-barcode MHC multimer approach, we have identified several neoepitope-responsive T cell populations in tissues from non-small cell lung carcinoma patients. We demonstrate that the technology can be applied for parallel T cell detection in limited biological samples, such as uncultured tumor material, and for simultaneous assessment of target recognition and functional capabilities of cancer-responsive T cells.
Conclusions: This technology enables true high-throughput detection of antigen-responsive T cells and will advance our understanding of immune recognition from model antigens to genome-wide immune assessments on a personalized basis.

Poster Abstracts

Clinical

Poster # 47
Cytomegalovirus-specific CD8+ and CD4+ T-cells are associated with cellular markers of immune senescence and immune activation, but not with systemic inflammation, in HIV-positive individuals on ART
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Introduction: Coinfection with cytomegalovirus (CMV) is highly prevalent among HIV-positive individuals, and has been associated with increased risk of non-AIDS related comorbidity and a senescent immunologic phenotype (immune risk profile) characterized by a reduced CD4+/CD8+ T-cell ratio and increased levels of terminal differentiated and senescent T-cells. In this study, we investigated the association between the immune response against CMV and presence of the immune risk profile, in a well-characterized cohort of HIV-positive individuals on suppressive ART. We further characterized CMV-specific T-cell polyfunctionality by measuring co-expression of cytokines.
Methods: CMV-specific CD4+ and CD8+ T-cell responses were examined by flow cytometry measuring three cytokines (i.e., intracellular interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ)) after stimulation with three representative CMV peptide pools (CMV-pp65, CMV-IE1 and CMV-gB). Furthermore, CMV IgG antibodies and plasma levels of Interleukin-6 (IL-6), TNF-α, high-sensitivity C-reactive protein (hsCRP) and

soluble-CD14 (sCD14) were measured. Chronic activated (CD38+HLA-DR+), terminal differentiated (CD45RA+CD27-CCR7-), naïve (CD45RA+CD27+CCR7+) and senescent (CD28-CD57+) T-cells were measured by flow cytometry.
Results: CMV-specific immune responses, measured as levels of CMV-IgG antibodies and proportions of CD4+ and CD8+ CMV-specific T-cells (% of CD4+ and CD8+ T-cells), were negatively associated with the CD4+/CD8+ T-cell ratio in HIV-positive individuals and positively associated with the ratio of terminal differentiated versus naïve CD8+ T-cells. In addition, CMV-specific CD4+ and CD8+ T-cells were associated with senescent CD8+ T-cells, and CMV-specific CD4+ T-cells were associated with chronic activated CD4+ and CD8+ T-cells. Coexpression of TNF-α and IFN-γ dominated the polyfunctional T-cell response with CMV-specific CD8+ T-cells having the highest proportion of TNF-α/IFN-γ polyfunctional cells. CMV-specific immune responses were not associated with systemic inflammation.
Conclusion: The magnitude of the CMV-specific immune response measured by CMV IgG and proportions of CMV-specific CD4+ and CD8+ T-cells was associated with development of an immune risk profile in HIV-positive individuals on ART.

Poster # 58

Implementation of the fully automated AQUIOS CL flow cytometer in a routine laboratory from Stockholm, Sweden

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Introduction: Accurate high throughput flow cytometric analysis of lymphocytes is a challenge in routine laboratories. At the Clinical Immunology laboratory at Karolinska University Hospital, Stockholm, Sweden, we analyze frequency and absolute counts of T-cells, B-cells and NK-cells (TBNK-analysis) in 9000+ patient samples each year. To stream line the work flow and add even better patient safety we implemented the fully automated AQUIOS CL flow cytometer (Beckman Coulter) where sample loading, preparation and analysis is integrated in one instrument.
Method: We compared TBNK-analysis using the AQUIOS CL flow cytometer to our semi-automated system which includes a PrepPlus2 pipetting robot, TQ-prep lyse and fix module and a Navios flow cytometer (Beckman Coulter).
Results: Implementation of the fully automated AQUIOS CL flow has freed a lot of time and reduced monotonous tasks. In our laboratory, this system works well for patients with TBNK-cell counts within the reference range as well as patients with deficiency of certain lymphocyte subsets but otherwise normal leukocyte counts (eg. HIV-patients or patients undergoing anti-CD20 therapy). On the other hand, we are not able to analyze samples with very low or high leukocyte count correctly with the AQUIOS CL flow cytometer and need to re-analyze such samples on the less limited Navios flow cytometer. Every now and then we come across a sample that is not analyzed correctly on the AQUIOS CL flow cytometer despite having normal lymphocyte counts.
Conclusion: In our opinion, the implementation of the AQUIOS CL flow cytometer at our laboratory is a success. The biggest drawback is that some samples need to be reanalyzed on the Navios flow cytometer but on the whole, we can now analyze T-, B-, and NK-cells in more samples in less time.

Poster # 60

Correlation of CLEC12A expression with FLT3 and NPM1 mutations in acute myeloid leukemia

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Introduction: Flow cytometry is crucial in diagnosing acute myeloid leukemia (AML) by the identification of leukemia-associated phenotypes (LAIP), and in post-treatment prognostication by the detection of minimal residual disease (MRD). We and others have identified the C-type lectin domain family 12, member A (CLEC12A) as a reliable AML blast marker at diagnosis, with up to 92 % of patients being CLEC12A+, as well as a potential MRD handle, since the CLEC12A expression is preserved in most patients suffering from relapse. Remarkably the amount of CLEC12A+ blasts ranges from hardly any to almost 100%. The most solid prognostic tools in AML are cytogenetics and identification of molecular aberrations, of which internal tandem duplication (ITD) and point mutations affecting aspartic acid 835 (D835) in FMS-like tyrosine kinase 3 (FLT3), and mutated nucleophosmin (NPM1) are the most important. FLT3 mutation, especially FLT-ITD, is considered as a predictor of poor outcome, whereas NPM1 is associated with a positive prognosis, except when accompanied by an FLT3 mutation. We hypothesized that the varying CLEC12A expression was associated with specific molecular aberrations and evaluated the immunophenotype and mutational status at time of diagnosis in a cohort of 140 AML patients.

Methods: Patients were retrospectively identified from the records of Hemodiagnostic Laboratory, Department of Hematology, Aarhus University Hospital. 140 consecutive cases of newly diagnosed AML with FLT3 and NPM1 genotyping performed between 2009-2013 were included. Demographic data and lab results were drawn from the Danish AML database and medical records. Six-color multiparametric flow cytometry was performed on a Canto II (BD Biosciences, San Jose, CA, USA) in the routine laboratory at time of diagnosis, using a combination of the following antibodies: CD34 APC, CD123 FITC, CD45 PerCP-Cy5.5, CD117 PE-Cy7, CD14 APC-H7, and anti-hMICL PE. All samples were reanalyzed using FlowJo 10.0.8 software (Ashland, OR, USA) at time of inclusion. Capillary fragment analysis was performed to confirm the presence of mutations in a semi-quantitative way. For correlation analysis FLT3-ITD and FLT3-D835 patients were pooled. Analysis of marker expression was performed using binary regression taking the number of live cells as the total count applying a log-link and risk ratios are reported. Pairwise Spearman correlations were calculated to illustrate inter-marker dependencies. ROC analysis was performed to establish cut off levels for single markers.
Results: Out of 140 patients, 17 were found to be positive for either FLT3-ITD or FLT3-D835, 23 had mutated NPM1, and 14 had mutations in both FLT3 and NPM1. When looking at the expression of single markers on CD45low/SSClow blasts, the median of both CD123 and CLEC12A expression, were significantly higher for FLT3, NPM1 and double mutated patients compared to wild type patients (table 1). However, the correlation was strongest for CLEC12A. On the contrary, NPM1 and FLT3NPM1 positive patients were characterized by a significantly lower CD34 expression, which is in accordance with previously reported data. Furthermore we tested inter-marker dependencies, and found a weak positive association between expression of CD123 and CLEC12A and CD34 (rs = 0.14 and rs = 0.10, respectively). A positive association between CD123 and CLEC12A was found (rs = 0.66). When using a ROC analysis to establish a cut off level of marker expression and mutational status, only CD34+ in NPM1 positive patients was suitable for this. A cut off of 2.1 % SSClowCD45lowCD34+ cells yielded a satisfactory sensitivity of 86 % and a specificity of 77 %.
Conclusion: Our results indicate, that the heterogeneous level of CLEC12A expression in AML patients may in part be explained by FLT3 and NPM1 mutational status. The heterogeneous group of FLT3 and NPM1 negative AML patients can be characterized by low CLEC12A and CD123 expression, however due to the low sensitivities and specificities, none of these immunophenotypes can be used as surrogate markers in screening for FLT3 and/or NPM1 mutations.

Poster # 70

Exercise-dependent regulation of immune cell function in a clinical cancer perspective

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INTRODUCTION. Currently, exercise plays an increasing role as supportive therapy for cancer patients, aiming to improve patients' physical functioning, quality-of-life and cancer-related fatigue. In addition, emerging evidence suggests that exercise may also have direct anti-oncogenic effects on disease progression. Yet, in order to pursue 'exercise as cancer medicine', detailed insight into the underlying mechanistic effects is warranted. Exercise is known to mobilize cytotoxic NK and T cells. We have recently performed a study in healthy young men, performing either 2 hours of interval-based cycling (INT) or 2 hours of cycling at contrast Watt intensity, matched for the intensity in the INT trial. Here, we observed a rapid mobilization of NK cells to the blood, which was evident already after 10 min of exercise, as well as increased concentration of the cytotoxic effector molecule Granzyme B, lowered proliferation (as evident by a decrease in Ki-67), and increased cellular size reflecting increased metabolic activity of the mobilized NK cells (unpublished data). In light of these results, we hypothesized that exercise in cancer patients induces similar changes in NK cell mobilization, as well as increases in cytotoxic effector molecules, lowering of cellular proliferation and enhancement of cellular metabolism in the mobilized NK and T cells, as we have seen in healthy men.
METHODS. We studied immune cell mobilization during an acute bout of exercise in patients with gastro-esophageal (ECV) cancer. Blood was collected before training, after warm-up, at selected time points during training, and at 1 hour after cessation of training.
The blood samples were by flow cytometry evaluated for:
1) Differential blood cell counts at Dept. Clinical Biochemistry
2) Differential blood cell markers by flow cytometry. Here, we stain for CD3, CD4, CD8,
CD19, CD14, CD16, CD56, and HLA-DR to allow for differentiation of T cells, B cells, and NK cells.

3) Cytotoxic profile by abundance of Granzyme B
4) Proliferation status by expression of Ki-67
RESULTS. So far we have studied the NK-cell recruitment in 4 ECV patients, and we find up to 8-fold increase in circulating NK-cells after 35 min of interval-based cycling.
CONCLUSION. Based on our preliminary data, we conclude that it is possible to obtain NK-cell recruitment to the circulation with exercise in cancer patients undergoing neo-adjuvant chemotherapy. Furthermore, this recruitment is comparable (if not better) than what we find can be obtained by healthy subjects.

Poster # 72

Immune activation status as predictive marker for cancer progression

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Introduction: Recent progress in understanding the interplay between the immune system and cancer has highlighted the importance of immune activation in the elimination of cancer cells. Immune activation is essential for successful elimination of cancer cells, and the naturally mounted
immune response (prior to any treatment) is crucial for the disease prognosis. Patients with minimal immune activation have increased risk of disease progression. We aim to identify an immune signature associated with disease progression in patients with cervical neoplasia (cervical intraepithelial neoplasia, CIN) and cancer. The hypothesis is that by identification of patients with insufficient natural immune activation in early disease, we can intervene with novel immunomodulatory therapies and thereby block the progression of disease. Extensive screening programs provide access to premalignant stages of cervical cancer – this offers an ideal opportunity to assess immune recognition of cancer and immune cell infiltration throughout different stages of disease. We will analyze premalignant stages (CIN), early cervical cancer (local disease) and metastatic cervical cancer. We hypothesize that the combination of local immune reactivity and specific tumor-directed immune recognition is essential for disease elimination at premalignant stages. Upon identification of an immune profile predictive for disease progression, patients with insufficient immune activation can be identified and offered for early immune activationthrough novel immunomodulatory therapeutics.
Method: Assessing the prognostic value of IMMUNOSCORE in cervical cancer development. Through retrospective analyses of immune infiltration in premalignant CIN 3 lesions, Formalin Fixed Paraffin Embedded (FFPE) specimens from two cohorts: A) 30 patients with no signs of disease after removal of the primary CIN 3 lesion and B) 30 patients that progressed to cervical cancer after removal of their primary CIN 3 lesion. Immune infiltration will be analyzed according to the IMMUNOSCORE guidelines using state-of-the-art immunohistological equipment available at the diagnostics department at DTU VET. Density and location of T cells will be analyzed in the two patient cohorts. Mapping of T cell reactivity towards the HPV 16 proteins, E2, E6 and E7. Based on our recent advances in detection of antigen-responsive T cells using DNAbarcode labelled MHC multimers, we aim to assess the T cell reactivity towards HPV infection and the impact of such T cell response on progression from early stage to metastatic disease. We will fully characterize the T cell recognition and such T cell recognition profiling will be a major advantage in determining the immunological fingerprint associated with favorable disease outcome, and may serve as a biomarker for therapeutically induced immune activation. We will use material from both cervical cancer patients and patients with precancerous lesions to assess T cell recognition systemically (blood) and locally (tumor/premalignant tissues). Studies will be conducted prospectively as viable cell preparations are required to describe the T cell reactivity both in terms of specificity, phenotype and functionality. Analyses of immune recognition in other cancer diseases have shown evidence for early immune suppression initiated by the (few) cancer cells present – and that the depth of such immune suppression correlates with tumor burden. All the above experimental analyses will be taken into account, alongside with all clinical parameters of potential interest: age, health status, other diseases (especially immune mediated disease), history of smoking, and other environmental factors.

Basic Research

Poster # 4

Lack of filaggrin in the thymus results in an increased development of IL-17A-producing γδ T cells

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Introduction: Loss-of-function mutations in the human filaggrin gene (FLG), which encodes the skin barrier protein filaggrin, are the most significant risk factors for atopic dermatitis (AD). Recently, it has been shown that mutations in FLG result in an increased systemic level of Th17 cells in both humans and mice. Th17 cells secrete IL-17 that primarily induces signaling in cells of non-hematopoietic origin, including epithelial cells. In these cells, IL-17 stimulates the production of a range of pro-inflammatory cytokines and chemokines. IL-17 is produced by a variety of cells, including γδ T cells. Preliminary data from our lab show that there is an increased amount of IL-17-producing γδ T cells in the periphery of filaggrin-deficient mice. As the functional differentiation of γδ T cells to either IFNγ-producing or IL-17-producing is determined in the thymus, we speculated that filaggrin might be expressed in the thymus and that filaggrin deficiency affects the development of γδ T cells.
Methods: Using the flaky tail (ft/ft) mouse as a model of filaggrin deficiency, we determined the distribution of IL-17A-producing γδ T cells in the thymus and periphery by flow cytometry. Furthermore, we investigated different developmental stages of T cells by including various markers in the experiments. Finally, transcriptional and translational expression of filaggrin in the thymus was determined using qPCR, histology and western blotting.
Results: We found that there are an increased number of IL-17A-producing γδ T cells in the periphery of ft/ft mice compared to wild type mice. Furthermore, we demonstrate that filaggrin is expressed in the thymus of wild type mice, and that filaggrin expression is decreased and the amount of IL-17A-producing γδ T cells increased in the thymus of ft/ft mice.
Conclusion: These data indicate that lack of filaggrin in the thymus of ft/ft mice leads to an altered development of γδ T cells, with a phenotype skewed towards IL-17A-production. Thus, carriers of filaggrin mutations might not only be affected in the skin, but in all tissues inhabited by IL-17-producing γδ T cells regardless of filaggrin status in the specific tissue.

Poster # 8

Targeted Redox-sensitive liposomes for glioblastoma treatment

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Introduction: Treatment of glioblastoma remains a challenge due to inability of the drug to reach the intracellular target. Invasive glioblastoma is associated with high grade vascularization and break-down of the blood-brain barrier (BBB), which could aid in delivering drugs to the tumor site. However, once at the tumor site, the drug has to be internalized and transported to the specific target. To address these issues, we have created a targeted DDS consisting of cationic liposomes with a redox-sensitive PEG-coating and functionalized with an antibody. After uptake into tumour cells, the PEG-coating is shed from the liposomes in a reducing environment, revealing a positive charge for endosomal escape and drug release. We have created three different redox-sensitive lipopeptides (RSLs) for comparison of reactivity and a fluoro-RSL with the ability to change emission spectrum upon cleavage. The aim of the current study is to characterize the system in vitro with regards to cleavage and charge-reversal in buffer. In addition uptake, and cleavage patterns of targeted RSL liposomes is investigated using a model cell line and antibody.
Methods: HPLC, MALDI, DLS and phase analysis light scattering was used for the initial characterization of the system. Flow cytometry was exploited to assess the effect of cleavage on uptake and antibody based uptake in the chosen cell line. Flow cytometry, fluorometry, and confocal microscopy was used to assess cleavage of the fluoro-RSL by the cells at defined time points.
Results: DTT cleavage revealed that RSL3 had the fastest cleavage kinetics. In uptake studies DTT cleavage of RSL2 and RSL3 liposomes resulted in increase in uptake for both saturated (sat) and unsaturated (unsat) liposomes. RSL1 liposomes showed different uptake patterns in sat and unsat liposomes; non-cleaved sat liposomes were taken up to a higher degree, while uptake of cleaved unsat liposomes was only observed at low DTT concentrations. In general the liposomes were stable after cleavage and zeta-potential revealed a charge-reversal mechanism for all

formulations except for unsat RSL1 liposomes, where only the lowest amount of DTT resulted in a positive charge. Determining cleavage patterns is ongoing work.
Conclusion: Characterization of the system revealed a structural impact on cleavage kinetics and uptake. While RSL2 and RSL3 would be positively charged after cleavage, RSL1 could be neutral or slightly negative due to the possibility of carboxylic acid formation. Based on the characterization results, RSL3 was chosen for further experiments as the faster cleavage could be an advantage for intracellular drug delivery.

Poster # 15
Investigating uptake of fluorescently labeled liposomes in vivo
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Introduction: Immune evasion by tumors is amongst the main reasons for both establishment and progression of tumors. The immune evasion is largely due to the tumor microenvironment containing a large portion of suppressive immune cells. The suppressive state can be affected to be more antitumorigenic whereby the immune system potentially can eradicate the tumor. In my project, immunemodulation is approached by a variety of liposomal formulation to deliver cargo to the tumor microenvironment.
Methods: Rhodamine B labeled liposomes of different formulations and containing different cargo were prepared. Liposomal uptake by tumor cells and several immune cell types from spleens, livers, and tumors were assessed by up to 11-color flow cytometry to supplement efficacy studies and PET tracking of circulation using the most promising formulations.
Results: The flow cytometry panels are able to distinguish the relevant populations and determine how the uptake is affected by how the liposomes are formulated.
Conclusion: Flow cytometry allows for a unique insight into why and how liposomal drug delivery is useful in the treatment of cancer.

Poster # 24
Combinations of immune checkpoint inhibitors and liposomes containing chemotherapy
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For many years radiotherapy and surgery have been the main methods used for treatment of cancer. However, the possible treatment methods have changed considerably during the last decades, and the tumor microenvironment appears to be the cornerstone of new cancer therapies, including immunotherapy.
Cancer immunotherapy aims at re-establishing or improving the ability of the immune system to fight cancer. One example of immunotherapy is immune checkpoint inhibitors which prevent cancer cells from suppressing immune cells and thereby enhance the anti-tumor immune response. Checkpoint inhibitors that target the programmed-death 1 (PD-1) receptor or the PD-1 receptor ligand (PD-L1) have been shown to induce tumor regression and improve overall survival in cancer patients, and many studies are combining these antibodies with chemotherapy with great effect.
The aim of this study is to combine liposomes containing chemotherapy with anti-PD-1 or anti-PD-L1 therapy, and study whether the anticancer effect of the liposomes can be augmented by combining the treatments. Furthermore, the changes that the treatments induce in the tumor microenvironment will be examined by flow cytometry.

Poster # 26
Clearance mechanisms of cationic liposomes in whole human blood
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Introduction: Nanoparticles are a promising tool in cancer immunotherapy as these can be used to deliver immunostimulating compounds into antigen-presenting dendritic cells. If these cells are activated properly, such a treatment could induce an effective and long-lasting anti-tumor immune response, that may even make it possible to treat metastasis.Instead of targeting dendritic cells directly in the tissue, monocytes can instead be targeted in the blood through vascular injection, as these cells has the ability to differentiate into dendritic cells. It has earlier been observed that cationic liposomes seem to be specifically taken up by monocytes over other types of leukocytes

when incubated with whole human blood. The mechanism behind this apparent specificity to monocytes was investigated.

Method: A range of fluorescently labelled liposomes formulated with various amounts of cationic lipids were incubated with fresh whole human blood for 1 hour. Leukocytes were then isolated from the blood by centrifugation followed by lysis of the erythrocytes. Leukocytes were stained for CD14 and the association with granulocytes, lymphocytes and CD14 positive monocytes was studied with flow cytometry. In order to differentiate surface binding from actual uptake, cells were washed with heparin.For investigation of the uptake mechanisms, two of the main pathways suspected to be involved in clearance of nanoparticles from circulation, scavenger receptors and the complement system, were inhibited. The blood was thus pre-incubated with inhibitors of the complement system or scavenger receptor B-blocking antibodies before adding the liposomes. Also, the CD14 receptor was blocked using specific antibodies. Changes in uptake in the three cell populations mentioned above was investigated upon treatment with these inhibitors.

Results: Blocking the Scavenger Receptor B (CD36) resulted in a decrease in the uptake in monocytes, but also led to a large drop in the number of monocytes being acquired. This concentration dependent drop in monocyte number being caused by the anti-CD36 antibody was also seen in control samples without any liposomes added, but not in samples treated with isotype antibodies. We suspect that these data indicate that the blocking antibodies result in an activation of the monocytes. Inhibition of the complement system reduced the uptake in granulocytes, but led to an increase in the uptake in monocytes. This indicate that the complement system is not involved in the monocyte-mediated clearance of the liposomes investigated, but rather promotes the uptake in granulocytes. The uptake in monocytes could instead be efficiently inhibited when blocking the CD14 receptor, and reductions in the uptake down to 25% of the uptake for liposomes without inhibitor were observed.

Conclusion: Our results show that monocytes are the main population of peripheral blood leukocytes involved in clearance of cationic liposomes from circulation. The data illustrate that the complement system is not involved in this specificity to monocytes, but is mediating the clearance by granulocytes instead. Instead, the CD14 receptor seem to be a main component in the mechanism behind the specificity to monocytes of the cationic liposomes investigated.

Poster # 27

Monocyte-subsets and their expression of Tie2 in metastatic renal cell carcinoma

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Monocyte-subsets and their Tie2 expression have been shown to be associated with increased angiogenesis and progression in human cancers. However, the importance of circulating monocyte-subsets and their Tie2 expression has not been investigated in metastatic renal cell carcinoma (mRCC). Thus, this was the aim of this study to investigate.

Peripheral blood mononuclear cells (PBMC) from 89 patients enrolled in a randomized clinical trial investigating the therapeutic efficacy of bevacizumab (anti-VEGF) as well as 20 age- and gender-matched healthy controls (HC) were analyzed. Patients were stratified by the Motzer scoring system and only patients with a favorable prognosis (FP) or an intermediate prognosis (IP) were included in the study. The distribution of classical (CD14++CD16-, Mϕ-Cl), intermediate (CD14+CD16+, Mϕ-IM) and non-classical (CD14-CD16+, Mϕ-NC) monocytes together with the expression of the angiopoietin receptor Tie2 were assessed by multi-parameter flow cytometry on PBMC samples collected at baseline and following start of therapy at 5 weeks, at 9 months and/or at time of progression.

The Tie2 expression on all monocyte-subsets was found to be increased in mRCC patients compared to HC as indicated by a 3.28 (95% CI: 2.83-3.87), 2.87 (95% CI:2.36-3.47) and 2.97 (95% CI: 2.45-3.59) fold increase on Mϕ-Cl, Mϕ-IM and Mϕ-NC monocytes, respectively. No differences were observed between the FP and the IP groups.

The IP group showed a skewed monocyte distribution with an increase in the Mϕ-Cl fraction and a decrease in the Mϕ-NC fraction. The Mϕ-Cl and Mϕ-NC compromised a 0.9 and 0.06 fraction of all monocytes in the IP group vs. 0.86 and 0.1 in the HC group. The difference in the Mϕ-Cl fraction between the IP group and HC was of 0.038 (95% CI: 0.009-0.06) while in the Mϕ-NC fraction it was of -0.037 (95% CI: -0.06-(-0.01)).

Longitudinal analysis following start of therapy showed an increased Mϕ-Cl/ Mϕ-NC ratio which tended to normalize towards baseline level at progression. The largest changes in Tie2 expression during treatment was observed in the Mϕ-IM and the Mϕ-NC subsets with an increase of 9.3 % (95 % CI: 3.8-14.9 %) and a decrease of

10 % (95 % CI: 5.7-14.1 %) respectively. These changes also tended to normalize towards baseline levels at progression.
In conclusion, our results showed an increased expression of Tie2 on circulating monocytes in mRCC compared to HC, and the distribution of monocyte-subsets was skewed toward classical monocytes in the intermediate prognosis group. Also, the normalization of circulating monocyte-subsets and their Tie2 expression at progression might be a candidate biomarker of progression in mRCC. Our findings support earlier observations indicating changes of the myeloid subsets in cancer patients which might be of clinical importance due to their association to angiogenesis and immunomodulatory functions.

Poster #39

Liposome based vaccines in cancer immunotherapy: delivery of tumor antigens in combination with immunostimulatory compounds to antigen presenting cells for induction of antitumor response

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The millions of cancer-related deaths happening every year emphasize the importance of identifying novel anticancer therapies that also prevents relapse in patients. The immune system plays a pivotal role in the pathogenesis of cancer and much attention is being given to the development of immunotherapy based cancer treatments that stimulate the patient's own immune system to fight the cancer cells. Interestingly, preclinical and clinical data suggests that the delivery of immunostimulatory compounds enclosed in liposomes can elicit an increased immune response towards the cancer cells compared to the same compounds delivered in solution.
The aim of this project is to develop a liposome based delivery system of immunogenic tumor antigens in combination with immunostimulatory compounds to antigen presenting cells (APCs) of the immune system. We hypothesize that the combined delivery of tumor antigens and adjuvant to APCs via liposomes will elicit an effective antitumor immune response through the activation of T cells.
Liposomes containing immunogenic tumor antigens in combination with immunostimulatory compounds are formulated in house. Initially, the ability of the liposomes to deliver antigens to APCs will be assessed and compared to delivery of soluble antigens in vitro using co-culture assays with APCs and T cells. The T cell activation will be assessed using CFSE staining in flow cytometry.
The liposome formulations will be administered as a cancer immunotherapy in vivo using syngenic mouse models. To characterize the tumor microenvironment during the stages from therapy initiation to tumor elimination, the composition and quantity of innate and adaptive immune cells in tumor biopsies will be analyzed using flow cytometry combined with cytokine profiling. The tumor microenvironments in tumors from mice that have received different treatment regimens and combinations will be compared using these assays. This set-up will provide important information about the characteristics of the immune response under different circumstances with regards to therapy and tumor elimination.
Preliminary results demonstrate the ability of our liposome formulation to deliver several variants of the model antigen SIINFEKL to APCs in vitro for subsequent presentation on MHC I molecules. Furthermore, SIINFEKL variants delivered in our liposome formulation is presented to a higher extend than the same antigens delivered in solution.
The development of this liposome based delivery system of immunogenic tumor antigens to APCs will allow us to characterize the immune effectors that act at specific time points during the process of tumor elimination in vivo. This will provide us with an important tool to analyze how an immunological memory response against cancer cells is generated. Furthermore, the development of this cancer immunotherapy will enable the characterization of the tumor microenvironment and the state of the immune system during cancer initiation, progression and elimination. Importantly, the project has great translational potential with regards to development of novel anticancer therapies.

Poster # 40

Regenerative properties of adipose derived stromal cells – mode of action in ischemic heart disease

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At Cardiology Stem Cell Centre, Rigshospitalet, 4 clinical trials are performed where patients with ischemic heart disease receive direct intramyocardial injections with Adipose derived Stromal Cells (ASCs) for regenerative purposes.

The aim of this PhD project is to determine the regenerative mechanism/mode of action (MoA) of ASCs in the ischemic heart, using in vitro models. Human cell-based co-culture models are established at ischemic conditions in order to determine juxtacrine versus paracrine effects of ASCs on cells resident in the ischemic myocardium. Models of cardiomyocyte survival, fibrosis, cell proliferation and angiogenesis will be established and evaluated using flow cytometry and microscopy. Once key regenerative MoAs are determined, possible surrogate markers will be identified, and the assays will be translated into flow cytometry-based potency assays. These potency assays will aid donor selection and quality control of ASC batches for clinical efficiency.

Poster # 45

Lipid based carrier systems for delivery of TLR agonists to the tumour microenvironment

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Introduction: Tumour immune escape is the primary reason for cancer appearance and progression. Evidence suggests that the immune system interacts with the tumor throughout cancer development [1]. Thus, the complex cross talk between the immune system and the tumor can both promote and inhibit cancer progression. Cells of the innate immune system, such as the monocytes, macrophages (Mø) and dendritic cells (DC), are often negatively affected by the immunosuppressive environment within the tumour [2]. Providing activated immune cells within tumour microenvironment (TME) could thus be a possible way to break the immune suppression and re-establish cancer immune surveillance and revert tumour development [3]. We therefore suggest the development of a lipid-based carrier system containing toll like receptor (TLR) activating agents for activation of immune cells present within the TME. The targeting and distribution of the drug delivery system will be investigated in vitro, as well as in vivo by flow cytometry and positron emission tomography (PET) imaging.
Methods: Rhodamine labelled lipid based nanoparticles will be prepared using the lyophilization method, where after agonists will be post inserted. The lipid based nanoparticles will be characterized using dynamic light scattering (DLS) and measuring the zeta potential. The content of the encapsulated agonist will be determined using ICP-MS and HPLC. Immune cells will be obtained from human blood and treated with the suggested nano carrier system. Supernatants will be collected to determine the secretion of key cytokines indicative of immune cells activation. Tumours from mice, will be collected post treatment administration and further analysed. The obtained immune cells will be analysed using flow cytometry for uptake and association with the suggested nano carrier system.
Results: This PhD project is too new to have yielded any results of importance yet. Furthermore, the results obtained during this PhD project are confidential and any obtained data can therefore not be published in this abstract.
Conclusion: No conclusion can be drawn due to the before mentioned confidentiality.
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Poster # 43

The Aldefluor assay – pitfalls in a multicolor setup

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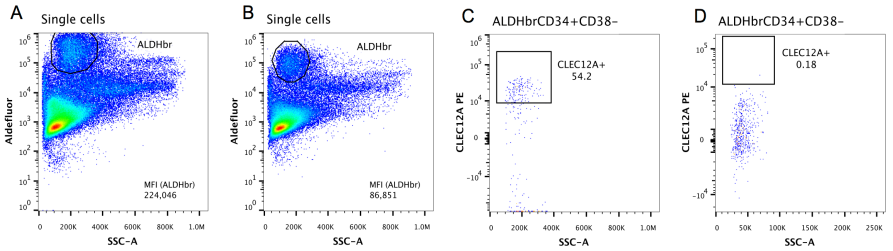
Introduction: The Aldefluor™ assay (StemCell Technologies) is an enzymatic assay, which measures the activity of aldehyde dehydrogenase (ALDH) in live cells. In brief, an aminoacetaldehyde (substrate) which is linked to a fluorescent BODIPY® dye (BODIPY® FL,SE) diffuses across cell membranes where ALDH converts it to fluorescent BODIPY-aminoacetat. This product retained inside cells is excited by a 488 nm laser and has a green fluorescent emission detected in the standard “FITC”-channel. Stem- and progenitor cells are characterized by a high activity of ALDH (named ALDHbr cells) and the assay is widely used in the evaluation of cancer stem cells in solid tumors and hematological malignancies. We are employing the Aldefluor™ assay together with both prevalent stem cell markers and the upcoming marker of cancer stem cells in myeloid malignancies, CLEC12A. The hallmark of CLEC12A

is its complete absence on the normal hematopoietic CD34+CD38- stem cell subset. Until recently, CLEC12A was only commercially available conjugated to PE, thereby limiting possibilities for panel design. In the following, we aim to point out possible pitfalls when employing the Aldefluor™ assay in a multicolor set-up.

Methods: The Aldefluor™ reagent (StemCell Technologies) was titrated and for each tube 2 x 10^6 thawed bone marrow (BM) cells from a healthy donor was stained with 2.5 µl Aldefluor™ reagent in Aldefluor™ Buffer (StemCell Technologies) and incubated at 37°C for 45 minutes. Cells were washed, resuspended in Aldefluor™ Buffer, kept on ice and subsequently stained with CLEC12A PE (clone HB3), CD34 PerCP-Cy5.5 (clone 581) and CD38 PE-Cy7 (clone HB-7). Data acquisition was performed on a Navios flow cytometer equipped with three lasers (488nm, 638 nm and 405 nm) and a FACSAria III equipped with four lasers (488 nm, 561 nm, 633 nm, and 405 nm). Compensation was set using unstained- and Aldefluor™ single stained cells combined with UltraComp eBeads together with the relevant fluorochrome conjugated antibodies. Data analysis was done in FlowJo, version X.0.7 (FlowJo).

Results: Being an enzymatic assay, the Aldefluor™ staining is highly sensitive to day-to-day variation. Exemplified, we have experienced a pronounced difference in median fluorescence intensity (MFI) for the ALDHbr subset of BM cells of the same healthy donor run twice on the same cytometer in the same protocol, but on different days (Fig. 1, panels A and B). Since quality controls are performed on the instrument on a daily basis, these differences in MFI cannot be attributed to the hardware. Furthermore, due to the intense brightness of the Aldefluor™ signal, the photomultiplier tube voltage in the FITC channel (FL1, 525/40) had to be low in order to avoid getting the ALDHbr subset off scale. As a consequence, this phenomenon can be a challenge in terms of obtaining correctly compensated data in the adjacent channels (FL2, 575/30 and FL3, 620/30), which became evident to us when we observed false positive CLEC12A+ events in the ALDHbrCD34+CD38- cell subset of BM from a healthy donor in a compensated sample analyzed on the Navios (Fig. 1C). When the same staining of the same donor was investigated on the FACSAria III equipped with a 561 nm laser, no CLEC12A+ events was evident (Fig. 1D).

Conclusion: A high level of awareness in terms of available lasers and corresponding filters is crucial when using the Aldefluor™ assay in a multicolor set-up. If the use of PE and PE-Texas red conjugated MoAbs is needed, we would recommend to use cytometers equipped with e.g. a 561 nm laser. In our opinion, MFI is not a valid measurement for comparison of ALDH activity due to a high level of inter- and intra-experimental variation caused by the functional nature of the assay. Taken together, this variation adds to the complexity in terms of correct compensation and standardization of the assay and more importantly, calls for detailed information on methodology and hardware in resulting publications.



Poster # 48

Immunomodulatory influence of adipose tissue-derived stromal cells on assays of proliferation

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Introduction: Since the discovery of the Mesenchymal Stromal Cell (MSC) in the early 1970s, a broad range of their cellular functions have been elucidated, including tissue regeneration and angiogenesis. Around the turn of the century, yet another intriguing attribute was added to the continuously expanding list of properties linked to the cell type: immunosuppression. Since then, research has painted a more nuanced picture of the immunomodulatory plasticity of MSCs, demonstrating pro-inflammatory capabilities as well as the often highlighted anti-inflammatory potential. In the present study, we sought to establish a platform for investigation of the effects of MSC on proliferation of peripheral blood mononuclear cells (PBMCs). For starters, a robust model was based upon the use of CarboxyFluorescein Succinimidyl Ester (CFSE)-staining of cryopreserved PBMCs stimulated with the unspecific but very potent mitogen phytohemagglutinin. MSC derived from adipose tissue (adipose tissue-derived stromal cell, ASC) was introduced to the assay in a dose-dependent manner.

Method: ASCs were isolated and cultured as previously described. In brief, lipoaspirate was harvested from healthy voluntary donors and ASCs were separated and cultured manually in MEM-alpha (Gibco) containing 5% human platelet lysate (Cook Regentek), penicillin and streptomycin (P/S. 100 U/ml and 100 µg/ml, respectively. Gibco). PBMCs were isolated from buffycoats by density centrifugation on Lymphoprep (1.077 g/ml. Axis-Shield) in 50 ml LeucoSep tubes (Greiner Bio-One). PBMC were resuspended at a density of 2-3×107 cells/ml in pre-cooled FBS (Gibco) with 10% DMSO and cryopreserved in a rate-controlled CoolCell container (Biocision) overnight, then transferred to liquid N2 and stored for a minimum of 2 weeks. Assay cultures were set up in RPMI 1640 (Sigma-Aldrich) supplemented with 2% human AB serum (Sigma-Aldrich) and P/S and allowed to develop for 5 days in the presence or absence of 5 µg/ml PHA (Sigma-Aldrich). Cultures were collected, stained with Fixable Viability Dye 780 (BD) and analyzed on a Navios flow cytometer (Beckman Coulter). Data was analyzed in FlowLogic 7.
Results: The PHA stimulation protocol proved highly reproducible and suited for quantification, even between blood donors. ASC demonstrated an evident dose-dependent decrease in proliferation. Especially the fraction of PBMCs which would typically undergo several divisions was inhibited.
Conclusion: Proliferation assays based cryopreserved PBMC stained with CFSE holds the potential to demonstrate and quantify the immunosuppressive potential of ASC. It allows for detailed analysis of several subpopulations, and possibly identification of mechanisms of action.

Poster # 49

Using a high sensitivity flow cytometer for the enumeration of bacterial cells in environmental samples

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Quantification of total bacterial cells is an essential task in microbiological studies, especially from the concerns of public health and environmental issues. Due to the limitations of traditional detection methods such as microscopy and culture techniques, enumeration methods by flow cytometry is increasingly popular offering rapid and reliable quantification of individual cells. However, detection of bacterial cells is challenging because of their small size and minute contents of structural molecules prompting for a strategy to separate background noise from target particles. To overcome this challenge, a high sensitivity flow cytometer is introduced to improve the detection sensitivity of bacteria.
This study aims to enumerate populations of airborne and marine bacteria in environmental samples using a high sensitivity flow cytometer and qPCR, and to analyze the cell characteristics by the flow cytometer, and to evaluate the applicability of the flow cytometer in comparison to qPCR estimation. Air samples were collected from outdoor environments in Denmark and marine water samples were collected from Roskilde Fjord. The Cytosense (Cytobouy.com) benchtop flow cytometer was used to analyze stained bacterial cells from environmental samples and references against fluorescent beads.
The results so far indicated that average bacterial cell density measured by Cytosense ranged from 2×10^4 to 5×10^4cells/m^3 in air samples and 1×10^5 to 4×10^5 cells/ml in marine water samples. A significant linear correlation was found between bacterial concentrations obtained by the two methods. Overall, the results demonstrated that Cytosense can be used to determine the bacterial cells in different types of environmental samples. The present study is ongoing and recent results will be presented.

Poster # 51

Comparison of two dissociation methods to achieve single cell solution in murine syngeneic tumors

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Introduction: In our group we have used a gentleMACS™ Octo Dissociatior from Miltenyi in addition to Miltenyis tumor dissocation kit to achieve single cells solution of syngeneic tumors before analysis. Due to limited capacity of the octo dissociator we wanted to investigate how the gentleMACS™ compared to a water bath with agitation.
Method: Two syngeneic murine tumors were weighed and split in halves. All tumors were chopped with scalpels and added to enzyme solution. The tumors were then dissociated with either gentleMACS™ or agitated waterbath at 37 °C for 40 min. Following dissociation the tumors were passed through a 70 µm filter and washed by centrifugation (400xg, 5 minutes, 4 °C) before being passed through a 70 µm filter again. The cells were counted using Muse® Cell analyzer and following stained with Fixable Viability Dye efluor780, CD45 (PE-Cy7) and CD11b(BV711).

Results: There were no clear differences between the two dissociation methods when comparing cell count (not shown), viability, Leukocyte or myeloid populations as seen in figure 1(I am not able to upload the figure)
Conclusion: For future setups we will use the waterbath to dissociate murine syngeneic tumors. This would allow us to dissociate approx. 40 tumors as compared to 8 tumors every 40 minutes.

Poster # 52

Analysis of the co-presence of HLA-DR in the plasma membrane and cytoplasm of CD3+ T cells and CD19+ B cells

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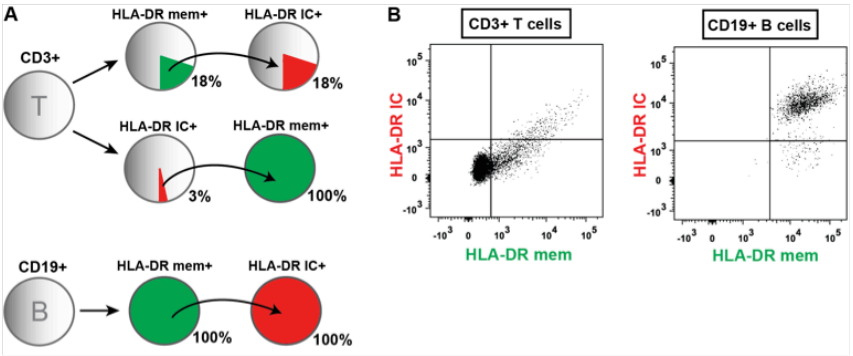
Introduction: The MHC class II or HLA-DR molecules are pivotal receptors in the immune system, facilitating the presentation of exogenous antigens to activate T cells. Professional antigen presenting cells (APCs), such as B cells, express HLA-DR constitutively. However, for several decades HLA-DR has also been used as an activation marker for T cells, yet a possible functionality remains to be identified. To characterize the presence of HLA-DR in the context of both B and T cells, we developed a staining and gating strategy to accurately identify and separate the protein found in the plasma membrane from that in the cytoplasm.
Method: Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood from 10 healthy donors using Lymphoprep density centrifugation. PBMCs were stained with anti-CD3-PE, anti-CD19-PerCP-Cy5.5, and anti-HLA-DR-AF488 (clone L243) to target plasma membrane-bound HLA-DR (HLA-DR mem). For the fluorescence minus one (FMO) control, the corresponding isotype antibody was applied instead of anti-HLA-DR. After fixation, the cells were permeabilized and incubated with a second anti-HLA-DR-AF647 (L243) to target intracellular HLA-DR (HLA-DR IC). The cells of interest were gated as: Lymphocytes (in FSC/SSC plot); T cells or B cells (CD3/FSC or CD19/FSC); HLA-DR mem+ (FSC/HLA-DR-AF488, positive gate defined with FMO control allowing 1% in double positive quadrant); HLA-DR IC+ (FSC/HLA-DR-AF647, positive gate also defined with FMO). A saturation control was included to ensure that plasma membrane-bound HLA-DR was saturated with antibody after initial incubation (full staining procedure, omitting permeabilization).
Results: The presence of HLA-DR in the plasma membrane (HLA-DR mem) and intracellular compartment (HLA-DR IC) differed considerably between the two types of lymphocytes. As such, approximately 18% of CD3+ presented HLA-DR mem and only a fraction of these cells also contained HLA-DR IC (figure, panel A). No T cells solely presented HLA-DR IC. All CD19+ B cells were double positive for HLA-DR mem and HLA-DR IC.
Conclusion: With the newly developed staining and gating strategy we revealed that in healthy individuals the presence of HLA-DR on APCs, here CD19+ B cells, differs significantly when compared to HLA-DR on CD3+ T cells. The identified percentage of HLA-DR mem+ CD3+ T cells was slightly higher than generally reported (5-10% vs. 18% in current study). However, the staining and gating strategy presented here was optimized to remove any user-bias in the identification of HLA-DR+ T cells, which is a heterogeneous population (figure, panel B). Therefore, the applied strategy could be used for future identification of the true HLA-DR mem+ T cells, with a benefit in both research and clinical applications.



Poster # 53

T cell receptor repertoire and thymic export after immune reconstitution in children with HIV

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Introduction: In children, the T cell receptor (TCR) repertoire is established in the thymus and naive T cells are then released into the periphery with a receptor diversity estimated in different clonotypes. T cells made in the thymus establish the peripheral naïve T cell pool during the first years of life. Thymic export then gradually declines from its peak at 2 year of age to much lower levels by early adulthood resulting in a dramatic reduction of TCR diversity and increased susceptibility to infection. In children with HIV infection, there is a loss of naive T cells that can be restored to a large extent by antiretroviral therapy. The resultant clonal diversity is largely unknown and will depend on the balance between output of naive cells from the thymus and homeostatic peripheral cell division.
Method: The novelty in this project lies in the design of the combination of high throughput sequencing of T cell receptors with novel bioinformatic and mathematical techniques. With this method we will investigate T cell reconstitution and receptor diversity in HIV infected children following antiretroviral therapy (ART). Further we will combine Flow Cytometry with mathematical modeling to estimate thymic output and correlate this with T cell reconstitution and receptor diversity. The cohorts of HIV infected children available for this study are from PENTA 11 that was a randomized controlled study where one cohort remained on ART and the other had treatment withdrawn for one year.
Results: So far, we have via Flow Cytometry measured IL-8 producing naïve CD4 T cells. It has recently been shown that this is an important anti-inflammatory cytokine in infants directly correlated to thymic output. Interestingly we see that IL-8 production quickly increases when the HIV infected children are coming off treatment suggesting that the proportion of naive thymic emigrants increases as a response to increased viral load. Very soon we will have the T cell repertoire diversity measurements to correlate with these results.
Conclusion: The increased IL-8 production could be a sign towards the dynamics of the T cell subsets changing as a response to ART being interrupted. This project will enable us to make more rational decisions about how to optimize patient management. For children with HIV, it will influence the choice of drugs and when to start antiretroviral therapy. It will also help answer the question whether periods of treatment interruption have an impact on subsequent homeostatic reconstitution of T cells and the antigen receptor repertoire and most importantly to guide HIV therapy in children to optimize their immunity into adulthood.

Poster # 55

Thyroid hormones and mitochondrial function in women with subclinical hypothyroidism and their offspring compared to euthyroid controls.

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Introduction: To investigate the level of thyroid hormones and MMP at birth in a group of euthyroid and subhypo mothers and their offspring.
Methods: In total, 45 pregnant women were enrolled to this study by blood collections in prior to a planned caesarean section. Thyroid hormones were measured, and 15 of the women presented with subhypo defined by a TSH>3.0 mU/L and normal level of fT3 and fT4. Of these, 30 women were euthyroid. As the child was born by caesarean section, cord blood was collected. Cord blood volume was adequate to measure thyroid hormones in 29 offspring of euthyroid women and 13 of subhypo women. Maternal and cord samples were kept cold overnight and the following day, flow cytometry was performed to measure MMP.
Results: Though the level of TSH was increased in the subhypo women group, the level of fT3 and fT4 did not differ from the euthyroid women. Thyroid hormones were the same in cord blood of subhypo and euthyroid offspring.

The level of maternal MMP (reflected as the fluorescence intensity of TMRM-stained lymfocytes) was significantly increased in the subhypo group (p=0.016) and almost significantly increased in the subhypo offspring group (p=0.056).
Conclusion: A higher level of MMP was present in a group of at-term pregnant women with subhypo and their offspring compared to euthyroid controls. The cord level of thyroid hormones did not differ between subhypo and euthyroid offspring. This indicates that maternal mitochondrial function might affect the child´s MMP through a pathway independent of thyroid hormones measurable in the fetal circulation.

Poster # 56

GLP-1R signalling in the immune response in psoriasis

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The skin is the body's first barrier of defence towards the environment and consists of both epithelial and immune cells. Psoriasis is a common, chronic inflammatory skin disease that is driven by a dysregulated immune response. Treatment of psoriasis aims at reducing inflammation either through general immunosuppression or by a direct inhibition of key mediators of inflammation. Interestingly, administration of glucagon-like peptide 1 (GLP-1) analogues to patients with psoriasis who concurrently have developed type 2 diabetes have been reported to improve the severity of psoriasis. The beneficial effect of GLP-1 analogue treatment was seen prior to obtaining glycaemic control indicating a direct effect of GLP-1 analogues on the immune system. However, it is not clear if cells of the immune system express a functional GLP-1R and if GLP-1 treatment could have an effect on the immune response in psoriasis.
Interestingly, recent studies have found the GLP-1R to be expressed in lymphocytes of the gut and adipose tissue in mice, further supporting a possible direct effect of GLP-1 on the immune system. We hypothesize that signalling through the GLP-1R can affect the inflammatory immune response in psoriasis. This potential immunoregulatory effect could be responsible for the alleviation of psoriasis seen in patients treated with GLP-1 analogues and could lead to further application uses for GLP-1 analogues.
In this project we want to investigate how GLP-1R signalling affects the immune response in psoriasis. In order to do this we will induce psoriasis-like skin inflammation in mice using imiquimod which is an established mouse model for psoriasis. We will identify different immune cell population in both the circulation and in the skin by flow cytometry and sort them by FACS for analysis of their GLP-1R expression. Furthermore, imiquimod-treated mice will receive systemic administration of GLP-1 analogues to evaluate the effect of GLP-1R signalling on the severity of the psoriasis-like skin inflammation and on the immune response involved in the inflammation.

Poster # 57

Does NF-κB activity predict recovery from acute illness in elderly patients?

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Introduction: In Denmark, elderly individuals (≥ 65 years) accounted for 43% of all acute hospital admissions in 2015, while they represented only 19% of the total population. Ageing is characterized by a decline in resilience, the capacity to recover from stressors. Many elderly patients do not regain health status and exhibit worsening disability and loss of independence following acute illness and hospitalization. The mechanisms and factors of that contribute to resilience are not well established. This challenges the ability to provide appropriate care and to predict responses to treatment and interventions. During acute illness, adequate regulation of immune and inflammatory responses is determinant for recovery and survival. The transcription factor NF-κB is central in the regulation of these responses. However, NF-κB signaling is dysregulated in the elderly, and may be a factor for poor recovery. We aim to investigate whether the activity of NF-κB during acute illness predicts recovery in acutely admitted elderly patients. We hypothesize that the degree of NF-κB activation during acute illness is associated with the ability to maintain or gain health after acute hospitalization in elderly patients.
Methods: The study is on-going and designed to include 54 elderly (≥ 65 years) patients acutely admitted to the Emergency Department at Hvidovre Hospital. Blood samples and data on physical and cognitive ability are collected at admission and 4-weeks after discharge. Whole blood unstimulated and stimulated with LPS and TNF-α is cryopreserved in proteomic stabilizer for phospho-flow cytometry analysis. Phospho-NF-κB p65 (pS529) is

measured in classical (CD14++CD16−), non-classical (CD14+CD16++), and intermediate monocytes (CD14++CD16+). Recovery from acute illness is assessed by the change in inflammation, physical and cognitive ability, and physiological function between admission and 4-weeks after discharge. Plasma levels of the inflammation biomarkers interleukin-6 and soluble urokinase plasminogen activator receptor are measured using ELISA. Physical and cognitive ability are measured using hand grip strength and the Orientation Memory Concentration test. Physiological function is measured by the frailty index (FI)-OutRef. Associations between NF-κB activity and measures of recovery are assessed using linear regression models.

Results: Forty-six patients have been included in the study and have completed the 4-weeks follow-up between November 2016 and June 2017. Stimulations of whole blood and protocols for flow cytometry analysis have been tested and optimized. Data is currently being collected and analysis is expected to take place in early 2018.

Conclusions: By exploring how immune responses to acute illness are involved in maintaining or regaining health, we can increase the understanding of why some individuals are able to recover from stressors better than others. This knowledge can be used to design tools that can predict the health trajectory of elderly individuals, and give the possibility to target interventions and treatments to those who will benefit from them, and avoid harming those who will not.

Poster # 69

Flow cytometric and transcriptomic analysis of immune cell phenotyping in breast cancer patients

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Introduction: Breast cancer accounts for 13.8% of all cancer cases and remains the most common cause of cancer deaths in European women. Interactions between the immune system and tumours are highly reciprocal in nature leading to speculation that tumour recurrence or therapeutic resistance could be influenced or predicted by immune events. The aim of this study was to identify markers that are reflective of disease state and potential responses to therapy by observing immune phenotypes in the periphery of patients with breast cancer, both prior to and following neoadjuvant therapy.

Methods: Multi-parameter flow cytometry was used to examine the prevalence and phenotype of natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), monocyte subsets and regulatory T (Treg) cells in the peripheral blood of 67 patients with breast cancer (31 of which were assessed both before and after one round of anthracycline-based chemotherapy) as well as 23 control volunteers. Transcriptomic profiles of peripheral blood mononuclear cells (PBMCs) in 23 of the patients were generated using a nanoString nCounter™ FLEX amplification-free gene profiling platform. Findings were validated using survival data with an opensource genomic data set.

Results: Patients exhibited a more immunosuppressive phenotype compared to control volunteers; with increased prevalence of CD127negCD25highFoxP3+ Treg cells, granulocytic MDSCs and intermediate CD14++CD16+ monocytes, and Treg cells exhibiting a more suppressive, activated phenotype (ICOS+ or CD39+). Following neoadjuvant chemotherapy, B cell numbers decreased, whereas monocyte numbers increased. Although chemotherapy had no effect on Treg, MDSC and NK cell counts, the NK cell receptor profile was altered to be overall more inhibitory, with the proportion of CD56dim NK cells expressing NKp44 and the intensity of NKp30, LAIR, NKG2A and 2B4 expression on these cells being increased. The proportion of CD56bright NK cell expressing CD85j and NKp44 was increased and the proportion of cells expressing DNAM-1 and NKp46 was decreased, coupled with an upregulated intensity of NKp46, LAIR, NKG2A and 2B4 expression and downregulated level of DNAM-1 expression. Transcriptomic profiling demonstrated that a distinct group of 3 patients with triple negative breast cancer (TNBC) expressed distinctly high levels of mRNA encoding genes involved in monocyte/macrophage functions. Expression profiles of three genes (CD163, IFNGR1, CXCR4) were found to significantly predict the survival in patients with TNBC, but not in other breast cancer subtypes.

Conclusion: Phenotypic profiling coupled with transcriptomic analyses of peripheral blood cells can reflect the disease status in a subgroup of patients with triple negative breast cancer, which may have the potential to inform clinical decisions and help predict therapeutic response.

Poster # 71

Characterisation of atherosclerotic plaques in diabetic LDLR-/- mice

Anne Midtgaard-Thomsen

Introduction: Cardiovascular disease is a leading course of death worldwide. People with diabetes have approximately 2-3 times increased risk of dying from cardiovascular events and diabetes mellitus is considered an independent cardiovascular risk factor. Cardiovascular disease is caused by atherosclerosis. The atherosclerotic plaque is characterized by an accumulation of lipids in the artery wall, infiltration of immune cells and the formation of a fibrous cap. Mice are the most frequently used species for atherosclerotic studies and plaque size is widely used as a measure of plaque progression. The aim of this pilot study was to characterize the atherosclerotic immune cells composition by flow cytometry of a diabetic mice model as compared to a non-diabetic mice model.
Method: A 17-week animal study was performed on LDLR-/- mice that develop atherosclerosis when fed a high-fat diet. One group was treated with streptozotocin at week 6 to induce diabetes and maintained on a high-fat diet (n=6) and a non-diabetic group was solely on a high- fat diet (n=6). After 17 weeks, the animals were anesthetized, the aortas were perfused, surrounding adipose tissue was removed and the aorta dissected. A single cell suspension of the murine aorta was prepared by enzymatic digestion, and the immune composition of the murine aortas was analyzed by flow cytometry.
Result: This animal pilot study showed no difference in total number of leukocytes (viable CD45+ cells) per aorta between the groups with a mean difference of 200.5(95%CI -432;833) and p=0.49. However, we observed a mean increase of 11854 (95%CI: -193.3; 5325) in median florescence intensity of the macrophage marker Galectin-3 (Mac2) on viable Cd45+Cd11b+ cells for the diabetic group (p=0.05). Furthermore, we observed a small non-significant increase in Cd68+ cells/aorta (gated as viable Cd45+Cd11b+CD68+ cells) with a mean difference of 111.5 (95% CI:-123.2;346.2) and p=0.31. For Cd8+ T cells (viable CD45+ CD11b+ cells) as percentage of Cd45+ cell we observed a non-significant (p=0.18) mean decrease for the diabetic group of - 11.9 (95%CI -30.5; 6.7). No change was observed for Ly6c+ cells (gated as CD45+, Cd11b+ cells).
Conclusion: This pilot study show indications of an increased presence and activation of macrophages in the diabetic mice model and an alteration in the percentage of Cd8 T cells. The results indicate that diabetes in the mice model can cause changes to the plaque composition and could potentially be an interesting model for diabetic atherosclerosis. However, more studies and inclusion of more markers need to be included in order to confirm this.

Other Topics

Poster # 5

An archaeal Cdc6 protein activating its own expression upon DNA damage and mediating DNA damage tolerance

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Two distinct classes of archaeal Cdc6/Orc1 (hereafter Cdc6) paralogues co-exist in a number of archaea; one functions in replication initiation whereas the other does not. Here the function of a non-initiator Cdc6 protein, the Sulfolobus islandicus Cdc6-2, was investigated. Depletion of Cdc6-2 by gene deletion rendered the mutant hypersensitive to 4-nitroquinoline 1-oxide (NQO), a UV-mimicking agent, compared to the wild-type strain. Upon DNA damage treatment, the expression of the cdc6-2 gene was strongly up regulated and the activation of gene expression required a DNA-damage responsive element (DDRE1) present in the promoter and the cdc6-2 gene. Electrophoretic mobility shift assay (EMSA) showed that Cdc6-2 recombinant protein bound specifically to its own promoter and DNase I footprinting identified 29-nt long stretch of DNA in the DDRE1 region which was protected by Cdc6-2. Mutagenesis of the protected DNA segment revealed the core binding site are essential for the DNA binding activity in vitro and the DNA damage-inducible expression in vivo. Together, these results indicated that Cdc6-2 mediates DNA damage tolerance in S. islandicus and the regulation involves the binding of Cdc6-2 to its own promoter.

Poster # 6

Construction of multiple functional expression systems in *Lactobacillus casei*

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Introduction: Lactic acid bacteria (LAB) represent one of the dominant groups of microorganisms in the human gastrointestinal tract and play important roles in this ecosystem. They are organisms generally regarded as safe and have been used widespread in food and fe-edstock industries. Much research has been devoted to developing gene expression systems for LAB. Lactococcus (especially Lc. lactis), has been developed into useful cell factories for producing recombinant proteins. However, others LAB gene expression systems, especially those of lactobacilli, have not been sufficiently studied.
Results: We isolated a new Lactobacillus strain, *L. casei* MCJ from a starter culture for a traditional yoghurt product in China, and analyzed its plasmid pMC11. The results indicated that this plasmid contains dual replicons and each replicon was employed to construct expression vectors and these vectors could drove the expression of enhanced green fluorescent protein, mannanase, catalase and superoxide dismutase in different Lactobacillus species. We also developed a *Bacillus pumilus* β-1, 4-mannanase gene reporter system in *L. casei*. Furthermore, the signal peptide for the secreted protein Usp45 from Lc. lactis and the full-length SlpA protein from the S-layer of *L. acidophilus* NCFM were tested to drive the secretion of heterologous proteins in *L. casei*.
Conclusion: 1. Constructing the intracellular expression vectors pELX1 and pELX2, secretion expression vectors pELSH and pELSPH, and cell surface-anchor expression vector pELWH. 2. Developing a *Bacillus pumilus* β-1, 4-mannanase gene reporter system in *L. casei*.

Poster # 9

Identification of putative durable targets in the tumor initiating cells of glioblastoma

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Background: Effective treatment of glioblastoma (GBM) remains a major hurdle as standard treatment, including surgery, radio- and chemo-therapy, provides only palliation. GBM patients experience median survival rates of 12-15 months. A small subset of cells referred to as GBM stem cells (GSCs) have been shown to drive the multidrug resistance observed in GBM.
Aim: In this study, we aim to identify novel genes involved in DNA repair and chromatin remodeling, thereby crucial to GSC maintenance after radiotherapy.
Methods: GBM primary cultures were characterized in regards to expression of CD133, sensitivity to IR and proliferation rates. Using siRNA library, we will perform a screen to identify novel factors sustaining GSCs' radio-resistant phenotype. The top genes causing cell cycle arrest and increased DNA damage response (gamma H2AX foci counts) will be scored and used for 'hit' identification.
Results: Characterization of GBM primaries prior to the screen showed heterogeneity in: i) sensitivity to IR; ii) percentage of GSCs based on CD133 staining; and iii) viability of cells over 72 hours post IR. Prior to the siRNA screen, cells cultured as neurospheres were transduced to stably express a SORE6-GFP reporter, driven by both SOX2 and OCT4. The resulting phenotype of knockdown will be assessed in both GSCs (GFP positive cells) and non GSCs (GFP negative cells) obtained from both radiosensitive and radioresistant GBM primary cultures..
Conclusions: Our findings will contribute to better understanding of the underlying mechanisms employed by GSCs to evade radiotherapy. The elucidation of multiple druggable targets in GSCs could aid the possibility of providing a curative treatment option for patients affected by GBM.

Poster # 11

New molecular assay for detection of latent tuberculosis infection using IP-10 as biomarker

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Introduction: The current gold standard assay for diagnosis of latent TB infection (LTBI) is the ELISA-based Interferon gamma release assays (IGRAs). IP-10 is an alternative biomarker with a 100 fold higher expression level compared to IFN-γ. This high expression of IP-10 allows for different analysis platforms including molecular platforms. In this case-control study, we investigated the diagnostic sensitivity and specificity of a molecular assay detecting IP-10 mRNA expression.

Method: We included 90 confirmed TB patients and 99 healthy controls. Blood was drawn in QuantiFERON-TB (QFT) tubes and 8 hours post stimulation IP-10 mRNA expression was analyzed. 20 hour post stimulation IP-10 protein and IFN-γ protein was analyzed using an in-house IP-10 ELISA and the official QFT ELISA respectively.
Results: The IP-10 mRNA assay provided a high specificity (98%) and sensitivity (80%) and AUC= 0.97 however it did not best the QFT assay with specificity (100%) and sensitivity (90%) and AUC=0.99. The IP-10 protein performed at par with the QFT assay with specificity (98%) and sensitivity (87%) and AUC=0.98.
Conclusion: We have provided proof of a robust molecular assay for detection of IP-10 mRNA. As a diagnostic tool, this assay would gain from optimization especially on the kinetics of IP-10 mRNA expression.

Poster # 18

Genetic engineering of filamentous fungi. Studies on self-resistance mechanisms in natural lovastatin producer Aspergillus terreus

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Application of statins is a first choice therapy in treatment of hypercholesterolemia in humans. It is evaluated to lower frequency of heart attacks and deaths caused by overall cardiac events by 25-30% worldwide [1, 2]. Statins competitively inhibit rate-limiting enzyme in cholesterol biosynthesis pathway – HMG-CoA Reductase [3] and increase the expression of LDL receptors in hepatocytes, thus lowering concentration of LDL and its precursors circulating in the bloodstream [4]. Global sales of statins in 2005 reached $25 billion [1], in 2011 $20.5 billion [7]. Available statins are of natural, semi-synthetic and synthetic origin. Naturally produced statins are compactin and lovastatin. Large scale production process of natural and semi-synthetic statins is performed via fermentation (submerged and solid state) with naturally producing filamentous fungi. Several species of filamentous fungi from Aspergillus, Monascus, Penicillium and Trichoderma genera are known for statins production with A. terreus being the most often used producer of lovastatin [5]. Efficient production of bioactive secondary metabolite is inevitably connected with development of self-resistance mechanisms in the natural producer. Revealing and understanding self-resistance mechanisms towards the secondary metabolite, can give bioengineers opportunity to construct cell factories of increased tolerance to the produced metabolite of interest. Increased tolerance can result in higher production yields obtained in fermentation, thus lower production costs. In case of lovastatin producer A. terreus, an efflux pump is encoded in the biosynthetic gene cluster and active form of lovastatin is secreted into the medium. Secondly, four copies of genes coding for statins target protein (HMGR-like) are found in the genome of A. terreus, which may suggest increased resistance to the inhibitor by increased copy number of the protein target. On the other hand, as it was studied before in case of compactin gene cluster [6], one of the additional HMGR-like proteins may show increased resistance towards statins. The goal of this project is to characterize additional copies of the statins target enzyme by deletions, fluorescent proteins tagging, protein expression and purification and enzymatic assays. We aim to explain, deepen the understanding of the existing and non-revealed statins self-resistance mechanisms in A. terreus.

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Poster # 20

Functional analyses of CRISPR-Cas Type III-B system in Sulfolobus islandicus E233S using virus challenging

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CRISPR-Cas system provides an adaptive immune defense against invading genetic elements in Archaea and Bacteria. In the current classification, there are six different types CRISPR-Cas systems among which Type III exhibits DNA/RNA dual targeting activity. The crenarchaeon Sulfolobus islandicus encodes three distinct CRISPR interference modules, which are a Type IA system and two Type IIIB systems: Cmr-α and Cmr-β. To reveal the in vivo DNA interference in S. islandicus, we employ Sulfolobus monocaudavirus1 (SMV1) to challenge S. islandicus E233S with or without Artificial Mini-CRISPR Array. The procedure of producing large amounts of infected S. islandicus cells with SMV1 infection was established at an appropriate MOI. Infected and uninfected cells were collected at different time points and analyzed for DNA content distribution by flow cytometry, and released virus from the supernatant of infected cells had been determined by plaque assay. The strain without Artificial Mini-CRISPR Array had a very high DNA content (>2 genomes) after 108 hpi (hours post infection), whereas cultures harboring type III-B DNA targeting activity could keep 2 genomes. We could not obtain the plaque from the strain with Artificial Mini-CRISPR Array harboring type III-B DNA interference at 108 hpi, 116 hpi, 132 hpi and 140 hpi, respectively, however the supernatant from the strain without this array could form plaques at these corresponding time points. The results indicate that the in vivo interference could function well by the antiviral immune system.

Poster # 22

Optimization of a human growth inhibition assay using virulent mycobacterium tuberculosis

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Introduction: Mycobacterial growth inhibition assays (MGIAs) measure the summative effect of a range of immunological mechanisms and could potentially provide an early measure of vaccine-induced immunity against mycobacterium tuberculosis (mtb). However, MGIA is still a novel technology and the assay pitfalls are poorly described. In this study we describe the optimization process of a human MGIA and try to investigate some of the underlying immunological growth inhibitory mechanisms.
Method: Optimized MGIA: 1 million human PBMCs were co-cultured with 100 CFU mycobacterial strain H37Rv. After 96 hours cells were lysed and mycobacterial growth was determined as time-to-detection by the Bactec MGIT System. The MGIA was evaluated in regard to the variability of the mtb incolum, mtb growth readout, PBMC viability, monocyte and T-cell ratios and media enrichment.
The effects of Bacillus-Calmette-Guerin (BCG) vaccination is currently being evaluated in groups of healthy vaccinated or non-vaccinated volunteers (n= 15+15). PBMCs, plasma and culture supernatants from the study volunteers will be analyzed by flow cytometry, multiplex analysis and antibody-ELISA before the conference date.
Results: Preliminary results include an improvement of PBMC viability from 22% in using state-of-art MGIA protocols to 69% under optimized conditions. Variability was low in individual donor replicates (average CV < 13 %, range 0-20%) as well as between experiments. Proof-of-concept analyses on growth inhibition in BCG-vaccinees vs. non-vaccinated as well as immunological analyses are still ongoing.
Conclusion: Analyses are still ongoing.

Poster # 29

Development of liposomal drug delivery system for targeted delivery of immunomodulating agents to leukocytes

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Introduction: The immune system plays a central role in controlling cancer development. Boosting the power of the immune system to fight cancer using immunotherapy represents a very promising approach to cancer

treatment. Liposomes have been extensively studied for their application as vaccines for targeted delivery of antigens and immunomodulatory agents to specific immune cells as an anticancer treatment. With the aim of delivering immunomodulating agents to specific immune cell subsets, the ability of negatively charged liposomes of various compositions to specifically target subsets of immune cells in whole human blood (WHB) was investigated.

Method: Various anionic liposome formulations were prepared. Compositions were varied with regard to the degree of charge and saturation, presence of cholesterol and polyethylene glycol (PEG), and size. Liposomes were incubated with fresh WHB and analyzed for leukocyte associations by flow cytometry. The involvement of complement proteins in the association of the liposomes with immune cells was studied by heat-inactivating heat-labile serum proteins as well as by specific inhibition of the central complement protein C3 using a selective peptide inhibitor. Furthermore, an immunomodulating agent was incorporated into the liposomes, and its ability to induce secretion of inflammatory cytokines IL12p40 and IL6 in WHB was studied using ELISA.

Results: Cholesterol-rich saturated liposomes with varying degree of charge demonstrated charge-dependent superior association with monocytes however; the same liposomes also showed some association with granulocytes though not to the same extent. Low lymphocyte association was observed irrespective of liposome formulation. Inhibition of complement proteins reduced the association of liposomes with granulocytes and monocytes. The anionic liposomes proved potent in delivering the immunostimulating agent to target cells and in inducing IL-12p40 and IL-6.

Conclusion: These results demonstrate that immune cell targeting can be obtained with liposomes possessing a negatively charged surface, and that complement proteins are involved in the cellular associations observed. The findings support that this drug delivery system could be useful for future application for anticancer immunotherapy.

Poster # 32

T cell recognition of breast cancer antigens

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Introduction: Breast cancer remains the leading cause of cancer death among women worldwide, rendering conventional therapies insufficient despite decades of research. Immunotherapy has excelled as an outstanding breakthrough, utilizing or enhancing the immune system to efficiently fight several types of cancer. Despite originally considered an immunologically silent malignancy, recent studies are encouraging research of breast cancer immunogenicity to evaluate the applicability of immunotherapy as a treatment strategy. The epitope landscape in breast cancer is minimally described, thus it is necessary to identify T cell targets to develop immune mediated therapies. This project investigates four proteins commonly upregulated in breast cancer and thus probable tumor associated antigens (TAAs). Aromatase, prolactin, never in mitosis a related kinase 3 (NEK3), and protein inhibitor of activated STAT3 (PIAS3) contribute to increase growth, survival, and motility of malignant cells.

Method: Aspiring to uncover novel epitopes for cytotoxic T cells, a reverse immunology approach is applied. In silico screening via NetMHC is used to detect peptides within the full length of each of the four proteins that are predicted to bind to HLA-A*0201 and HLA-B*0702. An MHC ELISA is then applied to experimentally confirm which of the peptides are indeed HLA-A*0201 and HLA-B*0702 binders. Hereafter, a novel method for high throughout detection of antigen specific T cells is applied. Via DNA barcode labeled MHC multimer technology parallel screening for T cell recognition of all MHC binding peptides is performed. A cohort of breast cancer patient samples and healthy donor samples are included and compared.

Results: Via in silico screening of the protein sequences, 415 peptides were predicted as HLA-A*0201 and HLA-B*0702 binders. Subsequent in vitro binding analysis in a MHC ELISA platform confirmed binding for 147 of the 415 predicted binders. The 147 peptides were evaluated for T cell recognition utilizing DNA barcode labeled MHC multimers to screen peripheral blood lymphocytes from breast cancer patients and healthy donor samples. Significantly more TAA specific T cell responses were detected in breast cancer patients than healthy donors for both HLA-A*0201 ($p<0.0039$) and HLA-B*0702 ($p<0.001$) restricted peptides. Importantly, several of the identified responses were towards peptides that were predicted as poor or intermediate affinity binders. This is indicative of the importance of inclusion these in the search for epitopes within shared TAAs.

Conclusion: Thus, the inspected proteins aromatase, prolactin, NEK3 and PIAS3, indeed contain targets for T cell reactivity. Further research will include functional testing of peptide specific T cell cultures to validate the peptides as true T cell epitopes through demonstration of intracellular processing and presentation at the cell surface.

Poster # 34

Evaluating prediction strategies for identification of immunogenic mutation-derived neo-epitopes in melanoma

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A number of recent reports point to an important role of mutation-derived neo-antigens in immune recognition of cancer, as predictors of clinical outcome and as potential targets in personalized immunotherapeutic strategies. Mutagen-induced cancer types as melanoma and lung cancer carry a large number of mutations, from which putative neo-epitopes can be predicted. Since the vast majority of mutations are patient specific, identification of neo-epitopes requires prediction and generation of large personalized peptide libraries. Previous reports of neo-epitope identification have however demonstrated that only a minority of peptides (<1%) elicit T cell recognition at a detectable level. Consequently, there is an unmet need to understand the rules identifying immunogenic neo-epitopes.

In this study we evaluate different neo-epitope prediction approaches in three melanoma patients. Peptide libraries of 200-800 peptides for each patient were generated from whole exome sequencing in combination with HLA binding predictions. A comparison of prediction from autologous tumor cell lines (TCL) and snap-frozen tumor fragments (TF) resulted in an overlap in prediction of 29-81%, 13-68% predicted solely from TCL and 0-5% predicted solely from TF. Furthermore, we included both RNA expression values and immunopeptidome analysis by mass spectrometry, as additional tools to identify potential immunogenic neo-epitopes.

T cell recognition in autologous patient material of each personalized peptide library were investigated (ongoing studies) by use of a novel technology based on DNA-barcode labeled MHC multimers, enabling high-throughput screening for >1000 neo-epitope specific T cell populations in a single sample. This broad assessment of neo-epitope reactivity, with minimal preselection, allows identification of the strongest predictive strategies for identification of immunogenic neo-epitopes.

Identification of precise and effective prediction approaches will provide an important step towards the use of neo-epitopes as therapeutic targets and predictors of response to immunotherapy.

Poster # 41

Combination of radiation therapy and liposome encapsulated TLR agonists can induce synergistic anti-tumor effects in cancer mouse models

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Introduction: Cancer remains one of the leading causes of death worldwide with 8.2 million cancer-related deaths in 2012. Each year 14 million new cases of cancer occur and this number is expected to increase by 50% within the next two decades. Development of novel cancer therapeutics and treatment regimens is, therefore, highly desired. It is increasingly recognized that the immune system plays a pivotal role in the development of cancer. Ideally, the tumor is recognized by the immune system and eradicated; however, tumors have developed mechanisms to avoid detection and induce an immune suppressive state that promotes tumor growth. Consequently, the field of immunotherapeutics is expanding with the goal of modulating the immune system to induce an anti-tumor response that will eventually cure the cancer. Multiple immunotherapeutic agents can be used to boost the immune system by several mechanisms e.g. TLR agonists. TLR agonists bind to TLR receptors in various cell types, leading to activation of dendritic cells and priming of specific T cell responses thereby activating the adaptive immune response. Combining radiation with TLR agonist have the potential of creating an in-situ vaccine since radiation promotes the generation of neo-antigens through the introduction of mutations and the immunotherapeutic act as an adjuvant that boosts the immune response. Systemic administration of TLR agonist has been shown to induce adverse effects in patients. Drug delivery systems have the potential to circumvent these toxicity issues and increase the circulation time of the drug. The aim of this project is, therefore, to develop liposomal drug delivery systems for the administration of TLR agonist and combine them with radiation.

Methods: The syngeneic murine tumor model CT26 will be treated with different regimens of radiation and liposome encapsulated TLR agonists administered systemically. To evaluate the anti-tumor effect of the treatments, tumor size will be monitored every other day. Additionally, multi-color flow cytometry analysis will be applied at certain time points to investigate the effect of the treatment on the composition of the tumor microenvironment and on the immune system. Two flow cytometry panels with cell markers identifying the myeloid-derived cells (dendritic cells, tumor-associated macrophages and myeloid derived suppressor cells) and the T-cells (CD4+ T-cells, CD8+ T-cells, and regulatory T-cells) will be used to examine the immune cell subsets in tumor and spleen.
Results and Conclusion: The project is in its initial phase and no results have been obtained yet.

Poster # 44

A functional study of AMBRA1 mutations in human cancer

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Introduction: Autophagy is a tightly-regulated degradation process in which intracellular material is sequestered in double-membrane vesicles (autophagosomes) and delivered to the lysosomes for degradation. The process serves to maintain a homeostatic balance between catabolic and anabolic activities, a regulatory role that in a cancer setting can lead to the following outcomes: on one side, autophagy helps to eliminate oncogenic proteins or damaged organelles, while on the other side, it can mediate the supply of metabolic substrates in an established tumour. Activating molecule in BECN1-regulated autophagy 1 (AMBRA1) has been shown to act as an early regulator of the autophagic process, however it has also been found involved in the regulation of cell proliferation and apoptosis. The intrinsically-disordered nature of AMBRA1, along with its high plasticity, due to its several protein-protein interactions and post-translational modifications (PTMs), appoints AMBRA1 to act as a molecular switch linking different intracellular processes to autophagy. Furthermore, AMBRA1 shows to be frequently mutated in many different cancer types, with 8 % of all melanoma patients possessing the highest frequency of AMBRA1 mutations among the cancer types listed on the cBioportal.org. Based on this, we speculate that AMBRA1 can act as a molecular mediator of the cross talk between different cellular processes known to be important in the development of cancer.
Methods: With this project, we have combined in silico and in vitro approaches to assess the impact of a number of AMBRA1 mutations found in cancer patients, in order to shed light on AMBRA1's contribution to the comprehensive signalling network that leads to impaired regulation of cancer-related processes. Mutations were extracted from cBioportal.org, and the functional impact of each mutation were scored and ranked by the application of various online prediction tools. Moreover, PTMs and interaction domains of AMBRA1 were identified, and a short-list of 20 mutations were compiled by the integration of the mentioned parameters. These mutations will be further examined in vitro by large-scale analyses, covering the cellular processes that AMBRA1 shows to be implicated in, namely autophagy, proliferation and apoptosis.
Results: Presently, 20 mutations that have been predicted to posses a structural or functional impact on AMBRA1 by the application of a broad range bioinformatical prediction tools are being assessed in relation to their effect on autophagy, cell proliferation, and apoptosis. For this purpose, a number of different cell-based model systems have been developed using the CRISPR/Cas9 technology.
Conclusion: The study is ongoing and no absolute conclusions can presently be drawn.

Poster # 46

Insights into giant algal viruses and their potential virophages in a freshwater lake

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Virophages are closely related with mimiviruses and amoeba. Infection by virophages reduced reproduction rate of mimiviruses, which inversely protected eukaryotic host. Virophages are widely distributed in the environment. Studies had shown virophages share homologous genes with giant virus in the family of Phycodnaviridae.
In this study, we analyzed sequences of virophages and giant viruses from Dishui Lake metagenome. Sequence assembly was performed by map-to-reference method. Accuracy of sequence assembly was checked by both observation and Poymerase Chain Reaction (PCR) experiment. Genomic sequences analyses included ORF prediction, gene annotation and phylogenetic tree construction.

Through sequence analysis, we found number of prasinovirus homolog genes (41.4%) was almost double of chlorovirus (21.5%). Phylogenetic analysis based on giant virus related MCP and PolB revealed that Dishui Lake sequences were closely related with Prasinovirus. Some sequences represent new members of Prasinovirus. Besides, we obtained a nearly complete genomic sequence of giant algal virus after sequence assembly. The genome size of giant algal virus was 181,117bp and named as Dishui Lake phycodnavirus 1 (DSLPV1). Genomic analysis indicated DSLPV1 was closely related with Prasinovirus. Diversity of virophages in Dishui Lake was studied by metagenomics analysis. 47 virophage related contigs were obtained in previous study. Phylogenetic analysis based on virophage conserved gene MCP revealed that Dishui Lake virophage related sequences were clustered with Organic Lake virophage (OLV) and Yellowstone Lake virophages YSLV1-7. A complete genomic sequence of virophage was obtained by sequence assembly and named as Dishui Lake virophage 2 (DSLV2). Genome size of DSLV2 was 31,238bp, which hold the largest genome record of virophage. G+C content of DSLV2 was 38.3%. Genomic sequence analysis showed DSLV2 was closely related with YSLV4. DSLV2 contained all virophage core genes.In addition, we found four genes of Dishui Lake virophages (DSLV1, -2) were homologous to DSLPV1 genes. In conclusion, genomic study was performed to give insight into giant viruses and virophages in Dishui Lake.

Poster # 54

SWOFF - the unrecognized sibling of FMO

Michael Kapinsky
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Background: Fluorescence-Minus-One (FMO) controls are widely used as controls for assessment of positive-negative thresholds of specific phenotypes within a given multicolor panel. However, typical FMO analysis will not reveal “false negative” signal intensities that may be disguised by data spreading through spillover emissions.
Methods: FMO controls were conducted in five 10color datasets and each of the resulting datasets was analyzed for the impact of the “minus one”-conjugate on the detection limit of all other conjugates. As this approach could be interpreted as to “switch off” spillover emissions it is proposed to call the method “switch off” or SWOFF analysis.
Results: Between the different conjugate combinations SWOFF analysis clearly indicated differences in the percentage of events that were gated positive for modulated and dim markers. Thus, the most sensitive combination of bright and discrete markers among the five combinations that would provide the highest sensitivity could be identified.
Conclusion: While FMO analysis determines only the detection limit of a modulated marker within the spillover pattern of a given panel, SWOFF analysis indicates unfavorable conjugate combinations with compromised sensitivity for modulated and dim markers. Moreover, the SWOFF concept allows to assess the accuracy of %positive-results obtained for modulated or dimly expressed antigens.

Poster # 59

Molecular characterization of atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease which affects both children and adults all over the world. The complex genetic, immunologic and environmental interplay in this disease has led to a still incomplete understanding of the pathology of AD. A better understanding of the molecular mechanisms behind the disease might give insights into the pathology, which hopefully will lead to better treatment of patients.
To achieve this molecular understanding we will use various bioinformatics methods combined with immunoassays and flow cytometry to get a better understanding of the genetic and cellular mechanisms involved in AD. We hope to create a thorough characterization of the disease integrating many types of data to improve the current knowledge in the field.

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