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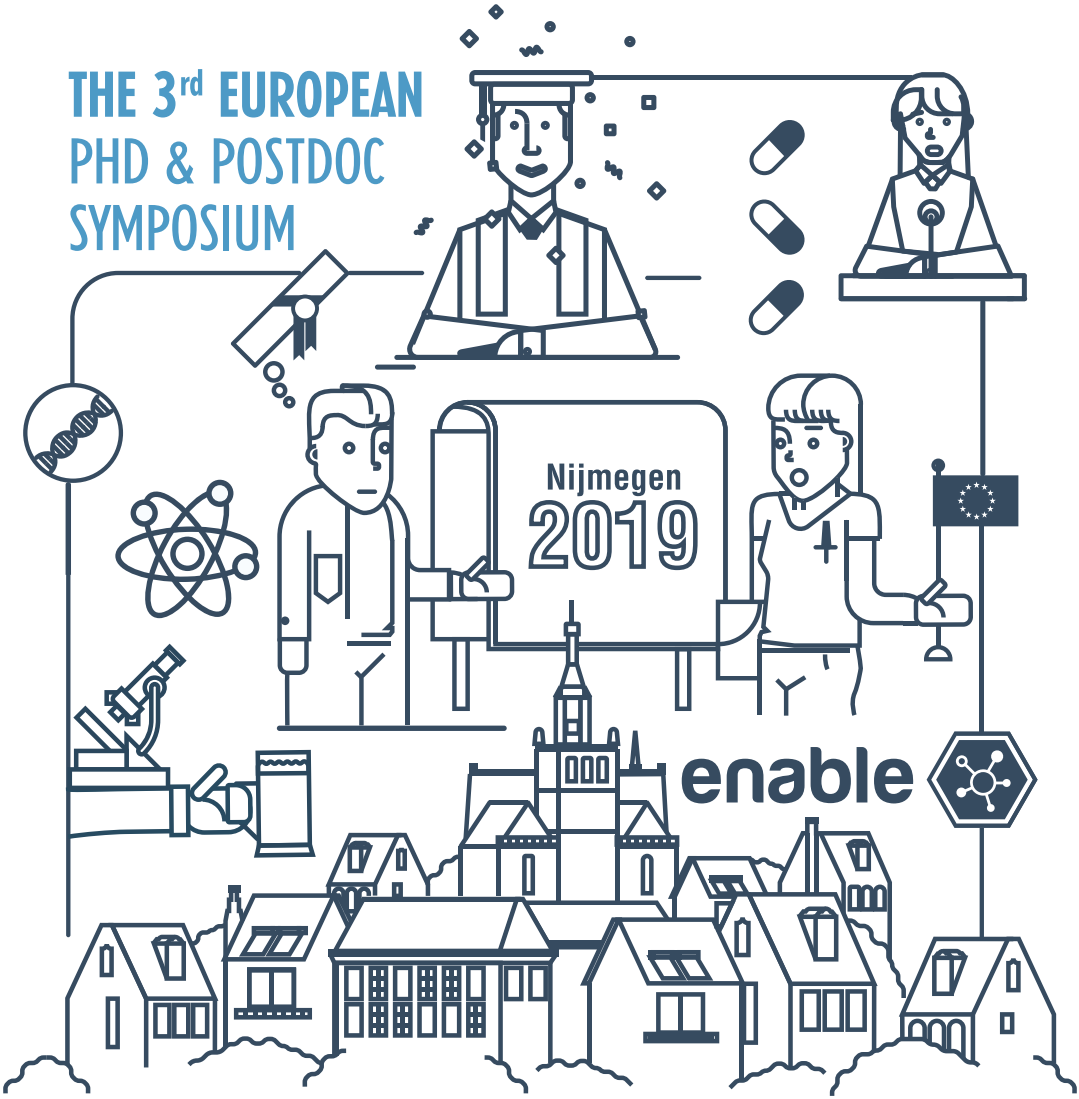

Next-generation life scientists
Side by side to break new ground

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Contents

Talks

Thu, 09:50 - 10:05

Targeting pathogenic Lafora bodies in Lafora disease using an antibody-enzyme fusion

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Lafora disease (LD) is a fatal childhood epilepsy and a non-classical glycogen storage disorder with no effective therapy or cure. LD is caused by recessive mutations in the EPM2A or EPM2B genes that encode the glycogen phosphatase laforin and the E3 ubiquitin ligase malin, respectively. A hallmark of LD is the intracellular accumulation of abnormal and insoluble a-linked polysaccharide deposits known as Lafora bodies (LBs) in several tissues, including most regions of the brain. In mouse models of LD, genetic reduction of glycogen synthesis eliminates LB formation and rescues the neurological phenotype. Since multiple groups have confirmed that neurodegeneration and epilepsy result from LB accumulation, a major focus in the field has shifted toward the development of therapies that reduce glycogen synthesis or target LBs for degradation with the goal of treating LD. In this study, we identify the optimal enzymes for degrading LBs, and we develop a novel therapeutic agent by fusing human pancreatic a-amylase to a cell-penetrating antibody fragment. This antibody-enzyme fusion (VAL-0417) degrades LBs in vitro, shows robust cellular uptake, and significantly reduces the LB load in vivo in Epm2a-/- mice. Furthermore, we demonstrate that wild-type (WT) and Epm2a-/- mice possess unique brain polar metabolite profiles. VAL-0417 treatment of Epm2a-/- mice yields brain polar metabolite profiles indistinguishable from WT animals by multivariate analysis. VAL-0417 is a promising drug for the treatment of LD and a putative precision therapy for an intractable epilepsy. Antibody-enzyme fusions represent a new class of antibody-based drugs that could be utilized to treat glycogen storage disorders and other diseases. Additionally, targeted metabolomics is a powerful and robust method for monitoring disease progression and response to treatment.

Thu, 10:05 - 10:20

Immobilized synthetic polymers as a novel cancer immunotherapy to activate and expand tumor-reactive T cells

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Traditional tumor vaccination approaches mostly focus on activating dendritic cells (DCs) by providing them with a source of tumor antigens and/or adjuvants, which in turn activate tumor-reactive T cells. Novel biomaterial-based cancer immunotherapeutic strategies focus on directly activating and stimulating T cells through molecular cues presented on synthetic constructs with the aim to improve T cell survival, more precisely steer T cell activation and direct T cell differentiation. Artifical antigen-presenting cells (aAPCs) decorated with T cell-activating ligands are being developed to induce robust tumor-specific T cell responses, essentially bypassing DCs.Filamentous polyisocyanopeptide polymers (PICs) are highly promising synthetic aAPCs that display unique semiflexibility and strain-stiffening behavior. PIC polymers (200-400 nm in size) can be functionalized with multiple biomolecules such as antibodies and cytokines that can be presented to T cells in a multivalent manner to evoke robust T cell responses (1-3). Here, we developed a novel system which enables site-specific attachment of end-functional PIC polymers to a surface (4). This system allows for immobilization of PIC polymers on (magnetic) beads or plate surfaces to create a unique platform to deliver the activating cues to T cells on a semiflexible brush. The PIC-modified particles induced higher proliferation and especially improved cytokine production by T cells in vitro when compared to beads directly modified with antibodies or commercially available Dynabeads. In addition, this system is highly modular and allows for attachment of a wide range of biomolecules, which can for instance be used to expand antigen-specific T cells using peptide:MHC complexes. The introduction of cargo-loaded particles into the system also allows for the delivery of small molecules, such as cytokines or TGF-beta inhibitors, to T cells. All in all, this approach is widely applicable and holds great promise for both in vitro and in vivo cancer immune T cell-therapies.

Thu, 12:10 - 12:25

Mast cells- derived osteopontin protects from neuroendocrine prostate cancer

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Cancer development is influenced by the interactions between tumor cells and the microenvironment. Using the TRAMP mouse model of prostate cancer we have recently identified Mast Cells (MCs) as promoters of adenocarcinoma growth, whereas their inhibition or genetic deletion (by crossing TRAMP mice with MC-deficient KitWsh mice) lead to unexpected increase of tumors with neuroendocrine (NE) features. In prostate cancer patients, these tumors emerge after androgen deprivation therapy and correlate with poor outcome. NE tumors can arise also as de novo entities, albeit very rarely. We observed an abnormal frequency of NE tumors also in TRAMP mice genetically deficient for the non-structural matricellular protein osteopontin (OPN), and found that prostate-infiltrating MCs were able to produce OPN. We therefore hypothesized that MCs can limit the growth of NE prostate cancer through OPN production. Accordingly, in vitro experiments showed that OPN sufficient (WT), but not OPN-deficient, bone marrow-derived MCs inhibited the proliferation of NE prostate cancer cell lines, while they did not affect adenocarcinoma cell growth. Moreover, adoptive transfer of WT, but not OPN-deficient, MCs in KitWsh-TRAMP mice reduced NE tumors to a frequency similar to that observed in TRAMP mice. We are now investigating which molecular pathways are involved in the control of NE differentiation mediated by MC-derived OPN. Our data indicate a dual role of MCs in promoting or preventing prostate adenocarcinoma or NE tumors, respectively, and warns that therapeutic targeting of MCs could be effective against prostate adenocarcinoma, but detrimental if not combined with approaches directed against NE variants.

Thu, 12:25 - 12:40

Towards a personalized eye-on-a-chip for age-related macular degeneration

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One of the major hallmarks of age-related macular degeneration (AMD) is the accumulation of protein-lipid deposits, known as drusen, in the tissues of the outer blood-retinal barrier (BRB). The key cells within the BRB are the retinal pigment epithelium (RPE) and the endothelial cells (ECs) forming the choroidal capillaries. The recent development of human-induced pluripotent stem cell (hiPSC)-derived RPE and ECs has led to their use as in vitro models in drug development. However, such models typically rely on simplified monolayer cultures that insufficiently capture the tissue dysfunction of AMD. In order to overcome this limitation, microfluidic organ-on-chip technology represents a promising technology for the development of 3D in vitro models. These microfluidic cell culture devices have engineered microchannels that are continuously perfused and inhabited by living cells to form tissues that exhibit organ-level physiology.The aim of this project is therefore to develop an organ-on-chip model of the outer BRB, fully based on hiPSC-derived cells.We are currently generating ECs and RPE from hiPSC lines obtained from control and AMD-affected individuals. All individuals were genotyped for 52 AMD-associated variants, and 13 AMD-related genes were sequenced to detect rare coding variants. Differentiation and maturation of hiPSCs-derived cells is assessed using immunostaining of several markers specific to each cell type. Characterized cells are then incorporated into an organ-on a-chip device containing a microchannel and an open top culture chamber, separated by a polyester membrane. ECs are seeded in the microchannel in order to mimic a capillary-like structure, and RPE cells are seeded in the open top culture chamber. For both cell types survival and maturation in their respective microenvironment is assessed.This new in vitro model will provide new knowledge on how various molecular, cellular and physical aspects interact in AMD, and can be used for testing new therapeutic molecules.

Thu, 14:50 - 15:05

Structural insight into TRPV5 channel function and modulation

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Introduction: TRPV5 (transient receptor potential vanilloid) is a calcium-selective ion channel that helps to maintain calcium homeostasis. Unlike other TRPV channels, TRPV5 does not exhibit thermosensitivity or ligand-dependent activation, but is constitutively opened at physiological membrane potentials. Channel function is tightly regulated by calcium and the calcium-sensing protein calmodulin (CaM). However, it is still unknown how CaM binds and inactivates TRPV5/6. Our study aims to understand the structural characteristics of CaM-dependent channel gating. Methods: Full length and truncated TRPV5, expressed in HEK293 cells, was affinity-purified and reconstituted into lipid nanodiscs or detergent micelles. After assessing the reconstitution efficiency with size-exclusion chromatography and negative stain EM, pure TRPV5 protein fractions were pooled, concentrated and used for cryo-EM analysis. Data collection took place on a Titan Krios electron microscope. Data analysis was carried out in CryoSPARC and RELION. Results: We report high-resolution cryo-EM structures of full length and truncated TRPV5 in lipid nanodiscs (2.8Å and 3.0Å), a TRPV5 W583A mutant structure (2.8Å), and a complex structure of TRPV5 with CaM (3.0Å). Our TRPV5 structures highlight several new features: an extended S1-S2 linker that stabilizes the upper pore and a tryptophan residue (W583) that controls closing of the lower pore. Indeed, the W583A mutant channel was resolved with an open lower gate. The TRPV5-CaM complex structure demonstrates that CaM interacts with two carboxy-terminal regions of TRPV5. During inhibition, CaM residue K115 inserts itself into the lower pore, where it is coordinated by the side chains of W583. This conformation sterically blocks ion permeation. Our data also demonstrates that CaM is able to bind TRPV5 either in a 1:1 or 1:2 stoichiometry. Conclusion: Our structures highlight channel features that diverge from other TRPV channels. Most notable, we provide insight into TRPV5 channel gating and propose a flexible stoichiometry model for CaM-dependent channel regulation.

Thu, 15:05 - 15:20

A tumor-on-a-chip approach for the investigation of novel cancer therapies

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To better understand and improve targeted drug delivery to tumor cells, new in vitro models simulating the complexity of the tumor microenvironment are needed. In this study, to assess the delivery of epithelial cell adhesion molecule (EpCAM) binding designed ankyrin repeat proteins (DARPins) to EpCAM-positive cells, a microfluidic tumor-on-a-chip system was used. The tumor-on-a-chip consists of a central chamber where tumor cells mixed with fibroblasts grow embedded in a collagen matrix. Two side channels serve as medium reservoirs to allow the diffusion of nutrients and the addition of DARPins. The diffusion into the collagen matrix and the delivery to EpCAM-positive cells of two EpCAM-binding DARPins with different affinities and one non-binding control were investigated. The experimental observations were then used to develop a mathematical model that helps to predict the penetration of the DARPins in the tumor-on-a-chip. Real-time 3D time-lapse videos of the three conditions were acquired using confocal microscopy, and variables including the diffusion coefficient of DARPins and a dextran as well as the rate of import into the collagen matrix were calculated based on the microscopy videos. Additionally, the receptor density on the surface of the cells was quantified using flow cytometry. The results demonstrate the potential of the tumor-on-a-chip system as a promising model system to study and predict drug delivery and penetration in complex 3D tumor microenvironments.

Thu, 17:20 - 17:35

Multimodal imaging of PD-L1 expression in a syngeneic mouse model

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Background: Cancer hijacks the immune system in order to survive. Recently, a new type of cancer treatment became available that blocks the inhibitory signals the tumor cells use to escape the immune system: 'checkpoint inhibitors'. These have been shown to be clinically effective even in the treatment of metastatic melanoma, a disease that was until recently untreatable. However, the treatment is not effective in all patients. One correlate of treatment outcome is the expression of checkpoint molecules by tumor cells, tumor-infiltrating lymphocytes and tumor-infiltrating myeloid cells. The solution: predictive and multimodal imaging of checkpoint expression in the tumor. Results: We developed a chemistry-based plug-and-play platform that allows us to design and synthesize a variety of constructs with different functionalities.We used CRISPR/Cas9 technology to generate antibodies and antibody fragments that can be modified site-specifically and are easy to purify. The disadvantage of a monoclonal antibody in imaging is the long half-life. This can be solved by using a Fab' fragment: because of its small size, the optimal tumor-blood ratio is achieved at an earlier time point compared to a mAb. Moreover, mAbs often give a high background signal because of liver uptake. It has been shown before that Fc-silent mAbs show a significantly reduced off-target uptake. To test these hypotheses, sortaggable Fab' fragments, mAbs and Fc-silent mAbs were successfully modified with a peptide that contains a fluorophore and a radioligand chelator to enable multimodal imaging. In vivo characteristics of the different constructs will be compared in an upcoming in vivo study.

Thu, 17:35 - 17:50

A mathematical perspective on glioma spread and migration in the brain

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Brain tumors, especially glioblastoma, are among the most aggressive and invasive type of cancer, with a poor prognosis and, often, a short life expectancy. In particular, glioma growth, spread and migration inside the brain tissue results in a complex evolutionary process involving several mechanisms on different spatial and temporal scales. One of the main problems associated to the diagnosis and the treatment concerns the difficulty to obtain a clear estimation of tumor infiltration and of its real outer border. Tumor cells, indeed, are highly motile and, exploiting the intrinsic characteristics of the nervous tissue, they spread inside the brain creating low density tumor regions that cannot be detected with the current medical imaging techniques, leading, often, to not completely successful treatments.On the basis of real clinical patient data, we formulate mathematical models for the description of tumor progression. From one side, starting from MRI scans and DTI data, we focus on an accurate reconstruction of the underlying nervous tissue geometry and of the fiber structures influencing tumor diffusion and leading to the emergence of heterogeneous patterns. From the other side, we translate into equations the cellular behaviors reported in the biological experiments and related to the chemical and mechanical processes driving cell migration and interaction with the extracellular environment.We perform numerical simulations of temporal evolution, growth and development of the tumor in order to show the effect of the modelled processes on its progression and its response to possible variations of the microenvironment conditions.As further step, we are going to use this computational framework to test the effect of potential therapies, changing dosages, combinations and time schedule.

Fri, 09:50 - 10:05

Molecular characterization of human T helper cell subsets using integrated analysis of multiple omics levels

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T helper cells play an important role in our adaptive immune system and have specialized to fulfill different tasks leading to efficient defense against harmful invaders. Those specialized for the same task are grouped into T helper cell subsets.We aim to describe T helper cell subsets on a deep molecular level and thus better characterize their phenotypes and functions by identifying a unique set of marker genes for each subset in a robust and unbiased way. To achieve this aim, we analyze gene expression and protein secretion among 79 human T helper cell clones.We cluster T helper cells based on their measured protein secretion profile by calculating a consensus of five different algorithms to obtain robust, uniform clusters. The computationally defined clusters are associated to known, biological T helper cell subsets or combinations thereof. Next, we use whole genome gene expression data to characterize T helper cell subsets on a molecular level. We build a consensus of six differential gene expression methods which uncovers a core set of T helper cell subset specific marker genes. The differential gene expression methods comprise standard tools, newly introduced methods (Aran et al., 2017) and regularized regression models. In regularized regression modeling measured gene expression levels are represented as features (predictors) and we perform feature selection using the elastic net penalty. In bionomial logistic regression, we control for multiple testing using stability selection. In multinomial regression models, we compare features selected by different lasso penalties.To set those uncovered subset specific marker genes in the right biological context and to determine possible targets for experimental validation, we additionally perform pathway analysis and investigate protein-protein interactions. In summary, our approach identifies known and novel marker genes for T helper cell subsets. Their implications for immune system functions still have to be experimentally tested.

Fri, 10:05 - 10:20

Biomarker-based Screening of Cervical Cancer for Potential Drug Targeting.

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To meet increased energy demands, metabolic activities of cancer cells changes to accommodate additional nutrients, using multiple mechanisms. This metabolic reprogramming leads to difference in metabolites along pathways through which they acquire and replenish their metabolic needs, when compared with those of normal cells.These differential metabolites are currently been evaluated as indicators for progression of precancerous lesions and therefore play significant role in early diagnosis of cancer. Serum protein samples from healthy control subjects, patients with Human Papilloma Virus infection (HPV), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and cervical cancer (CC) were profiled for differential proteome. Low abundant serum proteins were purified using AlbuVoidTM Immunodepletion kit. Enriched low-abundant proteins were subjected to electrophoresis and High performance liquid chromatography analysis to compare the proteome of HPV, LSIL, HSIL and CC group against control group followed by verification using target proteomics technology. Compared with the control group, both LSIL group and HSIL group showed 9 differential protein peaks; 5 differentially expressed proteins bands were identified in CC group. The molecular weight and HPR of the proteins showed obvious differential expressions in LSIL and HSIL groups compared with the control group, and could serve as potential biomarkers for the progression of cervical carcinoma. The expression of the differential peaks increased consistently with the lesion progression from LSIL to HSIL and CC, suggesting its value as a potential biomarker for the progression of cervical cancer. In Conclusion we identified 5 new protein biomarkers for cervical precancerous lesions and for prognostic evaluation of CC, and combined detection of these biomarkers may help in the evaluation of the development and progression of CC and also in improving the diagnostic sensitivity and specificity of cervical lesions.

Fri, 12:10 - 12:25

Brain organoids meta-analysis reveals model-specific timing of human cortical development recapitulation

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Brain organoid technology is transforming neurobiology by enabling the investigation of human neurodevelopment in vitro and thereby making its spatiotemporal dynamics accessible and experimentally tractable. While an increasing range of protocols, spanning undirected to patterning-driven differentiation, have achieved remarkable degrees of recapitulation of brain development, what is acutely lacking is a comprehensive analysis of how closely organoid models recapitulate the milestones of human fetal brain development. Here we meet this need through a comprehensive benchmarking of brain organoid protocols vis-a-vis in vivo corticogenesis, integrating: i) our in-house cohort of cortical brain organoids (CBO) from several individuals in multiple differentiation rounds profiled over 200 days, thereby covering the equivalent of early to mid-late gestation ii) an exhaustive meta-analysis of publicly available transcriptomic data from all organoid protocols in relation to the BrainSpan Atlas.Whole-transcriptome correlation analysis of CBO vis-a-vis fetal cortex revealed high and stage-specific concordance, establishing their across-lines reliability in recapitulating not only human cortical development but also its stereotypical timing. In contrast, the same analysis for other protocols unearthed strong correlations with advanced post-conceptional weeks already at early phases of organoid differentiation, indicating a protocol-dependent acceleration and compression of physiological corticogenesis, a finding confirmed also across all inter-protocols comparisons vis-a-vis patterned CBO. Consistently, time-wise differential expression analysis on CBO uncovered an initial rapid evolution of the system followed by subtler transcriptional modulations after 100 days, with alternative protocols showing an anticipated plateauing already at around 50 days.In sum, our work maps the current universe of brain organoid protocols onto a matrix of in vivo/in vitro developmental benchmarks, demonstrating for CBO a more precise recapitulation of the timing of human cortical development. This virtually complete alignment of in vivo/in vitro temporality makes CBO instrumental in dissecting stage-specific alterations of neurodevelopmental disorders.

Fri, 12:25 - 12:40

Disease modelling of core pre-mRNA splicing factor haploinsufficiency

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The craniofacial disorder Mandibulofacial Dysostosis Guion-Almedia type is caused by haploinsufficiency of the U5 snRNP gene EFTUD2/SNU114. However, it is unclear how reduced expression of this core pre-mRNA splicing factor leads to craniofacial defects. Here we use a CRISPR-Cas9 nickase strategy to generate a human EFTUD2-knockdown cell line, and show that reduced expression of EFTUD2 leads to diminished proliferative ability of these cells, increased sensitivity to endoplasmic reticulum (ER) stress and the mis-expression of several genes involved in the ER stress response. RNA-Seq analysis of the EFTUD2-knockdown cell line revealed transcriptome-wide changes in gene expression, with an enrichment for genes associated with processes involved in craniofacial development. Additionally, our RNA-Seq data identified widespread mis-splicing in EFTUD2-knockdown cells. Analysis of the functional and physical characteristics of mis-spliced pre-mRNAs highlighted conserved properties, including length and splice site strengths, of retained introns and skipped exons in our disease model. We also identified enriched processes associated with the affected genes, including cell death, cell and organ morphology and embryonic development. Together, these data support a model in which EFTUD2 haploinsufficiency leads to the mis-splicing of a distinct subset of pre-mRNAs with a widespread effect on gene expression, including altering the expression of ER stress response genes and genes involved in the development of the craniofacial region. The increased burden of unfolded proteins in the ER resulting from mis-splicing would exceed the capacity of the defective ER stress response, inducing apoptosis in cranial neural crest cells that would result in craniofacial abnormalities during development.

Fri, 15:05 - 15:20

Sensing of forces by asymmetric endothelial junctions in angiogenesis

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Collective migration of endothelial cells is required during angiogenesis, and critically relies on cell rearrangements through the remodeling of VE-cadherin-based cell-cell contacts couple to the dynamic actin cytoskeleton. During directional collective migration, an imbalance on pulling forces between cells leads to the formation of asymmetric adherens junctions (AJs) in which the F-BAR protein Pacsin2 specifically recognizes the positive curvature of the membrane generated in the front of follower cells. Our group previously showed that Pacsin2 locally inhibits VE-cadherin internalization to promote cell-cell adhesion. However, the mechanism behind junctional Pacsin2 signaling and the importance of asymmetric AJs for vascular physiology remain unknown. We have made use of the Pacsin2 and its effector knock-out mouse to further investigate the importance of asymmetric AJs in angiogenesis. Our results show that deletion of Pacsin2 leads to the formation of aberrant vascular sprouts characterized by the accumulation of endothelial cells, suggesting Pacsin2 is needed in endothelial collective behaviors in angiogenesis. Furthermore, we hypothesized that other curvature sensing proteins could be sensing and translating asymmetric forces in the junctions to control collective cell migration. To investigate that, we have performed a shRNA screen for a large library of curvature sensing BAR proteins and study their junctional implication in this process. Studying the molecular mechanisms responsible for asymmetry sensing would lead to a deeper knowledge of collective process as tissue morphogenesis, wound healing and cancer invasion.

Fri, 14:50 - 15:05

Differential functional roles for anterior and midcingulate cortex - implications for aggression and sociability

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Cingulate cortex is a hub for the control of emotional and cognitive processing and as such of interest to both clinical and preclinical research in a range of model species. However, translational research has been hampered by inconsistencies in anatomical definitions of cingulate cortex across species. While in humans, monkeys and rabbits the border between anterior cingulate cortex (ACC) and midcingulate cortex (MCC) is drawn along a rostro-caudal gradient, the most popular nomenclature in rats and mice draws the border perpendicular to that axis. A homologous definition of cingulate cortex for rats and mice exists, but is only applied in a small minority (< 10%) of studies. We present meta-analyses and experimental data demonstrating that the homologous nomenclature not only improves the translational utility of rodent studies, but also better reflects the structural and functional organization within rodent cingulate cortex itself. Utilizing the homologous definition in the BALB/cJ mouse model of aggression, we show that the volumes of ACC and MCC differentially predict aggressive behaviour, results that we would not have observed using the non-homologous definition. Interestingly, aggressive mice showed a high amount of pyknotic neurons in ACC and MCC, suggesting genetically induced apoptosis. In line with this, cFos activation levels were decreased twofold in ACC and MCC in these mice. We then related metrics of aggression and sociability in the same mice, and identified subgroups of animals displaying either sociable non-aggressive, sociable aggressive or non-sociable aggressive behaviour. These phenotypes were predicted differentially by the distribution of interneurons throughout cingulate cortex: Aggression, irrespective of sociability, was best predicted by a loss of interneurons in MCC, while social withdrawal, irrespective of aggression, was characterized by a flat, undifferentiated, distribution of interneurons across cingulate cortex. Together, these results highlight how the cytoarchitecture of ACC/MCC can shape both social and aggressive behaviour.

Posters

P1 (Session A)

PLGA nanoparticles for super-mega-ultra-sensitive in vivo cell tracking using SPECT/CT

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Nuclear imaging techniques are well-suited for in vivo tracking of injected cells, due to their high sensitivity, quantitative nature and whole-body imaging capability1-3. Previously, we have developed PLGA-based nanoparticles (PLGA-NPs), containing 19F for high resolution MRI and different fluorescent dyes for optical imaging. Here we describe the modification of PLGA-NPs for 111In-labelling to include SPECT in the multimodal tracking of cells in vivo.PLGA-NP modified with a diamine cap (PLGA-AA) were conjugated with DTPA and labelled with [111In]InCl3. Radiochemical yield was measured using a dose calibrator, radiochemical purity with iTLC, and stability was assessed in PBS or human serum. A range of 0.1-50mM EDTA was used for an overtime challenge experiment at 37oC. Lymphocytes and monocytes were incubated with 1.6-1.7MBq/mg [111In]In-PLGA-AA overnight at culture conditions and labelling efficiency was measured in a dose calibrator. CellTiterGlo was used for cell viability.PLGA-NPs (non-modified), PLGA-AA, and DTPA-PLGA-AA (PLGA-AA conjugated with DTPA) were made. The results showed specific 111In-labelling of PLGA-AA (Fig.1A). Labelling efficiency was 87%, 42%, and <10% for PLGA-AA, DTPA-PLGA-AA, and PLGA-NP respectively. 111In retention was >90% up to 3 days in PBS and serum (Fig.1B). 111In retention remained stable during EDTA challenge at low concentrations, but was released at higher concentrations (Fig.1C). Labelling of lymphocytes and monocytes with a range of [111In]In-PLGA-AA showed a labelling efficiency of 1.4-3.1%, with higher labelling efficiency for monocytes (Fig.2A). The specific activity increased with increasing concentration of particles: 10mg PLGA-AA resulted in a specific activity of >0.8MBq/10ˆ6 cells

(lymphocytes) and >3MBq/10ˆ6 cells (monocytes, Fig.2B). No variation in cell viability was seen (Fig.2C).We have shown efficient and specific 111In-labelling of PLGA-AA compared to PLGA-NP. Preliminary results indicate sufficient labelling efficiency for in vivo imaging. Cell viability was not affected after overnight incubation. Therefore, we conclude that PLGA-AA are suitable for cell labelling.

P2 (Session A)

Calcium-dependent Regulation of Transient Receptor Potential Vanilloid 5 by Calmodulin

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Calcium (Ca2+) homeostasis is vital to various physiological processes, like muscle contraction, nerve impulse propagation and intracellular signalling. In the kidney, the transient receptor potential vanilloid 5 (TRPV5) channel mediates Ca2+ reabsorption in the final part of the nephron, therefore being the gatekeeper of Ca2+ homeostasis. However, to prevent aberrant signalling and apoptosis-induction by an overload of intracellular Ca2+, a negative feedback mechanism mediated by calmodulin (CaM) inactivates TRPV5. Currently, the exact mechanism of this feedback remains elusive. The first aim of this project is, therefore, to investigate the Ca2+-dependency of the two lobes of CaM in the CaM-mediated inactivation of TRPV5. Additionally, the stoichiometry of the TRPV5-CaM complex will be investigated in vitro, since it can shed more light on the inactivation mechanism of TRPV5 by CaM. Using fluorescence lifetime imaging microscopy via fluorescence resonance energy transfer (FLIM-FRET) and Fura-2-mediated ratiometric Ca2+ imaging data was acquired on the occurrence and functionality of the interaction between TRPV5 and CaM respectively. It appears that the C-lobe of CaM mediates the interaction at basal Ca2+ concentration, whereas the N-lobe is essential for TRPV5 inactivation upon Ca2+ influx. Single molecule photobleaching using total internal reflection fluorescence microscopy was set up and extensively optimized for determining the stoichiometry of the TRPV5-CaM complex. As proof of principle, the tetrameric structure of TRPV5 was confirmed. In the future, the acquired knowledge could aid in elucidating TRPV5-associated disease pathogenesis and developing specific therapies modulating the negative feedback of TRPV5 by CaM.

P3 (Session A)

Towards a network-driven biological knowledge integration

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Biological data is steadily growing while its integration is becoming an increasingly cumbersome process. We have created a gigantic heterogeneous network (more than 500k nodes and 70M edges) that harmonizes and connects biomedical data from multiple kinds and sources. Overall, 12 types of biological entities (e.g. genes, diseases, drugs) were linked by 74 types of relationships (e.g. drug treats disease, gene interacts with gene). To provide fast and flexible querying of such a complex resource, we stored the full network as a graph database, appropriately handling the ontologies and vocabularies encountered throughout the >200 sources. Finally, we are able to encode this knowledge as numerical vectors, representing different biological contexts for each entity and enabling their use in state-of-the-art machine/deep learning approaches.

P5 (Session A)

Disentangling epigenetic regulatory mechanisms with interaction proteomics

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Disentangling epigenetic regulatory mechanisms with interaction proteomicsVelin Marita Sequeira1*, Michiel Vermeulen1Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Oncode Institute, Radboud University Nijmegen, 6525 GA Nijmegen, The Netherlands.Epigenetic modifications, such as DNA methylation and histone modifications alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. DNA methylation mostly occurs in a CpG dinucleotide context and involves the covalent addition of a methyl group at the 5-carbon position of cytosine (5mC). Dynamic changes in DNA methylation and demethylation orchestrate the transcriptional network during developmental processes like lineage specification. Alterations in these pathways often result in developmental disorders and cancer. CpG islands (CGIs), characterized by a high CpG density, are present in promoter regions of genes and are typically hypomethylated. Although methylation of CGIs is canonically associated with transcriptional silencing, recent research has provided evidence refuting this claim. Therefore, molecular mechanisms underlying the association between DNA methylation and regulation of gene expression have proven difficult to decipher. Towards this objective, the Vermeulen Lab has employed state-of-the-art quantitative mass spectrometry-based interaction proteomics technology to determine the binding affinities of proteins that bind to a genomic region of interest. This talk/ poster will illustrate the scope of employing this technology to identify high-affinity binding readers of biologically relevant methylated DNA sequences like developmentally regulated methylated regions or cancer-related methylated aberrations with relevant examples. Furthermore, the results from validation experiments and further characterisation will be discussed. The talk/poster will illustrate how blending proteomic and genomic approaches will help decode epigenetic regulatory mechanisms.

P6 (Session A)

Next-generation sequencing-based clonality assessment of immunoglobulin gene rearrangements distinguishes relapse from second primary classical Hodgkin lymphoma

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Background & Objective: Classical Hodgkin lymphoma (cHL) is highly curable, however relapse still occurs in up to 30% of (advanced) cHL cases. Case reports and small series have shown that some of these relapses appear to be a new primary cHL. Conventional clonality assays for cHL has thus far been hampered by low frequencies of Hodgkin and Reed-Sternberg cells and limited DNA quality obtained from formalin-fixed paraffin-embedded tissues. Within the EuroClonality-NGS Working Group, we developed a novel approach to detect immunoglobulin heavy chain (IGH) and k light chain (IGK) gene rearrangements. The objective of our study is to determine the clonal relationship between diagnosis and recurrent cHL to assess the incidence of second primary malignancies. Methods: We collected 70 paired diagnosis-recurrence cHL cases including early and late recurrences. Gene-specific IGH-VJ-FR3, IGHDJ, IGK-VJ and IGK-V/Intron-Kde primer sets were used to perform next-generation sequencing (NGS)-based clonality analysis with Ion Torrent PGM. Bioinformatics analysis is performed with the interactive web-based immunoprofiler ARResT/Interrogate. Results: Preliminary results of 7 paired diagnosis-relapse samples demonstrates the presence of identical clonotypes in 2 cases, while distinct clonotypes were observed in 3 other cases suggesting a second primary lymphoma. No specific clonotype were identified in either diagnosis and/or relapse of the remaining 2 samples. Additional cases of recurrent cHL have to be analyzed to reveal the true incidence of clonally unrelated lymphomas in recurrent cHL. Conclusions: This study is an important step towards establishment of NGS-based clonality assessment in clinical practice for cHL, and eventually the improvement of therapeutic management of recurrent cHL.

P7 (Session A)

High frequency of inactivating tetraspanin CD37 mutations in diffuse large B-cell lymphoma at immune-privileged sites

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Tetraspanin CD37 is predominantly expressed on the cell surface of mature B-lymphocytes, and is currently being studied as novel therapeutic target for B-cell lymphoma. Recently, we demonstrated that loss of CD37 induces spontaneous B-cell lymphoma in Cd37-knockout mice and correlates with inferior survival in patients with diffuse large B-cell lymphoma (DLBCL). Here, CD37 mutation analysis was performed in a cohort of 137 primary DLBCL, including 44 primary immune-privileged site-associated DLBCL (IP-DLBCL) originating in testis or central nervous system. CD37 mutations were exclusively identified in IP-DLBCL cases (10/44, 23%), but absent in non-IP-DLBCL cases. The aberrations included ten missense mutations, one deletion, and three splice-site CD37 mutations. Modeling and functional analysis of CD37 missense mutations revealed loss-of-function by impaired CD37 protein expression at the plasma membrane of human lymphoma B-cells. This study provides novel insight into the molecular pathogenesis of IP-DLBCL, and indicates that anti-CD37 therapies will be more beneficial for DLBCL patients without CD37 mutations.

P8 (Session A)

Understanding genome-wide cryptic intragenic transcription activation in pediatric acute myeloid leukemia

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Introduction: Acute myeloid leukemia (AML) is an aggressive heterogeneous disease. Pediatric AML is characterized by recurrent genetic abnormalities such as chromosomal translocations resulting in fusion genes. Translocations involving the mixed lineage leukemia (MLL) gene in chromosome 11q23 comprise 15-20% of all pediatric AML cases. In more than 50% of these cases, MLL is fused to the AF9 gene located on chromosome 9. The prognosis of patients with AML containing the MLL-AF9 fusion gene is very poor, with a 5-year overall survival (OS) of approximately of 50% for children and less than 10% for adults. Towards the understanding of the molecular pathogenic mechanisms of MLL-AF9 in AML patients, in 2018 the van der Reijden group showed that a subgroup of patients with MLL-AF9+ AML have a unique intragenic transcription activation pattern, defined by the presence of unusual epigenetic marks (H3K4me3/H3K27ac). Interestingly, both pediatric and adult MLL-AF9+ AML with this cryptic intragenic transcription are associated with a more favourable outcome. Objective: The goal of this project is to determine how this intragenic transcription activation is caused in pediatric AML and whether it contributes to the disease pathogenesis. This knowledge will be then used to define targets for future rational therapy, as the current treatments only include chemotherapy, radiotherapy and bone marrow transplantation. Methods: Patient derived iPSCs and patient primary material will be used as cellular models to study this phenomenon. Furthermore, novel proteomic techniques will be used to identify the transcription factors and chromatin remodelers that are responsible for the intragenic transcription initiation; high throughput sequencing techniques will be used to find new mutations that might be involved in transcription regulation.

P9 (Session A)

Evidence for homoplasy in ampC promotor region of Escherichia coli.

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Introduction: Escherichia coli is the leading pathogen in community, hospital-acquired, and healthcare-associated infections. Extended-spectrum beta-lactamase (ESBL) producing E. coli are spreading worldwide, restricting available treatment options. E. coli carries chromosomal ampC genes, mutations in de promotor and/or attenuator lead to hyperproduction of ampC resulting in enhanced hydrolyzes of penicillins and cephalosporins. With the use of Whole-genome sequencing (WGS) these mutations can be detected and further studies can be conducted. Currently, little is known about whether these mutations occur randomly or are part of an evolutionary process. An answer to this question could give us a hint on how to prevent certain events from happening. Methods: 172 E. coli strains were collected from multiple centers with a cefoxitin resistant phenotype (MIC > 8) and were subjected to WGS. Bioinformatics analyses were applied to detect mutations present in the ampC gene as well as its promotor and attenuator. From a single strain a reference genome was constructed using long-read PacBio sequencing. This reference was used as a template to call Single Nucleotide Polymorphisms (SNPs) of all 172 strains to eventually infer phylogeny. The Consistency Index of mutations in the promotor, attenuator, and ampC were calculated using the phylogeny to detect homoplasy. Results: Two positions on the promotor gave very low consistency index values which clearly indicates that these positions were subjected to homoplasy. Those two positions were previously described as being involved in the elevation of beta-lactamase resistance. Conclusion: The mutations which are linked to homoplasy combined with the knowledge that these mutations give rise to beta-lactamase resistance in Escherichia coli, could suggest that these mutations do not occur randomly.

P10 (Session A)

Unbiased characterization of Testicular Adrenal Rest Tumors using RNA sequencing

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Background: Testicular Adrenal Rest Tumors (TARTs) are a common complication in patients with Congenital Adrenal Hyperplasia (CAH), leading to irreversible damage of the testes and infertility. The origin and etiological features of these benign tumors are unclear. It was hypothesized that TART is derived in utero from adrenal rests that fail to regress due to the elevated adrenocorticotropin hormone (ACTH) levels in CAH patients. However, next to adrenal characteristics, also testicular characteristics have been reported for TART, suggesting a more pluripotent progenitor as the origin of TART.Objective: This study aims to unravel the origin and etiological features of TART. Methods: RNA sequencing data of 14 TART, 11 adult adrenal-, 10 adult testis-, 13 fetal adrenal -and 5 fetal testis-tissues was obtained. The origin of TART is explored using unsupervised hierarchical clustering analysis and principle component analysis. TART-specific functional characteristics and pathways were identified using differential expression analysis (DESeq2), followed by Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Results: Clustering analysis indicated highest similarity of TART with adult adrenal tissue and least similarity with fetal adrenal and fetal testis tissue. Differential expression analysis identified 747 genes differentially expressed in TART compared to adult adrenal tissue and 1901 genes differentially expressed in TART compared to adult testis tissue (fold-change > 2; adjusted p-value < 0.05). Functional annotation and pathway analysis of the 463 upregulated genes in TART compared to adult adrenal tissue revealed enriched functional annotations and pathways associated with steroid metabolism and receptor binding. Analysis of 503 genes upregulated in TART versus adult testis tissue highlighted GO-terms and pathways involved in extracellular matrix organization/remodeling. Conclusion: This study corroborates the similarity of TART with adult adrenal tissue. Gene Ontology enrichment analysis and pathway analysis confirm the adrenal steroidogenic and fibrotic properties of TART.

P12 (Session A)

New tools to induce specific-chromosome loss

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Aneuploidy, defined as the state of having a karyotype that deviates from a multiple of the haploid set of chromosomes, has been identified as a hallmark of cancer. Although aneuploidy and its consequences have been studied and modeled in multiple organisms, its causal relationship with cancer still remains unclear. In some cases it is shown to be a powerful tumor driver while in others it is reported to decrease cellular fitness and growth rate due to both metabolic and proteotoxic stress. Aneuploidy is currently modeled either by inducing chromosomial instability (CIN), defined as the high rate at which chromosomes are changed over time, or by inducing single or multiple chromosomial gain. Instead, chromosome loss and its consequences at the cellular level have not been specifically characterized yet. At what extent the effect of aneuploidy on tumor growth is due to the gene dosage imbalance activated pathway per se, and what is the contribution of a specific karyotype, in terms of gain or loss of specific chromosomes or gene clusters? Are there specific features that differ from chromosomial gain and loss which could be relevant? Finding cleaner and more precise tools to induce and model aneuploidy it is therefore a crucial point in answering these questions and dissecting aneuploidy's contribution to tumor development and growth. Here I test and validate in parallel two strategies to induce specific-chromosome arm loss in an epithelial tissue in Drosophila: the first one based on the Flp/FRT recombination system and the second one on the CRISPR/Cas9 system. In both systems the enzymes (Flipase and Cas9) are being expressed in a specific tissue or time window in order to generate big deletions in heterozygosis and therefore provide a model for conditional segmental aneuploidy.

P13 (Session A)

Dissecting piRNA 3' end formation and trailer piRNA production in Aedes mosquitoes

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PIWI interacting (pi)RNAs are small RNAs known for their role in transgenerational protection of genome integrity by silencing of transposable elements. Intriguingly, in the blood-feeding mosquito Aedes aegypti, somatic piRNAs are produced from cytoplasmic viral RNA, implying a role for piRNAs in antiviral immunity. It has recently been suggested that in Drosophila, trailer piRNA production is essential to diversify and expand the piRNA repertoire encoded in piRNA clusters. In contrast to piRNA cluster transcripts, viral RNA is produced de novo upon infection. It remains unknown whether the phased piRNA production mechanism is able to process such non-canonical substrates to expand the viral piRNA population in Aedes mosquitoes. We analyzed small RNA-deep sequencing libraries from Ae. aegypti cell lines and found that piRNAs derived from transposons as well as viral RNA have sharp 3' ends, which are predominantly followed by uridine residues. These signatures are suggestive of specific endonucleic cleavage defining piRNA 3' ends. To further investigate vpiRNA 5' and 3' end formation, we designed a reporter system based on recombinant Sindbis virus in which we introduced target sites for abundant endogenous piRNAs. Responder piRNAs from these viruses can readily be detected by northern blot and their production relies on the ping-pong partners Ago3 and Piwi5. Using this reporter system, we identified the endonuclease responsible for sharp 3' end formation of responder piRNAs. Furthermore, by placing uridine residues at regular intervals in the downstream sequence of the responder piRNA, we were able to promote production of trailer vpiRNAs. Altogether, our data indicate that phased piRNA production is conserved in the somatic piRNA pathway of Ae. aegypti. We propose that viruses can be targeted initially through recognition by host-derived piRNAs, followed by diversification of the piRNA repertoire through phasing, thus extending the antiviral potential of the mosquito piRNA pathway.

P14 (Session A)

Affinity Capture of a (Glyco)protein: Getting Your Sample Ready for the Mass Spectrometer

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Introduction: Extracting a pure sample of the target protein from a complex biological fluid is often crucial for reliable, repeatable and sensitive results in mass-spectrometry analysis. Special attention is to be paid to the targets with abundant PTMs, such as glycosylation. In this work we present a comparison of different affinity capturing techniques used to isolate a low-concentrated glycoprotein from biofluids, while minimizing protein background in the eluate. Objectives: Perform an evaluation study of low-concentrated protein enrichment strategies. Methods: This work explored the efficiency of affinity capturing methods exploiting various solid supports. Performances of sepharose-beads, amine-terminated and NHS-terminated magnetic beads, as well as monolith-immobilized gold nanoparticles were evaluated and compared in terms of capturing efficacy and background protein contamination. Key workflow steps, such as carrier vial choice, drying procedure and elution methods were determined and explored. The tests were conducted on a set of biological matrices, that differ in protein concentration and physical properties. Nanobodies (single-chain antibodies) were used in the majority of tests, showing potential as a low-cost and stable substitute of full-size monoclonal antibodies. As an extra development, we present aptamers-based approach: a promising affinity capturing ligand with unique properties and simplicity of production. Results: We state magnetic beads to be the preferred option for in-solution affinity capturing and highlight the value of monolithic column for background reduction in the eluate. The capturing efficacy with the method reaches 70% and the method is applicable for complex sample matrices. The resulting eluate yield is suitable for subsequent mass-spectrometry analysis. We also offer a roadmap for a capturing method development, with mapped pitfalls and points of attention. Conclusion: The methods we explored are valuable as a (glyco)protein enrichment step prior to mass spectrometry analysis and offer potential for exploring low-abundance protein targets in the protein-rich matrices.

P15 (Session A)

Tissue biology of Chromosomal Instability: dissecting the roles of apoptotic caspases

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Caspases have been always described to be death precursors, however, during the last years many studies have demonstrated that caspases can have many other functions and are crucial for development or wound healing processes (Fujisawa et al. 2019). Indeed, recently, the most interesting roles unravel for caspases are related to their non-lethal activity, like the ability to induce cell regeneration in tissues that have previously suffered damage (Wicovsky et al. 2006)How caspases are able to develop their diverse mechanisms it is still unknown. However, many studies have been trying to describe precisely the relationship between caspase activity, reactive oxygen species production and the activation of stress-related signaling pathways. On of the models that combines the full range of such distinct mechanisms are the events of Chromosomal Instability generation, also called, CIN events.Chromosomal instability (CIN) is described as changes in chromosome structure and/or number leading to the formation of aneuploid cells that can become pro-tumorigenic (Benhra et al 2018). CIN is a common feature of solid tumors, especially epithelial tumors, where the destabilization of correct cell division leads to the creation of cells with different genetic content that are not properly eliminated. The uncomplete clearance of these cells leads their maintenance in the tissues and, consequently, to a protumorigenic state where overgrowth takes place.During CIN events, the activation of pro-apoptotic genes takes place to ensure the proper elimination of the malfunctioning cells. These pro-apoptotic gene activate both the initiator and effect caspases that are then responsible for apoptosis event. However, in CIN derived tumors these effector caspases seem to have a determinant role, not in the activation of apoptosis but in the invasiveness of the tumor. Feature that would be important to unravel to fully understand if the migration of primary tumor cells to different sites.

P16 (Session A)

Determine the role of extracellular vesicles in mediating the therapeutic effects of mesenchymal stromal cells.

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Mesenchymal stromal cells and their extracellular vesicles (EVs) gained increasing in the field of stem cell-based Regenerative Medicine. The extracellular vesicles in particular are small particles excreted by most of the cells recapitulating several of the activities of the originating cells. The EV cargo is characterized by lipids, small RNAs and proteins. The mesenchymal stromal cell-derived extracellular vesicles were reported to protect the kidney from acute tubular injury. Therefore, Renaltoolbox project wants to examine an important mechanism by which stem cells ameliorate repair kidney injury.The goal is to characterise extracellular vesicles derived from different sources (bone marrow, umbilical cord, adipose tissue) of mesenchymal stromal cells. Ultracentrifugation is used to isolate extracellular vesicles. Two types of ultracentrifugation can be performed depending on the speed of the ultracentrifugation, to obtain small (microvesicles and apoptotic bodies) or large vesicles (exosomes). Potency tests for therapeutic efficacy and immunomodulatory capacity will be performed to better understand the limits and differences of the extracellular vesicles.All the results will be used in collaboration with other partners of the Renaltoolbox project and hopefully will generate insights for safe and effective application of stem cells or bioproducts in clinical practice, to ameliorate the life of the people with renal disease.This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 813839.

P17 (Session A)

Dynamic endothelial actin structures induce asymmetrical junctions to guide leukocyte extravasation

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During an inflammatory response, the activated endothelium, lining the inner layer of blood vessels attracts neutrophils that subsequently cross the vessel wall to resolve the inflammation, a process referred to as transendothelial migration (TEM). We found that the endothelium actively recruits and guides leukocytes in their exit. Upon TNF-induced inflammation, the endothelial membrane forms dynamic dorsal and lateral protrusions. Typically, we found that actin-mediated junctional protrusions serve as preferred exit sites for neutrophils. Based on a Rac1 FRET-based biosensor, we showed that these junctional protrusions are positive for Rac1 activity and that neutrophils prefer such areas for diapedesis. In an attempt to promote the extravasation of leukocytes by locally induce Rac1 activity, we used the photoactivatable Rac1 probe. Exogenous Rac1 activation leads to local and increased TEM while blocking Rac1 activity perturbed TEM. We hypothesized that for leukocytes to successfully cross cell-cell junctions, such junctions should display asymmetric activity of membrane protrusions. To test this, we transfected one endothelial cell population with the photoactivatable Rac1 probe or constitutively active Rac1 mutants and cultured them with a control population of endothelial cells. This resulted in an endothelial monolayer that showed asymmetric junctions, where one endothelial cell expressed the probe and the neighbouring one not. We found that almost 80% of all neutrophils had a preference to cross through the asymmetric junction by going underneath the protruding cell. These experiments show that endothelial cells generate dynamic actin structures at asymmetrical junctions to support diapedesis and not simply open cell-cell junctions as was suggested before. Our findings highlight the importance of the endothelium in recruitment and guidance of immune cells through the vessel wall and will have an impact on therapies that are devoted to controlling leukocyte extravasation by targeting the cell-cell junction regions.

P18 (Session A)

WISP1 expression is responsive to mechanical stress in human primary articular chondrocyte pellets

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Introduction: Chondrocytes are post mitotic cells residing in the articular cartilage in a maturational state, where they maintain tissue homeostasis. Upon challenging environmental changes, such as diseases or mechanical stress, chondrocytes highly depend on epigenetic mechanisms to dynamically alter gene expression. During osteoarthritis (OA), it has been consistently shown that control of epigenetic regulated transcription is lost, also for canonical Wnt signaling, which plays an important role in cartilage matrix homeostasis. This was further exemplified by a highly significant upregulation in the epigenetically regulated transcription of Wnt-1-induced signaling protein 1 (WISP1). Here, we set out to establish a model in which we can induce WISP1 expression through mechanical stress, causal to development of OA, and subsequently investigate epigenetic regulation of WISP1. Methods: Human primary articular chondrocytes were isolated from joints of patients (n=9) that underwent total joint replacement at end stage of OA (RAAK study). Cells were cultured for three passages and pellets were created by centrifugation. On day 11, pellets were subjected twice to mechanical stress (20% compression, 5 Hz) for 10 minutes, with a 10 min recovery interval. After two and twelve hours, pellets were harvested for RT-qPCR and immunohistochemistry to investigate WISP1 mRNA and protein expression, respectively. Results: WISP1 expression significantly increased (FC=1.43 P=0.009) in chondrocyte pellets two hours after subjection to mechanical stress, but decreased twelve hours after subjection (FC=0.51, P<0.001). On protein level, mechanical stress decreased WISP1 expression. Conclusion: Mechanical stress triggered upregulation of WISP1 in human chondrocyte pellets with a switch over time, while overall protein levels decreased. We hypothesize that the mechanically induced changes in WISP1 expression could be an important event resulting in the loss of control of the canonical Wnt signaling pathway. Currently, we are investigating whether the observed change in WISP1 expression is epigenetically regulated via DNA methylation.

P19 (Session A)

Method to develop allele-specific silencing: siRNAs screening and generation of artificial miRNAs

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Since its discovery RNA interference (RNAi) has aroused great interest as therapeutic approach and it has been used to design gene therapy strategies especially in the treatment of pathogenic gain-of-function dominant negative mutations. The use of siRNAs for RNAi is limited by their transient nature and cytotoxicity in mammalian cells. To overcome this limitation, siRNA can be sub-cloned into artificial Pol II transcribed miRNA expressing vectors that allow continuous and long term expression of pre-miRNA molecules. This system is better tolerated by the receiving cells since pre-miRNAs are processed through the endogenous RNAi pathway. One challenge in performing allele specific silencing (ASP-silencing) is the design of silencing molecule able to knock-down a mutant allele even if it differs from the wild-type by only one nucleotide. Our group has developed a routine method to identify molecules able to specifically target the mutant allele and to have no/low effect on the expression of the wild-type one. This method involves in vitro analysis of the effect of a set of siRNAs on the expression of reporter alleles that encodes chimeric proteins formed by a fluorescent gene, a protein tag and portion of wild-type or mutant allele cDNA. Best performing siRNA can be converted into an artificial miRNA that, once processed, encodes the original effector sequence. This molecule is subsequently validated applying the same method. We will show an example of the described strategy applied to genes involved in genetic arrhythmic syndromes.

P20 (Session A)

Renal flow sensing and electrolyte reabsorption: what is the molecular link?

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The kidneys are essential to maintain plasma electrolyte levels within a narrow concentration range. Following filtration, the tubular system adjusts reabsorption rates to match variable electrolyte loads associated with fluctuating prourine flow. Renal flow sensing is mediated, among others, by the primary cilium through a complex of polycystin-1 (PC1) and polycystin-2 (PC2). This complex induces mechanosensitive signalling pathways, including purinergic signalling. The physiological relevance of the PC1/2 complex is highlighted by the finding that mutations in PKD1 or PKD2, encoding PC1 and PC2, cause autosomal dominant polycystic kidney disease (ADPKD). This disease is characterized by the formation of fluid-filled cysts in the kidney in combination with electrolyte abnormalities, including hypocalcemia, hypomagnesemia and hyponatremia, due to renal losses. Although it is unknown what causes these electrolyte imbalances, it was recently shown that pre-cystic Pkd1-/-mice mimic the biochemical phenotype of patients with ADPDK. This suggests the involvement of PC1/2 in renal electrolyte handling. Importantly, PC1 was also demonstrated to be involved in flow-mediated purinergic signalling. We therefore hypothesize that disturbed renal flow sensinginduces the electrolyte disturbances observed in ADPKD. To address this hypothesis, the following three objectives will be studied during my PhD project:1) Relationship between tubular flow and electrolyte reabsorption Assess the role of flow on expression and function of electrolyte transporters in kidney cells isolated from pre-cystic Pkd1-/-mice, by combining a state-of-the-art biosorter with microfluidics. 2) Molecular signalling linking flow-induced ATP signalling and electrolyte reabsorptionUnravel the molecular pathway of flow-mediated ATP signalling and its role in electrolyte reabsorption by using Pkd1-/-cells in combination with microfluidics. 3) Flow-dependent electrolyte homeostasis in vivoDetermine the effect of impaired ATP signalling in vivo in pre-cystic Pkd1-/-mice. Together, this complementary project will elucidate the flow-mediated molecular mechanisms controlling renal electrolyte handling.

P21 (Session A)

Strategies to avoid isoflurane chemical shift artefacts in high sensitivity in vivo 19F MRI

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Introduction: 19F MRI is an increasingly popular imaging technique exploiting the benefits of background free imaging with the stable 19F isotope in imaging agents mainly consisting of inert perfluorocarbons[1]. Isoflurane (ISO) is the anaesthetic of choice for preclinical imaging studies, however, as this compound contains 19F, it can be a complicating factor, and may result in chemical shift artefacts (CSA). Methods: A phantom consisting of 50% PFCE and 50% ISO was imaged as a proof of concept. For in vivo imaging, mice were injected with 20mg PFCE containing nanoparticles[2] and imaged after 2 days. MRI was performed on an 11.7T BioSpec. A RARE sequence was used with 4 different parameter sets. 1) standard 2D 2) out of plane shift with an extremely small bandwidth 3) suppression pulse 4) 3D selective excitation. In vivo imaging time was 12:48min. Results: In vivoNMR spectra show two broad ISO peaks and one sharp PFCE peak. In vitro 19F MRI shows the CSA when using a standard sequence, a very narrow acquisition bandwidth shifts it out of plane. Figure 2c shows the ISO and PFCE signal a fair distance apart, either signal can be supressed using a frequency selective 90pulse before the excitation pulse. In vivo19F MRI with a standard sequence shows the ISO problem; ISO CSA ghosts confound image interpretation and quantification. Shifting these signals out of plane is feasible in vivo. An ISO suppression pulse results in effective in vivosuppression of ISO signals. Selective 3D slab excitation with a narrow excitation bandwidth results in artefact free images. Conclusion: Preclinical 19F MRI scan times are limed by isoflurane chemical shift artefacts, complicating imaging analysis and quantification. Here we show three strategies to avoid these chemical shift artefacts while maintaining a high SNR.

P22 (Session A)

Glycosylation signatures in stage II colorectal cancer elucidated by mass spectrometry imaging

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Changes in glycosylations, which is one of the most common protein post-translational-modifications, are considered to be a hallmark of cancer and the correlation between colorectal cancer (CRC) and change in the pattern of expression of N-glycans is studying in the search for new biomarkers. The diagnostic of CRC is based on risk-features, specific to each stage. However, due to the heterogeneity of CRC stageII, a portion of patient have greatest risk of recurrence. The challenge is how to best stratify them so that the right treatment can be identified. That why, we aim to compare the glycomic composition of stageII CRC and its microenvironment and investigate the glycomic changes based on patient prognosis.To address this issue, we made use of the MALDI-Mass-spectrometry imaging (MSI) technique, which simultaneously provides the spatial distribution of hundreds of N-glycan whilst maintaining morphological integrity of the tissue.A cohort of CRC stageII patient have been analyzed by MSI and an in-house derivatization method was applied in order to differentiate between, a2,6- and a2,3-linked sialic acids. Virtual microdissection was performed to compare N-glycan compositions of cancer, invasive front and stroma area interacting directly with the tumor.Our finding reveal specific glycosylation patterns for each of the different areas of the tissue. The most interesting N-glycan features are the increase level of high-mannose, of tri- and tetra-antenna and of both 2.6- and 2.3-linked sialic acid in cancer areas as well as the decrease in fucosylation compared to the normal epithelium cells. We also discovered that the cancer and the stroma touching the cancer part share almost the same N-glycan profile.Altogether, our finding the show the high potential of N-glycans as cancer markers and provide a solid base for further investigation into their role in CRC stageII progression.

P23 (Session A)

Differences in gene expression between susceptible and resistant Aspergillus fumigatus isolates after 48h exposure to azole compounds

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Over the last few decades, many azole resistant Aspergillus fumigatus isolates have been documented worldwide. In this study, it is intended to get more insight in differentially regulated cellular processes in clinical and environmental A. fumigatus isolates with low and high MICs for itraconazole (MIC>16 µg/ml), to uncover potential novel targets for future drug development. In this study, we aim to compare transcriptome differences between susceptible and resistant A. fumigatus isolates after 48h treatment with sublethal concentrations of itraconazole and the newest azole; isavuconazole. Our data shows that cellular stress induced by azoles results in the differential expression of several groups of genes. Furthermore, the pan-azole resistant isolate V181-30 with high MICs for all azoles showed 0 DEGs after 48h incubation with 8 µg/ml itraconazole, as opposed to the other azole resistant isolate V162-06 which still showed 318 DEGs. After addition of isavuconazole to the medium, resistant isolate V162-06 showed up-regulation of drug-efflux transporter abcA, whereas resistant isolate V181-30 showed strong up-regulation of drug-efflux transporter abcE. The basal gene expression of the isolates was highly variable between isolates. Transcriptomic analysis by RNAseq can give great insights in transcriptome changes, helping us understand how A. fumigatus adapts its expression to survive in the presence of azoles in the environment. These results suggest that there are differences in the transcriptomic response to azoles between clinical isolates, and that some azole resistant strains differentially regulate several cellular processes, to maintain optimal growth and fitness.

P24 (Session A)

Evaluation of the potential of novel vaccine candidates to induce an immune response that interrupts malaria parasite development

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Background: A critical part of the malaria lifecycle is the uptake of sexual stages (gametocytes) by mosquitoes from infected humans. These sexual stages develop in the mosquito making them infectious to other humans. Transmission blocking vaccines (TBV) aim to interrupt this development in the mosquito midgut and to prevent further spread of malaria throughout the population. Only a limited number of TBV candidates have been identified of which gametocyte antigens Pfs48/45 and Pfs230 have progressed most towards clinical development. Natural blocking of transmission has been observed in field studies, wherein naturally acquired antibodies from individuals block transmission in mosquito feeding assays. These individuals showed significantly higher antibody responses against 43 gametocyte antigens, other than Pfs48/45 and Pfs230, as determined by a protein microarray (Stone, W. et al 2018). Aim: In this study, we evaluated thirteen of these antigens as potential TBV candidates. Methods: Protein fragments were expressed in a Wheat Germ Cell Free system, injected in mice and antibody responses were evaluated. Results: All antigen immunisations resulted in antigen specific antibody responses. Purified total IgG from mice immunized with antigens Pf11.1 and liver stage antigen 3 (LSA3) exhibited reduction of oocyst development in two independent mosquito feeding assays. Antibodies against Pf11.1 showed recognition of the native antigen in the parasitophorous space of gametocytes. No recognition of whole parasites or parasite lysate by antibodies against LSA3 was observed. Conclusion: We identified Pf11.1 as a novel vaccine candidate that induced a malaria transmission reducing antibody response. Future work will include the evaluation of Pf11.1 in a rat immunisation study to further characterise the potency of the elicited antibodies. Additionally, more novel candidates are being tested in mice to evaluate their potential to induce a transmission blocking immune response.

P25 (Session A)

Complexome profiling reveals Plasmodium falciparum mitochondrial protein dynamics during sexual differentiation

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The Plasmodium falciparum mitochondrion has hallmarks of an attractive drug target as it is essential and sufficiently distinct from host mitochondria. Currently, atovaquone is the only validated antimalarial targeting the mitochondrion. Big contributors to this are the uncommon and poorly understood characteristics of this organelle in Plasmodium species. Among other peculiarities each parasite harbours only a single mitochondrion, which contains the smallest known mitochondrial genome and has limited significance for energy generation during blood-stage development. Rather, the organelle is critical in various anabolic pathways such as pyrimidine or Fe-S cluster biosynthesis. During gametocytogenesis the mitochondrion undergoes heavy expansion and de novo formation of cristae, which is accompanied by a shift of the parasite towards increased mitochondrial catabolism.Here, we used complexome profiling to obtain an unbiased view of the protein changes underlying this phenomenon. Complexome profiling is a technique that combines native gel electrophoresis and mass spectrometry to assess composition and relative abundance of protein complexes. We applied this technique to mitochondrial fractions of P. falciparum asexual and sexual blood stages. The presence of various previously characterized protein complexes validated our approach. We identified novel protein complex components as well as poorly recognizable orthologues of subunits known from eukaryotic model systems. Furthermore, we revealed remarkable differences in abundance and presence of mitochondrial complexes between gametocytes and asexual blood stages. These findings allow us to construct new hypotheses about the changing role of the mitochondrion during sexual differentiation of the parasite and beyond. Additionally, construction of a robust mitochondrial complexome will highlight divergent protein complexes as attractive drug targets not detectable by traditional proteomic approaches.

P26 (Session A)

Monitoring immune responses against neoantigens after dendritic cell vaccination in Lynch syndrome patients

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Germline aberrations in the DNA mismatch repair (MMR) genes cause an autosomal dominant syndrome called Lynch syndrome (LS) and increases a patient's lifetime risk for colorectal cancer (CRC) to 60-80%. Genes harboring microsatellites are extremely sensitive to insertions and deletions that lead to frameshift derived peptides (FSP) which are highly immunogenic and only expressed by malignant cells, hence an ideal candidate for immunotherapy. In a recent clinical trial, we administered dendritic cell (DC) vaccination to 3 LS associated CRC patients and to 20 healthy LS patients. From all patients, DCs were generated from monocytes and pulsed with CEA peptide and FSPs of CASP5 and TGFBR2. The vaccination did not have any severe adverse effects and produced antigen specific T cell responses in all three CRC patients and 17 out of 20 healthy LS patients. Determining cytotoxic potential of these neoantigen specific CD8+ T cells and the evaluation of the effect of the vaccination on patient adenomas along with immune landscape characterization are ongoing.

P27 (Session A)

ANGPT2 and CXCR4 upregulation induced by ERK1/2-signaling mediates liver metastasis from colon cancer

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Carcionoma development in colorectal cancer (CRC) is driven by genetic alterations in numerous signaling pathways. Alterations in the RAS-ERK1/2 pathway are associated with the shortest overall survival for patients after diagnosis of CRC metastatic disease, but how RAS-ERK signaling regulates CRC metastasis is still unknown. Here, we identified an ERK1/2-controlled metastatic gene set (EMGS) that is associated with increased recurrence and reduced survival for patients with CRC tumors. Higher levels of EMGS expression were detected in the CRC subsets Consensus Molecular Subtype (CMS)1 and CMS4. We show that the RAS-ERK1/2 axis controls the expression of the cytokine ANGPT2 and the cytokine receptor CXCR4 in CRC cells, and that this facilitates the development of liver but not lung metastases, suggesting that ANGPT2 and CXCR4 are essential for metastatic outgrowth in the liver. We also show that CXCR4 also controls the expression of the cytokines IL10 and CXCL1, and provide evidence for a causal role of IL10 in supporting liver colonization. In summary, we demonstrate that amplification of ERK1/2 signaling in KRAS-mutated CRC cells affects the cytokine milieu of the tumors, thus possibly affecting tumor-stroma interactions and favoring liver metastasis formation.

P28 (Session A)

Dissecting virus-host interactions in iPSC-based cell culture models for Zika and Dengue virus

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After several decades of being considered as non-pathogenic, the Zika virus (ZIKV) established itself as an important threat to human health. ZIKV is evolutionarily related to the dengue virus (DENV) and the cell types that these viruses infect partially overlap. Because of the similarities between both viruses, it is surprising that ZIKV only recently emerged and that the complications associated with ZIKV or DENV infection are considerably different. ZIKV infection can cause microcephaly in foetuses and Guillain-Barre syndrome in adults, whereas DENV infection can lead to haemorrhagic fever. A possible explanation is that ZIKV interacts with different host factors than DENV during the course of an infection. By identifying these host factors, we can not only learn about the molecular mechanisms of ZIKV and DENV infection, but also identify possible drug targets. To approach the in vivo situation as much as possible, we have differentiated human induced pluripotent stem cells into cells that are linked to ZIKV and DENV complications. For these cells, we will investigate which pathways are differentially regulated upon DENV or ZIKV infection and identify host proteins that interact with the viral proteins. These interaction partners will then be validated with CRISPR or RNAi. Finally, we are testing compounds with known anti-DENV activity as well as other compounds from the NIH clinical collection to identify novel antivirals against ZIKV.

P29 (Session A)

Altered ultrasonic vocalisations in the maternal immune activation mouse model of autism and its relation to serum cytokine profiles.

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The development of the nervous system is a tightly orchestrated process where small changes during the prenatal period can lead to neurodevelopmental disorders, such as in the case of autism spectrum disorder (ASD). One of the most important risk factors for ASD is maternal immune activation (MIA) induced by infection during pregnancy.In order to study the underlying mechanisms, a MIA mouse model was developed in our lab, in which pregnant mice are injected with poly (I:C). Our goal was to phenotype the model at an early stage of development and identify the cytokines involved in neurodevelopmental abnormalities. Compared to control pups, MIA newborns exhibited fewer and less complex ultrasonic vocalisations suggesting communication impairment at an early age. As a first step to investigate the role of immune system in this phenomenon, we measured the level of several cytokines in individual mice and performed association studies to assess their contribution to communication impairment after adjustment of covariates. Together with loss-of-function and gain-of-function experiments, our study may allow for a better understanding of the role of individual variables including immune parameters in ASD.

P30 (Session A)

Tick tock - How circadian clocks modulate aggression

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Conduct disorder, characterized by the display of antisocial behaviours including aggression and delinquency, is often associated with inattention and sleep disturbances. The suprachiasmatic nucleus (SCN) of the hypothalamus functions as the master circadian clock; it secretes a number of hormones including melatonin and cortisol that affect the sleep/wake cycle and it also modulates aggressive behaviour. To date, there is little evidence on the role of the circadian rhythm and function of SCN related to aggression. To study this mechanistically, animal models are needed. Conduct disorder can be modelled in BALB/cJ mice that show increased aggression and anxiety-like behaviour compared to the genetically related BALB/cByJ substrain. In the present study we aim to investigate the role of the circadian rhythm on aggression. Here, aggressive behaviour of 12 BALB/cJ and 12 BALB/cByJ mice is assessed for five consecutive days in the resident-intruder paradigm and related to changes in circadian locomotor activity during a 12-hours light/12-hours dark cycle and during a 24-hours dark cycle for 10 days. In addition, as glucocorticoids are known to influence the sleep/wake cycle, we further want to assess their influence on aggression by taking corticosterone measurements shortly after the last aggressive interaction in the resident-intruder and compare them to basal measurements. Furthermore, corticosterone measurements are taken before, during and after restraint stress to measure the corticosterone stress-response and subsequent recovery. Together these data can give important insight into how the different components of the circadian rhythm can influence aggression. This will help us in better understanding how disturbances in the sleep/wake cycle in patients might lead to aggression.

P31 (Session A)

TGF-beta-activated CAFs modulate the tumour immune landscape in colorectal cancer liver metastasis

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Colorectal cancer (CRC) is one of the leading causes of cancer related deaths. Patients suffering from CRC typically die from metastatic disease, which occurs mainly in the liver, and its growing incidence urges for devising new efficient therapeutic strategies. High levels of TGF-β play a major role on influencing CRC aggressiveness and progression to metastasis by 1) inducing a gene signature in cancer-associated fibroblasts (CAFs) that correlates with poor prognosis and 2) suppressing T cell infiltration and activation, which consequently obstructs effectiveness of immunotherapies against immune checkpoint receptors, such as PD-L1. However, the specific roles of TGF-β-activated CAFs in mediating immune suppression need clarification. To target the TGF-β pathway in CAFs from CRC liver metastasis, we transplanted CRC mouse organoids generated in our laboratory into mice with a floxed allele of the TGF-β Receptor II (TβR2), and that express the Cre recombinase under the promoter of Transgelin (Tagln), a TGF-β target gene expressed in CAFs. When Tamoxifen was administered in Tagln-Cre; TβR2+/[f,+] (WT) mice, we reported recombination in PDGFRβ+, Tagln+ CAFs exclusively. Deletion of the TβR2 in Tagln-Cre; TβR2f/f (KO) mice lead to a downregulation of the TGF-β program, but did not affect tumour growth compared to WT mice. Surprisingly, when recombination was combined with treatment using monoclonal antibodies against PD-L1, treated KO mice showed therapeutic responses up to 50% of cured mice. PD-L1 inhibition synergised with ablation of the TGF-β pathway by increasing the density of tumour infiltrating lymphocytes. Our results describe a novel subpopulation of TGF-β-activated, Tagln-expressing CAFs within CRC liver metastasis, which excert immunosuppressive roles. Specific deletion of the TβR2 in combination with anti-PD-L1 therapeutic antibodies induced successful curative effects driven by an increased response of the adaptive immune system.

P32 (Session A)

PTNS: still going strong or an ever ending story?

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Introduction: Percutaneous Tibial Nerve Stimulation (PTNS) is a form of minimal invasive neuromodulation. Usually it is used in the treatment of overactive bladder (OAB). However patients do need maintenance treatment with PTNS. Materials & Methods: In this retrospective study we included all patients who underwent PTNS from January 2008 till July 2018. We analyzed indication for starting treatment with PTNS, moment/reason of discontinuing of PTNS and next line of treatment after PTNS. Kaplan-Meier curves were used to calculate 'survival' of the treatment. Results of treatment were evaluated for 4 groups. Group A: all patients. Group B: all patients who continued PTNS after 12 weeks. Group C: patients with maintenance PTNS minus patients with initial good response who however quitted because of death, moving, successfully switch to TENS or without any problems of OAB after treatment. Group D: group C minus patients with initial good response but who have quitted their treatment because of physical strain or problems visiting the hospital. Results: In total we included 402 patients. Baseline criteria were as follows: 70% female, median age was 70 years (SD 15). Mostly indication for starting PTNS was OAB-wet (54%) or OAB-dry (29%). Median follow up (mFU) of the total group was 4 months (Fig. 1A, maximum follow up 112 months). 228 Patients (57%) continued treatment after 12 weeks (Fig. 1B). In group C 48 Patients discontinued PTNS (Fig. 1C, mFU18 months). Group D has a mFU of 46 months (Fig. 1D). Patients who quitted during maintenance PTNS mostly choose Botox (14%) or Mirabegron (10%) as next line of treatment in OAB, or nothing (57%). Conclusion: Our real life data show comparative percentages of success in the treatment of OAB by PTNS compared to former published data.

P33 (Session A)

Hepatitis C Elimination in the Netherlands (CELINE): a nationwide study retrieving lost to follow-up chronic hepatitis C patients.

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Background: Nowadays, hepatitis C virus (HCV) infection can be cured with the new direct acting antivirals (DAAs). HCV prevalence in the Netherlands is low (estimated at 0.16%), which makes this country a candidate for HCV elimination. Unfortunately, up to 30% of the diagnosed population is lost to follow-up (LTFU) before being successfully treated. The Hepatitis C Elimination in the Netherlands (CELINE) project aims to retrieve and re-evaluate LTFU HCV patients and link them to care. Methods: This nationwide multicentre cohort study identifies potential LTFU patients based on laboratory records. Subsequently, patient records are reviewed to identify current HCV-positive but untreated patients. Patients are invited for re-evaluation and are, if needed, treated with DAAs. Primary study endpoint is the number of LTFU patients who have been successfully linked to care. Results: CELINE started in 2018 and is scheduled to finish in 2021. So far, six hepatitis treatment centres have finished the identification phase and initiated the retrieval phase. Of 4654 potential ever chronically infected patients, 2756 (59%) have already been cured and 427 (9%) were LTFU and eligible for retrieval (alive and current address information available). Currently, 123 patients have been invited for re-evaluation, of which 38 (31%) were already (being) treated elsewhere, 8 (6%) had too severe comorbidity or were deceased, 18 (15%) refused to be re-evaluated and 32 (26%) could not yet be reached. So far, 17 patients (14%) have successfully been linked to care and an additional ten patients (8%) have an outpatient care appointment planned. Conclusion: These interim results show the first step in reaching HCV elimination in the Netherlands. Results are in line with those of the CELINE pilot project (REACH), which retrieved 16% of LTFU patients eligible for retrieval (n=47). CELINE can be used to serve as a blueprint for retrieval projects in other countries.

P34 (Session A)

Characterization of the role of Cdkl5 at the inhibitory synapses in a mouse model of CDKL5 Deficiency Disorder

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Mutations in the cyclin-dependent kinase like 5 gene (CDKL5) have been found in individuals with a rare neurodevelopmental disorder characterized by early-onset epileptic encephalopathy, severe intellectual disability, and intractable seizures. Mostly females, who are heterozygous for CDKL5 deficiency and mosaic for the mutated allele because of the random X-chromosome inactivation, are affected. Presently, no cure exists for patients with CDKL5 deficiency disorder (CDD). In this regard, seizure management represents a dramatic barrier since most patients are resistant to the conventional anti-epileptic drugs. Neuronal CDKL5 functions have been investigated in both primary neurons silenced for Cdkl5 expression and in Cdkl5-KO mice. Neurons devoid of Cdkl5 are characterized by filopodia-like immature dendritic spines, reduced excitatory synaptic puncta, decreased amplitude and frequency of miniature excitatory postsynaptic currents. Cdkl5-null mice recapitulate most features of the human disorder including autistic-like features, impaired learning and memory and motor control; these models are therefore useful for the study of the molecular basis of CDD and for preclinical drug-testing. Although the role of Cdkl5 at excitatory synapses is widely accepted, its possible role in regulating inhibitory neurotransmission is still unknown. The investigation of its possible function at the inhibitory synapses would allow characterizing, in more details, the molecular aspects underlying the epileptic, cognitive and autistic phenotypes. Our preliminary data suggest that Cdkl5 activities converge at both excitatory and inhibitory synapses and the loss of Cdkl5 impacts on the main molecular actors of post-synaptic inhibitory compartment. Importantly, we found that loss of Cdkl5 leads to reduced membrane-insertion of GABAA receptors (GABAARs). Moreover, we found a reduction in gephyrin expression levels in primary Cdkl5-KO neurons. We speculate that CDKL5 exerts an important control of GABAAR expression and functioning in part through its direct interaction with proteins at the post-synaptic sites.

P35 (Session A)

Identification of novel genetic determinants of arbovirus vector competence in Aedes aegypti mosquitoes

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Dengue virus is responsible for an alarming number of human morbidity and mortality worldwide. Nevertheless, the vector, Aedes aegypti mosquito, does not show signs of pathology when infected by this virus. Although the mosquito immune system is known to be the key regulator of viral replication, the underlying mechanism of the mosquito immune pathway is largely uncharacterized. The aim of this research is to identify and characterize new components of the Aedes mosquitoes immune pathway that affect arboviruses replication. We performed a RNA interference (RNAi) screening targeting RNA-binding proteins. For this screening, genes of interest were knocked down in Aedes aegypti mosquito cells using double stranded RNA (dsRNA), followed by infection with Sindbis reporter virus expressing a luciferase gene. Viral replication was measured by means of luminescence assays and the genes with antiviral activity were further tested in an infection with dengue virus 2 (DENV-2). Remarkably, we identified 7 genes with antiviral properties upon Sindbis and dengue virus infection. This result was confirmed by using two independent sets of dsRNA and analyzing the knockdown effect on RNA levels. These hits are currently being further characterized in-vitro and in-vivo. Our research shows that RNAi screening in mosquito cells is an effective approach to identify putative antiviral host factors. Therefore, allowing us to characterize, previously unknown, components involved in the underlying mechanisms of the mosquitoes immune pathway.

P36 (Session A)

Unravelling the suppressive effect of adenosine and CD137 agonism on tumor cell killing by cytotoxic T cells

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INTRODUCTION: Current immunotherapies still fail to create a successful anti-tumor response, caused by the ability of tumors to create a tolerant microenvironment, in which extracellular adenosine acts as a negative immune checkpoint molecule. AIM: This study aims to elucidate the effect of CD137 agonism on the efficacy and dynamics of tumor cell killing by cytotoxic T lymphocytes (CTLs) in vitro, either alone or combined with adenosine A2a receptor antagonism. This was studied in both immune permissive and adenosine-rich immunosuppressive microenvironments. METHODS: CTL-mediated tumor cell killing was studied in an three-dimensional organotypic cytotoxicity assay in which OT-I T-cells were confronted with ovalbumin-expressing B16F10 melanoma cells. The efficacy and dynamics of tumor cell killing was monitored by flow cytometry and time-lapse brightfield microscopy respectively. RESULTS: Here we demonstrate that agonistic CD137 therapy reduced the absolute number of CTLs while maintaining overall tumor cell killing, thereby enhancing tumor cell killing on a per T-cell basis. Anti-CD137 therapy stabilized CTL-target cell contacts and partly restored tumor cell killing in the presence of adenosine. Inhibition of the adenosine A2a receptor might cooperate with CD137 stimulation to enhance tumor eradication by CTLs within an adenosine-rich microenvironment. However, this combinatorial treatment strategy did not lead to further enhanced CTL-target cell contacts, as compared to the individual treatments alone. CONCLUSION: Our findings showed that adenosine-suppressedCTLs treated by anti-CD137 antibodies during their effector phase, clearly enhanced their anti-tumor cytotoxicity, which highlights that agonistic CD137 therapy represents a promising avenue to combat adenosine-induced immunosuppression in metabolically perturbed tumors.KeywordsImmunotherapy, CD137, adenosine, cytotoxic T lymphocyte, tumor cell killing

P38 (Session A)

Magnesium is essential for insulin-stimulated glucose uptake in insulin-sensitive cells

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Type 2 Diabetes Mellitus (T2DM) represents a major health problem that is characterized by a decreased insulin sensitivity and relative insulin shortage. Insufficient insulin action results in hyperglycemia and unfavorable changes in lipid metabolism. Screening 400 T2DM patients shows that 30% suffers magnesium (Mg^{2+}) deficiency (plasma Mg^{2+} concentration <0.7 mM). Although Mg^{2+} is established as an important co-factor in many bioenergetic pathways, its role in insulin action remains to be elucidated. The objective of this study is to determine whether Mg^{2+} is essential for insulin-stimulated glucose uptake. Insulin-mediated glucose uptake and translocation of the glucose transporter 4 (GLUT4) to the cell surface has been studied in vitro and ex vivo by incubating 3T3-L1 adipocytes, mouse primary adipocytes and C2C12 myotubes with a low or physiological concentration of Mg^{2+}. Radioactive-glucose labelling and immunocytochemistry were used to assess the insulin-sensitivity of cells. In 3T3-L1 adipocytes, the absence of Mg^{2+} compared to a physiological level of 1 mM Mg^{2+} decreases the insulin-stimulated uptake of glucose by 65%. The lack of Mg^{2+} in C2C12 myotubes and results in a decreased insulin-stimulated uptake of 25%. A high Mg^{2+} concentration of 1.5 mM Mg^{2+} compared to 0.5 mM Mg^{2+} does not increase the insulin-stimulated uptake of glucose in primary adipocytes. Preliminary data studied in 3T3-L1 adipocytes suggest that insulin-stimulated GLUT4 translocation towards the membrane is significantly impaired in the absence of Mg^{2+}. In conclusion, Mg^{2+} is required for insulin-stimulated glucose uptake in insulin-sensitive cell lines. Identifying the pathway mechanisms could give more insight into which factors or proteins are hampered by Mg^{2+} deficiency. Future experiments will reveal whether the increased glucose uptake occurs by activation of the IR/IRS1/PDPK1/AKT insulin signaling pathway.

P40 (Session A)

PLD activity as novel player in tumor-induced osteoclast-dependent podosome-mediated osteolysis

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Osteolytic bone metastasis frequently occurs in breast cancer patients, resulting in bone degradation. Existing therapies limit bone damage, but have serious side effects and do not cure the disease. One of the current hypotheses suggests that this osteolysis is mediated by osteoclasts, bone-resorbing cells of the body, that may become hyperactive upon cancer metastasis. Understanding this putative osteoclast-cancer cell interplay could provide new therapeutic targets. Osteoclasts resorb bone by excreting acids and enzymes in a secluded compartment, delimited by the sealing zone, a circular actin-based structure made of podosomes tightly adhering onto the bone. Recent studies have shown that phospholipase D (PLD) is essential for podosome formation, suggesting that PLD might be a useful therapeutic target in osteolytic bone disease. Here we aimed to study the role of PLD activity in tumor-induced osteoclast-mediated bone resorption. First, we sought to explore and improve cellular models of osteoclast differentiation. We carefully explored the murine RAW264.7 macrophage cell line and observed large variation in osteoclast yield depending on cell density, passages and substrate used. In addition, we tested two assays to determine the resorption capacity of the RAW-derived osteoclasts and found that not bone slices, but calcium-phosphate coated culture plates were allowed a standardized assessment. Furthermore, we explored the effect of breast cancer cells on osteoclast activity using co-cultures and revealed that the tumor cells promote proliferation of RAW264.7 cells, with subsequently reduced resorption. Finally, we used PLD inhibitors to determine whether PLD is important for osteoclast differentiation. Our results provide a critical view on the RAW264.7 osteoclast model and help in establishing a well-working, standardized method to assess osteoclast activity that is currently missing. Furthermore, we were first to study the role of PLD in osteoclasts and our results suggest a possible application for PLD as therapeutic target in osteolytic bone disease.

P41 (Session A)

Real-world use of the IBD Disk tool for evaluation of patient-reported disability in the outpatient clinic

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Background: The IBD disability index (IBD-DI) is a validated healthcare professional (HCP) administered tool that can assess the functional status of patients in trials. The IBD-Disk was adapted from the IBD-DI as a tool that patients can use to capture their functional status for HCPs to review. Methods: The IBD-Disk was constructed by an expert steering committee of 30 international gastroenterologists/nurses who ranked the IBD-DI items. An IBD-DI working group of 14 gastroenterologists used a modified Delphi process to agree on 10 IBD-Disk items. Patients were asked to rate their level of agreement for each item on the IBD Disk on a visual analogue scale of 0-10 (0 = absolutely NO, 10 = definitely YES). Results: A total of 200 patients took part. The mean age of the cohort was 41 years. 113 (58%) were female. Fifty per cent of patients had CD, 41% had UC and 9% were unclassified. Of the domains of the IBD disk (Table 1, Figure 1), energy levels and joint pain scored highest (most impairing) with mean values of 5.71 and 4.90, respectively, whereas interpersonal interactions and sexual functions were least affected, mean scores 2.54 and 2.62. The mean difficulty rating was 2.2. Significant correlation was found between abdominal pain and energy levels/sleep ($r = 0.60$ and $r = 0.55$; $p < 0.01$) and between joint pain and energy levels/sleep (both $r = 0.56$; $p < 0.01$). Clinicians highlighted that the IBD disk opened up conversations beyond GI issues and gave a good overview of well-being. Patients' feedback highlighted that they were glad they were able to express their functional statusMean (SD) scores for each IBD-disk item. Conclusion: Energy levels and joint pain were the most disabling for this unselected IBD cohort. Our first experience with the IBD-Disk proved very positive.

P42 (Session A)

Not all calciproteins are the same: differences in size, number of particles and calcification potency

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Chronic kidney disease (CKD) is associated with an increased risk for vascular calcification and cardiovascular disease. Recently, circulating calciprotein particles (CPP), precipitates of calcium and phosphate with incorporated proteins, were identified as drives of the calcification process in CKD. Currently, many different protocols to make CPP in vitro are used. However, it is unknown how the synthesis of CPP affects their morphology and function. Therefore, the present study aims to compare four differently synthesized CPP on morphology, composition, number of particles and calcification potency. The CPP were synthesized in mixtures containing 4.4 mM (CPP-A and B) or 6 mM (CPP-C and D) phosphate and 2.8 mM (CPP-A and B) or 10 mM (CPP-C and D) calcium. As protein source fetal bovine serum was used in CPP-A, B and D and CPP-C was made with fetuin A only. The mixtures were incubated for 7 (CPP-A), 14 days (CPP-B) or 12 hours (CPP-C and D). Transmission electron microscopy showed no visual differences between the CPP in density, only CPP-C are larger (306 $\pm$ 19 versus 192 $\pm$ 8, 195 $\pm$ 7 and 205 $\pm$ 6nm for CPP-A, B and D, respectively). Additionally, the number of particles of CPP-C (2.9x1011) was significantly increased compared to CPP-A, B and D (1.5, 2.0, 6.4x1010 respectively). By incubating human vascular smooth muscle cells (hVSMC) with CPP equivalent to 100 mg/ml calcium, calcification was induced. hVSMC calcification was significantly increased using CPP-B (655 $\pm$ 192) and CPP-C (624 $\pm$ 147), compared to control (6 $\pm$ 1), CPP-A (214 $\pm$ 43) and CPP-D (53 $\pm$ 8 mg Ca2+/mg protein). However, when normalizing CPP on particle number rather than calcium content, calcification was comparable among CPP-A, B and C. In both experiments CPP-D was not potent to calcify hVSMC. Differently synthesized CPP are morphological comparable, however calcification potency varies between CPP. Additionally, our results question the method of quantifying the amount of CPP based on calcium content.

P43 (Session A)

Genetic variations and expression changes in urgency urinary incontinence: a systematic review

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Context: Urgency urinary incontinence (UUI) is a common condition that negatively impacts quality of life (Kupelian et al. 2006). Patients sense a sudden, compelling desire to void that is difficult to defer combined with the involuntary leakage of urine (Haylen et al. 2010). Multiple studies have examined possible molecular mechanisms that might explain the occurrence of UUI, but thus far systematic analysis has been lacking. However, this knowledge is necessary for developing new treatments or prevention strategies. Objective: To perform a systematic review to summarize the current knowledge of protein and gene-expression changes and genetic variations in UUI. Evidence Acquisition: A systematic search was performed in Pubmed, Embase, Web of Science and Cochrane library using synonyms for UUI and genetic variations, gene- and protein expression changes and essay methods. Retrieved studies were screened on eligibility. The risk of bias was assessed using the Robins-I for clinical, Syrcle for animal, and modified risk of bias tools for in vitro studies.Results: 31/704 studies met the inclusion criteria. 25/31 scored medium high to high on risk of bias. Biological processes indicated included inflammation (e.g. upregulated adipokines and increased serum CRP), neuronal cell growth and proliferation (e.g. upregulated neurotropic factors), connective tissue remodeling e.g. (upregulated factors of extracellular matrix), and purinergic signaling (increased ATP-release and upregulated purinergic receptors). A meta-analysis on the value of urinary NGF showed increased concentrations in UUI patients compared to controls (Standardized mean difference 2.26 (95% confidence interval 1.25, 3.27)). Conclusion: The medium high to high risk of bias of the included studies limits their value in respect to the pathophysiology of UUI. Elevated urinary NGF levels are related to UUI, but how this relates to molecular changes is unclear. This systematic review will aid in good quality research and enhance our understanding of molecular mechanisms related to UUI.

P44 (Session A)

Optimizing identification ofLynch syndrome by unravelling the underlying cause of microsatellite instability in colorectal and endometrial cancers

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In approximately 15% of colorectal cancers (CRC) a high level of microsatellite instability (MSI) occurs. MSI is the result of an insufficient repair of incorrect insertions and deletions made during DNA replication. Inactivation of mismatch repair (MMR) machinery is usually caused by mutations in the MMR genes MLH1, MSH2, MSH6 and PMS2 or hypermethylation of the MLH1 promoter. Patients with a germline mutation in these genes have Lynch syndrome (LS), which is primarily associated with an increased risk of developing CRC and/or endometrial cancer (EC). Patients without a germline explanation, but with MSI-high cancers, have been referred to as Lynch-like patients. However, a substantial part of this latter group is explained by somatic inactivation of the MMR machinery. The aim of the current study is to identify the fraction of patients with a CRC or EC that developed by somatic inactivation of the MMR genes. By using the sensitive state-of-the-art single molecule molecular inversion probe technique and multiplex ligation-dependent probe amplification, the fraction of somatic mutations of yet unexplained Lynch-like patients, that present with an MSI-high CRC or EC, will be investigated. Patients with somatic mutations and patients that do not present with somatic mutations will be characterized and compared to LS patient characteristics. Cases that present without a germline or somatic explanation for their MSI-high cancer will be analyzed using long-read sequencing of MMR genes to access whether intronic germline mutations occur. Our data will contribute to a better understanding of MSI in CRC and EC and will help to optimize surveillance protocols of patients diagnosed with MSI-high tumors and their relatives.

P45 (Session A)

Circulating Tumour DNA as a prognostic biomarker for the treatment response in patients with stage IV melanoma

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Introduction: Until recently, the prospects for patients with melanoma were poor, especially in the advanced disease stages. The outcome of melanoma patients has drastically improved, since the introduction of immunotherapy (PD-1/CTLA-4) and targeted therapies (BRAF/MEK). Nonetheless, (mono)therapy is not always beneficial for all patients, and particular combinational regimens can induce severe side effects. This, in combination with the lack of conclusive treatment response measurements results in the urgent need to discover biomarkers that can help the clinical guidance of patients with advanced melanoma. Objectives: In this study, we aimed to evaluate the potential of circulating tumour DNA (ctDNA) as a response biomarker for patients with stage IV melanoma. The ctDNA is released into the bloodstream due to apoptosis and necrosis of the tumour cells and can harbour the aberrations present in the tumour. We aimed to investigate if the abundance of ctDNA during treatment corresponds to the treatment response of patients. Methods: Longitudinal blood samples were collected of 41 progressive stage IV melanoma patients that were enrolled in the COWBOY study (NCT02968303). All these patients were tested positive for a BRAFV600 mutation in their tumour biopsy. Using the droplet digital PCR (ddPCR), we analysed the abundance of the BRAFV600 mutation in the plasma during treatment. Results: We demonstrate that the ctDNA abundance in the plasma, represented by the BRAFV600 mutant fraction, corresponds to the radiographic response during treatment. Moreover, in some cases, changes in the ctDNA were earlier detectable than the radiographical changes later on. A complete molecular response, defined as zero BRAFV600 mutant copies in a plasma sample, was associated with a good long-term response to immunotherapy. Conclusion: Although the study included a limited number of patients, the results underlines the potential of ctDNA as a prognostic and possibly predictive biomarker for treatment response in stage IV melanoma patients.

P46 (Session A)

Fluid hydration to prevent post-ERCP pancreatitis in average- to high-risk patients receiving prophylactic rectal NSAIDs (FLUYT trial): study protocol for a randomized controlled trial.

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BACKGROUND: Post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP) is the most common complication of ERCP and may run a severe course. Evidence suggests that vigorous periprocedural hydration can prevent PEP, but studies to date have significant methodological drawbacks. Importantly, evidence for its added value in patients already receiving prophylactic rectal non-steroidal anti-inflammatory drugs (NSAIDs) is lacking and the cost-effectiveness of the approach has not been investigated. We hypothesize that combination therapy of rectal NSAIDs and periprocedural hydration would significantly lower the incidence of post-ERCP pancreatitis compared to rectal NSAIDs alone in moderate- to high-risk patients undergoing ERCP. METHODS: The FLUYT trial is a multicenter, parallel group, open label, superiority randomized controlled trial. A total of 826 moderate- to high-risk patients undergoing ERCP that receive prophylactic rectal NSAIDs will be randomized to a control group (no fluids or normal saline with a maximum of 1.5 mL/kg/h and 3 L/24 h) or intervention group (lactated Ringer's solution with 20 mL/kg over 60 min at start of ERCP, followed by 3 mL/kg/h for 8 h thereafter). The primary endpoint is the incidence of post-ERCP pancreatitis. Secondary endpoints include PEP severity, hydration-related complications, and cost-effectiveness. DISCUSSION: The FLUYT trial design, including hydration schedule, fluid type, and sample size, maximize its power of identifying a potential difference in post-ERCP pancreatitis incidence in patients receiving prophylactic rectal NSAIDs.

P47 (Session A)

Local application of lipoxin and doxycycline in a thermoreversible hydrogel for the treatment of periodontitis

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Periodontitis is an inflammatory disease caused by bacterial infections. Topical usage of antibiotics and anti-inflammatory agents have been proved to be effective to treat periodontitis. To facilitate local delivery of therapeutic agents, a drug carrier is required to preserve and retain the active ingredient in the periodontal pocket. The objective of this study therefore was to evaluate a novel injectable thermoreversible polyisocyanopeptide (PIC) based hydrogel for the application as a drug carrier for lipoxin and doxycycline.Methods: Three formulations of PIC gels, 0.2, 0.5, and 1% w/w, were prepared. As controls, commercially available poloxamer 407 (P407) gels of 20% and 26% w/w were used. Lipoxin A4 (LXA4) and doxycycline(Dox), was suspended into the gel solutions. The systems were evaluated regarding dynamic mechanical properties, injectability and stability, releasing pattern and cytocompatibility. Subsequently, the PIC gel was evaluated in the naturally occurring dog model of periodontitis, and the effect of two different treatment (Dox vs. LXA4) was compared.Results: In vitro results showed that the gelation temperature of PICs and P407s was around 13-23C. PIC gels were mechanically weaker than P407 due to the low polymer concentrations. However, PIC gels kept gel integrity for at least two weeks whereas P407 was eroded totally within one week. The release of LXA4 or Dox from 1% PIC can sustain for around 4-7 days. The extent of burst release was negatively related to the polymer concentration. LXA4 remained bioactive after release from PIC gel but not from P407. No cytotoxicity was observed for 1% P407 gel, however, 26% P407 inhibited periodontal ligament cell and gingival epithelial cell growth. The in vivo study in a naturally occurring beagle dog model of periodontitis is currently on going.Conclusion: The thermos-reversible PIC gel possesses appropriate properties to be used as a carrier for LXA4 and Doxycycline in the periodontal application.

P48 (Session A)

A PBPK modelling approach to simulate the rifampicin-moxifloxacin drug-drug interaction in TB patients

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Background: Moxifloxacin has an important role in the management of TB patients. Moxifloxacin is metabolized by phase II metabolic enzymes (glucuronidation and sulfation), and is a substrate for the efflux transporters P-gp and MRP2. The enzymes and transporters are induced by rifampicin, resulting in sub-optimal moxifloxacin plasma exposures (˜30% decrease in AUC0-24). Our aim was to gain mechanistic insight into the interaction between moxifloxacin and rifampicin. Methods: Simcyp's Population-based Simulator was used to run simulations. Moxifloxacin physicochemical parameters and enzyme activity were obtained from literature. Passive permeability and P-gp transporter kinetics for moxifloxacin were determined experimentally. Intrinsic clearance by MRP2 was predicted with sensitivity analysis. Rifampicin was already available as a substrate file in Simcyp. Three dosing scenarios were simulated: 400mg monotherapy moxifloxacin, 400mg moxifloxacin with rifampicin, and an adjusted 600mg moxifloxacin dose with rifampicin. The simulations were compared with available therapeutic drug monitoring (TDM) data. Results: A permeability-limited liver model, including P-gp and MRP2, resulted in an acceptable description of moxifloxacin PK profiles, with and without rifampicin. The AUC0-24 of 400 mg moxifloxacin in the TDM cohort was 41.7 mg*h/L, the simulated AUC0-24 was 36.7 mg*L/h. The simulated within-patient decrease upon rifampicin co-administration in moxifloxacin AUC0-24 was 40%. Modelling an adjusted 600 mg dose moxifloxacin with rifampicin resulted in an increased AUC0-24 of 33.5 mg*h/L, which is in accordance to the AUC0-24 in our TDM cohort (37.1 mg*h/L). Conclusion: Simcyp simulations showed a rifampicin-mediated decrease in moxifloxacin exposures comparable to that observed in our TDM patient cohort. Exposures restored upon increasing the dose of moxifloxacin from 400 to 600 mg in the TDM patient cohort and the PBPK model. Our next step will be to model moxifloxacin doses and exposures in other populations such children, pregnant women or elderly, to enable pharmacokinetically guided advices on dose adjustments.

P50 (Session A)

Rat model for functional analysis of the normal and wounded soft palate

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Introduction: Telemetric electromyography (EMG) is used for monitoring muscle function in conscious animals during daily activities. In this surgical model, electrodes are inserted in the soft palate of the rat. The velocity of contraction and the muscle activity during normal activities can be measured through EMG. Clinical relevance: Cleft lip and/or palate is a congenital disease that affects speech and swallowing. Though this defect can be corrected by surgery, dysfunctions in swallowing and speech persists in about 30% of cases, mainly due to muscle fibrosis. At present, no therapies are available to improve the function of the soft palate after surgery.Objective To evaluate the function of the normal and wounded soft palate in a rat model for muscle regeneration. Methods: To measure the function of the normal soft palate, two electrodes were inserted in the soft palate, and the transmitter was implanted in the upper region of the chest. The measurements are taken once a week during 56 days. To measure the function of the wounded soft palate, a surgical wound of 1mm is made in the soft palate two weeks after the implantation of the electrodes and the transmitter. The measurements are taken in the same way as in the normal soft palate. The function of the normal soft palate and the wounded soft palate will be compared. Results and conclusions: The rats survive the implantation. There was no bleeding during or after the surgery. A good healing process of surgical wounding was observed. There were no signs of infection or severe swelling.

P51 (Session B)

A multiplexed high-throughput strategy to investigate mitochondrial dysfunction in drug-induced acute kidney injury

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Acute kidney injury (AKI) is a major burden for healthcare, as it accounts for twenty percent of all hospitalized adults worldwide. Though depending on AKI severity, the average mortality rate is over fifty percent and even less severe manifestations are associated with profound short- and long-term adverse effects, including chronic kidney disease. A variety of commonly used drugs, such as antibiotics, antivirals and immunosuppressant agents have a nephrotoxic potential in twenty five percent of all cases. Mitochondrial dysfunction seems to play a pivotal role in drug-induced AKI, particularly in proximal tubular cells, but exact underlying mechanisms remain unknown. Therefore, we aim to develop and apply a multiplexed high-throughput microscopy strategy to understand the adverse effects of well-known nephrotoxic drugs on mitochondrial function. Our strategy uses human conditionally immortalized proximal tubule epithelial cells (ciPTEC), fluorescently stained for multiple mitochondrial functional parameters, including oxidative stress, mitochondrial membrane potential, intracellular calcium and oxygen levels. To distinguish between primary and secondary (i.e. mitochondrial dysfunction caused by other cellular pathways) mitochondrial toxicity, we also simultaneously assessed cell viability. To this end, we extended our fluorescence microscopy method to investigate true cell viability, as conventional viability assays such as MTT depend on cellular metabolic activity. Known nephrotoxicants, including cisplatin show reduced cytotoxicity in our fluorescent method compared to MTT, which demonstrates that the latter is not a good viability marker for compounds with a metabolic adverse effect. Combining our high-content imaging approach with future metabolomics-based metabolic network models will determine disturbances in mitochondrial function upon nephrotoxic drug exposure. Eventually, this will contribute to the development of safer drugs to reduce the incidence of drug-induced AKI.

P52 (Session B)

Imaging CD8+ T cell tumor infiltration following radiotherapy

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Introduction: Immunotherapy is considered a new cornerstone in cancer treatment. However, methods for accurate early response-monitoring are lacking. Noninvasive quantitative imaging of CD8+ cytotoxic T-cells can provide dynamic and spatial information of anti-tumor response. Therefore, we developed an method to image influx of CD8+ T-cells in tumors in a syngeneic mouse model. Methods: CT26 tumor bearing BALB/c mice were intravenously injected with 8.5 µg [111In]In-DTPA-CD8 antibody. SPECT/CT imaging was performed at 4h, 24h, 48h and 72h after injection. In a separate experiment, C57Bl/6 mice bearing bilateral B16-F1 tumors received right-side external beam irradiation (1x10Gy). After 24h, mice received either: 8.1 MBq [111In]In-DTPA-CD8 antibody or 8.7 MBq [111In]In-DTPA-Isotype control followed by SPECT/CT at 48h post irradiation. Results/Discussion: CD8+ T cell containing organs were clearly visible on SPECT scans at all time points. SPECT quantification showed significantly increased uptake of [111In]In-DTPA-CD8 antibody in irradiated B16-F1 tumors compared to non-irradiated tumors in the radiation naïve mice (13.65 $\pm$ 0.83 vs. 9.95 $\pm$ 1.65 %ID/g $\pm$ SD, p = 0.005). Uptake of control IgG showed no differences between irradiated or non-irradiated tumors (4.70 $\pm$ 1.04 vs. 5.06 $\pm$ 0,96). Further analysis of the SPECT scans indicated the presence of tertiary lymphoid structures in the tumor periphery also showing increased CD8 uptake in irradiated tumors. Conclusions: The anti-CD8 antibody showed specific uptake in CD8+ T-cell containing tissues in vivo, but tumor uptake was limited because of the low number of CD8+ T-cells present. We demonstrated that irradiation induces a significant increase in tracer uptake in B16-F1 tumors. These differences were shown to be specific for an increase in CD8+ T-cells. Concluding, this tracer has potential for in vivo evaluation of CD8+ T-cell infiltration and could assist in immunotherapy response monitoring.

P53 (Session B)

Computational Modeling of RNA

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RNA is an essential molecule for life and has characterized some of the latest revolutions in the biomedical and biotechnological sectors (e.g. CRISPR-CAS9 or iRNA), and this seems only the beginning. RNA is probably the biological polymer with the widest range of functions due to its structural diversity, but we can't completely link function and structure yet. What's more, structural data in RNA is rather limited (the Protein Data Bank has only one RNA structure for every ˜30 others) and predicting its structure still represents a challenge. Computational modeling has succeeded for a range of molecules when experimental restraints are available. However, the secrets of such structural complexity remain elusive. Herein, I will present the current state of the methods to model RNA and what can we expect in the near future.

P54 (Session B)

Development of an automatic pipeline for participation on the CELPP Challenge

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The prediction of how a ligand binds to a target is an essential step for Structure-Based Drug Design (SBDD) methods. Molecular docking is a standard tool in drug design for lead compound identification and in virtual screening to find novel biologically active molecules. However, docking programs not always find correct ligand poses and scoring functions are not always accurate when selecting the best pose. These issues have a direct impact when developing new therapeutic approaches as more and more projects are relying on these methodologies. On the other hand, the improvement in the throughput of many experimental techniques is generating large amounts of data that, if used correctly, could lead to an enhancement in the performance of computational tools. Nonetheless, handling this amount of data could be a challenge by itself. Here we present a fully automated pipeline for pose prediction validated by participating in CELPP Challenge. This pipeline is able to recover information from other protein-ligand complexes and apply it during the docking process. Given an initial protein sequence and the corresponding ligand, from which the binding mode is unknown, we performed some similarity searches in PDB to be able to find some constraints from similar protein-ligand complexes to guide the docking process. If with the constraints generated we are able to improve the predictions, the constraints will drive the docking process. Alternatively, docking without constraints is performed. During validation, we prove that the pipeline is able to generate predictions for most of the proposed targets as well as obtaining poses with low RMSD values when compared to the crystal structure.

P55 (Session B)

Identifying novel substrates of the APC/C during ciliary disassembly using proximity labeling

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Objective: The primary cilium is present on almost every cell of the human body and is essential for proper cell-cell signalling. Misregulation of the cilia can lead to a range of hereditary diseases, called ciliopathies. Assembly and disassembly of the primary cilium is closely linked to the cell cycle due to a double role for the centrosomes. Nonetheless, the precise mechanism of ciliary disassembly remains unclear to date. A large scale siRNA screen generated in our lab implicated a moonlighting role for the anaphase-promoting complex/cyclosome (APC/C) in disassembly of the primary cilium. Furthermore, knockdown of CDC20, a co-activator of the APC/C, resulted in elongated cilia. To gain insight into the role of the APC/C complex during disassembly of the cilium, we aim to identify novel substrates of this E3 ubiquitin ligase using BioID2 proximity labeling proteomics. Methods: Initial localization of the APC/C during disassembly was determined by immunofluorescence microscopy. Cell cultures were synchronized using serum starvation, followed by induction of ciliary disassembly by serum resupplementation. Subsequently, a screen for novel APC/C substrates will be performed, using BioID2 proximity labeling, followed by label-free mass spectrometry and statistical analysis using Perseus. Results: Several components of the APC/C localize at the base of the cilia prior to, and at the start of ciliary disassembly, as shown with IF microscopy. This localization was no longer observed after the initiation of disassembly. Analysis of the BioID2 mass spectrometry data might reveal possible substrates of the APC/C during ciliary disassembly. Conclusion: Localization of APC/C components at the ciliary base during disassembly of the primary cilium indicates that the complex might play a role in ciliary disassembly. Identification of novel substrates of the APC/C could shed more light on the ciliary moonlighting role of this complex.

P56 (Session B)

Maturation of blood-brain barrier drug efflux transporters in the pediatric brain

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Introduction: When drugs exert their effects in the brain, extrapolation of doses from adults could be harmful for children as the blood-brain barrier (BBB) and blood-CSF barrier (BCSFB) function is still immature. For example, P-glycoprotein (Pgp/MDR1)-mediated transport of drugs out of the BBB appears age-dependent. As human data is scarce, we studied developmental variation in human BBB and BCSFB transporters. Methods: Age-dependent variation in localization and staining intensity of the ABC transporters Pgp, breast cancer resistance protein (BCRP) and multidrug resistance proteins 1, 2, 4 and 5 (MRP1/2/4/5) was investigated using immunohistochemistry in post mortem brain tissue derived from 48 fetuses, neonates and children between gestational age 13-42 weeks and 0-3 years of postnatal age, and 4 adults. Staining intensity of transporters in cortex microvessels (BBB) and choroid plexus (BCSFB) was analyzed by semi-quantitative scoring. Results: Immunostaining was detectable for Pgp, BCRP, MRP1, and MRP2 in BBB. Staining intensity was higher for Pgp and BCRP in adult brain compared to fetuses, neonates and children. In contrast, MRP1 and MRP2 BBB staining intensity was higher in fetuses, neonates and children. BCSFB was positively stained for Pgp, MRP1, and MRP2 and did not show age-related differences. MRP4 and MRP5 were neither detected in BBB nor in BCSFB. Conclusion: BCRP, Pgp, MRP1, and MRP2 were detected in BBB and BCSFB of human fetal and pediatric brain and staining patterns appeared to be dependent on brain location and age.

P57 (Session B)

Expression and functional analysis of lncRNAs NAALADL2-AS2 and AC012531.25 in castration-resistant prostate cancer

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Prostate cancer (PCa) the third leading cancer by morbidity and mortality. Aberrant activation of androgen receptor (AR) signalling pathway is the dominant mechanism of PCa carcinogenesis, which leads to tumor growth and disease progression. Current strategies for antitumor therapy are based on androgen deprivation with the aim to disrupt AR signalling. However, a multitude of resistance mechanisms leading to AR-independent growth are known to arise in PCa, resulting in development of lethal castration-resistance PCa (CRPC).In CRPC, the AR pathway is modulated by long non-coding RNAs (lncRNAs), single-stranded RNA molecules longer than 200 nucleotides that regulate cell growth and differentiation, and are aberrantly expressed in malignancies. LncRNA expression is also deregulated in PCa, where it promotes tumor growth and proliferation through oncogenic signalling pathways and repression of tumor suppressor genes. High expression specificity across tissues and tumors allows for the use of LncRNAs as effective biomarkers, as evidenced by use of lncRNA PCA3 for PCa diagnosis, but no molecular biomarkers are currently available for CRPC.lncRNAs NAALADL2-AS2 and AC012531.25 are CRPC-specific, and their expression correlates with high Gleason score, loss of tumor differentiation and poor survival. A bioinformatic prediction of NAALADL2-AS2 and AC012531.25 mechanisms of action was performed, and yielded potential regulatory targets. NAALADL2-AS2 and AC012531.25 were shown to be upregulated upon androgen deprivation, and loss of their expression affected PCa cell viability and apoptosis, suggesting a potential role in mediating PCa cell survival and therapy resistance. AC012531.25 was demonstrated to regulate expression of HOXC4 and HOXC6 transcription factors, and could potentially inhibit PCa cell death through repressing the cyclin-dependent kinase inhibitor CDKN1A/p21.LncRNAs NAALADL2-AS2 and AC012531.25 provide potential novel biomarkers for screening for and staging of aggressive PCa and CRPC. Further elucidation of their regulatory targets and involvement in intracellular signalling could yield possible therapeutic targets.

P58 (Session B)

Clonal evolution and mutational landscape of DLBCL recurrences

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm in adults. Despite improvement in therapy strategies, 30 to 40% of DLBCL patients relapse and 10% display refractory diseases, where outcome of relapsed/refractory DLBCL remains poor. The clonal relationship between the tumor at initial diagnosis and its recurrence(s) is not routinely assessed in diagnostics, although the existence of clonally unrelated recurrences have been reported, which may influence treatment strategies. Moreover, the molecular mechanisms that underlie DLBCL heterogeneity in therapy response, are still poorly understood. Objective: To improve prognosis and treatment of DLBCL, we aim to define the clonal relationship and mutational landscape of DLBCL recurrences. Methods: For assessing the clonal relationship, we make use of a novel approach to detect immunoglobulin (IG) light and heavy chain gene rearrangements by next-generation sequencing (NGS) as developed by the EuroClonality-NGS Working Group. By performing IG-NGS clonality analysis on a large cohort of 50-70 paired DLBCL diagnosis-relapse cases, the true incidence of clonally unrelated second primary lymphoma can be established. For the cases that are clonally related, extensive mutational analysis through whole exome and targeted sequencing will be performed, to define the clonal evolution patterns in relapsed DLBCL and identify genes related to therapy resistance. Patients with evidence of second primary lymphoma, will be further investigated to assess potential links to genetic predisposition of lymphoma development. Results & Conclusion: IG-NGS clonality analyses on the first 9 matched DLBCL diagnosis-relapse samples revealed a clonal relationship in 8 out of 9 cases, while one case was clonally unrelated. These results indicate the existence of an independent second primary lymphoma at relapse even in this small cohort, however validation in a larger patient cohort is required. Our studies will improve the understanding on the genetic landscape contributing to the development of DLBCL recurrences

P59 (Session B)

Liquid biopsies in diagnostics of non-small cell lung cancer patients: measuring the mutational profile of the tumor in the circulating tumor DNA

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Background: Liquid biopsies involve the analysis of cell-free nucleic acids in bodily fluids such as blood. Genomic profiling of circulating tumor DNA (ctDNA) in plasma can offer a noninvasive way to characterize the tumor, enabling precision oncology. Highly sensitive methods are required for optimal clinical use of these liquid biopsies, complementing histology findings from tissue biopsies. However, to enable objective assessment of assay performance, detailed analytical validation is required, which was the aim of the present study. Methods: In this study 90 patients suspected of having lung cancer were included. From these patients blood and tumor tissue samples were collected at time of diagnosis. The tumor mutational profile of KRAS and EGFR was measured in the tissue biopsies with a targeted next-generation sequencing (NGS) approach. This was compared to the mutational profile of the plasma ctDNA measured with droplet digital PCR (ddPCR). Results: Of the 44 patients that had non-small cell lung cancer (NSCLC) or a lung adenocarcinoma, tissue NGS was available for 36 patients. In these patients, seventeen activating mutations were found in EGFR and KRAS. Seven out of these seventeen mutations were also found in plasma with ddPCR, giving a concordance of 41%. However, when focusing on patients with stage IV disease, presence and absence of detected mutations in ctDNA corresponded with tissue NGS, data resulting in concordance of 100%. Conclusion: Although tissue biopsies remain essential, plasma ctDNA analysis can be used to detect the mutational profile of the tumor. Since sensitivity differs per disease stage, a pre-selection of suitable patients for plasma analysis is required. Larger clinical studies are needed to elucidate the validity and utility of these liquid biopsies.

P61 (Session B)

Regulation of blood pressure: linking sodium and magnesium in the distal convoluted tubule

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Renal Na+ reabsorption is a tightly regulated mechanism controlling the body volume and accordingly, blood pressure. Recently, the putative Mg2+ sensor in the distal convoluted tubule (DCT), CNNM2, has been postulated to regulate blood pressure based on GWAS analyses and mouse models. However, to our knowledge, no previous studies have investigated how Mg2+ transport affects Na+ reabsorption in the DCT and thus blood pressure. In this study, we aim to analyze whether CNNM2 affects the trafficking and/or activity of NCC, the Na+/Cl–cotransporter located in the DCT. To study the trafficking of NCC to the plasma membrane, we performed cell surface biotinylation specific to tag proteins located at the cell surface. By coexpressing NCC with either wild-type or mutant CNNM2, we showed that NCC trafficking was not altered in the presence of CNNM2. Next, as NCC activity is dependent on its phosphorylation, we stimulated NCC phosphorylation by treating cells with either isotonic or hypotonic low Cl- buffer in the presence of CNNM2. Our results revealed that CNNM2 did not affect the phosphorylation of NCC and thus its activity remained unchanged. However, CNNM2 WT expression reduced the total expression of NCC WT in both assays. Lastly, we established a stable cell line that expresses fluorescent proteins YFP-E2A-mKate. In the future, this model could be used to observe the influence of CNNM2 on NCC activity more directly using a YFP-halide-sensitive live-cell imaging system. Taken together, this study uncovers a potential regulatory role of CNNM2 on NCC expression, which might be important in blood pressure regulation.

P62 (Session B)

Elucidating how Drosophila Wnt1 expression is controlled in wing development, regeneration and tumorigenesis

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Wnt1 is a member of the Wnt family present in almost all the organisms, vertebrates and invertebrates. The fact that Wnt1 is conserved in organisms that evolutionary are considerably far away means that plays a crucial role. In fact, Wnt1 has been implicated in very different processes such as organ and tissue development, regeneration and tumorigenesis. This makes us wonder how is Wnt1 expression controlled in processes that are so different. To respond to this question we have used Drosophila melanogaster imaginal wing disc as a model. A good candidate to regulate Wingless (Wg, Drosophila Wnt1) in these three processes was the enhancer within wg1 deletion, wing-enhancer. The deletion of this enhancer induces the emergence of completely normal functional adults that only lack wings. Furthermore, this enhancer has been involved in regeneration and tumorigenesis too. In the case of wing development, we have been capable to demonstrate that the wing-enhancer is responsible to trigger the wing development and we have narrowed down the minimal functional region required for it. Moreover, we have been capable to elucidate the molecular mechanisms behind the enhancer: Hedgehog (Hh) and Vestigial (Vg). We have been capable to detect wing-enhancer expression in regeneration and tumorigenesis too but the regions of the enhancer required in these two processes differ from the ones required in development. In addition, our results indicate that wing-enhancer in tumorigenesis and regeneration is not controlled by Hh but it is controlled by the JNK pathway.

P63 (Session B)

Characterizing the regulation and function of the hGID complex in cell proliferation and differentiation

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The ubiquitin-proteasome system (UPS) and particularly E3 enzymes have recently gained special attention due to its direct involvement in multiple diseases including cancer and neurodegenerative disorders. We recently discovered that the conserved multi-subunit E3 ligase complex GID (glucose-induced degradation deficient) regulates cell proliferation of mammalian cells at least in part by targeting the cell cycle regulator Hbp1. In my PhD project,I combine cell biological and biochemical approaches to investigate the role and regulation of GID-dependent degradation of Hbp1 and other substrates for cell cycle exit and ROS signaling during differentiation and development. In particular, I use CRISPR-Cas9 technology and the d-TAG system to study the molecular function of different GID subunits, with the aim to understand the mechanism of ubiquitin-transfer and substrate recognition.

P64 (Session B)

Pioneering the use of novel tools to unravel actin isoform specificity

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β- and y-cytosolic actin are ubiquitously expressed isoforms of actin that, despite high similarity, have specific non-redundant functions. Studying these differences is complicated because of problems with specifically labelling each isoform. We aimed to overcome this by pursuing the following objectives: exploring the possibilities of internally tagging β- and y-actin, generating cell lines endogenously expressing these tagged variants of the two isoforms, and studying β- and y-actin knockout cells. In this study, we successfully generated cell lines expressing internally tagged β- or y-actin using CRISPR-Cas9, and showed that these tagged variants normally integrate in actin filaments. With these cell lines, we were able to perform an isoform-specific co-immunoprecipitation that can be used to determine β- and y-actin binding proteins. In addition, we explored the possibilities of inserting probes for live cell imaging in this internal tag position, but both GFP- and tetracysteine-based imaging proved to be suboptimal. Lastly, we generated CRISPR-Cas9-mediated β- and y-actin knockout cells that can be used to study various functions of the two isoforms. In conclusion, we have generated multiple tools to independently study actin isoforms, which can be used to determine the specific characteristics and functions of β- and y-actin.

P65 (Session B)

Characterization of PIWI proteins and viral piRNA biogenesis in the mosquito vector, Aedes albopictus

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Multiple arboviruses like dengue, Zika and chikungunya are transmitted by Aedes mosquitoes, where the siRNA pathway is the chief mediator of antiviral immunity. Recently, the PIWI-interacting RNA (piRNA) pathway was also shown to have a similar role. As compared to 3 PIWI-family proteins in Drosophila, the PIWI gene family has undergone expansion to 8 proteins (PIWI 1-7 and Argonaute 3) in Aedes mosquitoes. Earlier, PIWI5 and Ago3 were shown to be responsible for biogenesis of viral piRNAs (vpiRNAs) in Ae. aegypti. We sought to elucidate piRNA biogenesis and PIWI dependence in another important mosquito vector, Ae. albopictus. Using the recently annotated Ae. albopictus genome, orthologs corresponding to the Ae. aegypti PIWI proteins were identified. Surprisingly, the Ae. albopictus genome possesses multiple seemingly redundant copies of Ago3, PIWI4, PIWI5 and PIWI7. PIWI protein knockdown in Ae. albopictus U4.4 cells through dsRNA-mediated silencing revealed that production of Sindbis virus (SINV)-derived vpiRNAs is also dependent on PIWI5 and Ago3. Unlike that observed for Ae. aegypti-derived Aag2 cells, no antiviral effect of PIWI4 knockdown was seen against two different alphaviruses. Drosophila CRISPR-Cas9 reagents were optimized for use in U4.4 cells and functional knockouts were generated for PIWI5 and PIWI6. However, knocking out either Ago3 or PIWI4 seemed to be lethal. In the context of a PIWI5 knockout, vpiRNAs derived from SINV or dengue virus infections were reduced to negligible levels and a putative antiviral function could be uncovered. Ae. albopictus PIWI5 therefore seems to have an important role, not only in vpiRNA biogenesis, but also as an antiviral effector.

P66 (Session B)

How Chromosome Imbalances Shape the Proteome

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Aneuploidy, a status characterised by an incorrect chromosome number, is a hallmark of cancer. Remarkably, the aneuploid state is highly detrimental for untransformed cell fitness. The effects of these abnormal karyotypes span from proliferation defects and DNA damage to metabolic alterations and proteotoxic stress.To shed light on how aneuploidy affects cell physiology, our work aims to uncover the immediate consequences of mitotic errors in untransformed human cells. By interfering with the mitotic apparatus, we are able to induce chromosome segregation errors, generating a heterogeneous population of cells with random chromosome gains and losses, useful to address the acute effects of aneuploidy.Accordingly, the presence of extra chromosomes should lead to an increase in transcription, mRNA translation and protein accumulation. Surprisingly, we have observed that the levels of some crucial proteins and of their mRNAs are significantly reduced in aneuploid cells. Chaperones, whose activity and levels have been reported to be reduced in aneuploidy, may have roles in this altered proteostasis. The severe impact on the proteome can be due to an impaired protein synthesis triggered at different levels.Our working hypothesis hinges on the idea that chaperones are limiting in aneuploid cells and this might trigger stalled protein translation. In turn, this will lead to mRNA degradation of transcripts whose translation has been halted. At the same time, stalled polysomes can act as scaffolds for the assembly of dynamic structures such as stress granules, which might protect specific mRNAs from degradation. Concerning this aspect, it will be interesting to address whether or not feedback mechanisms are active at the transcriptional level and potentially targeting nascent mRNA molecules.By testing these hypotheses, we hope to better understand how chromosome segregation errors shape cell proteome and, more in general, cell proliferation, with crucial implications for both basic and cancer biology.

P67 (Session B)

Role of non clathrin-mediated endocytosis in CD147 regulation and cellular response

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Epidermal growth factor receptor (EGFR) has essential role in cell proliferation, survival, differentiation and migration. Endocytosis is a master regulator of EGFR activity. Upon stimulation with high doses of EGF, EGFR can be internalized through clathrin-mediated endocytosis (CME) and non clathrin-mediated endocytosis (NCE). While CME mainly targets EGFR to recycling, NCE mostly leads to its degradation. Together with specific NCE functional regulators, we have recently identified CD147 as an NCE cargo, co-trafficking with the EGFR through this pathway. CD147 overexpression at the surface of different cancer cells types is associated with tumor progression/invasion and poor prognosis. Thus, the characterization of mechanisms able to downmodulate its levels/activity could be of great interest. We aim at elucidating the regulation of CD147 internalization via NCE, and its fate after internalization through this pathway. We are currently investigating if CD147 directly interacts with EGFR or simply co-traffic with it, and how EGFR signaling affects the internalization and the destiny of CD147. We are also exploring if NCE exists in different cell contexts and if it is triggered by other ligands and receptor tyrosine kinases. We discovered that, in addition to the EGF, also saturating doses of other EGFR ligands (i.e. transforming growth factor a and amphiregulin) can trigger CD147 internalization via NCE. Our research will allow to uncover how broad is NCE impact on cell physiology and its role in EGFR/CD147-mediated cellular responses.

P68 (Session B)

Development of tools to study 3-O sulfated heparan sulfate involved in (patho-)physiological processes

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Heparan sulfate (HS) is a linear polysaccharide with high functional diversity, resulting from different sulfation motifs. The sulfation of the C3-hydroxyl group of the glucosamine moiety modulates many protein interactions and is associated with several pathologies. This modification is generated by seven HS glucosaminyl 3-O-sulfotransferases resulting in motifs that can be classified either as AT-type (GlcA-GlcNS3S) or gD-type (IdoA2S-GlcNS3S). Since the number of isoenzymes exceeds the number of motifs, larger, more complex motifs are anticipated. Previously, we obtained the single chain antibody (scFv) HS4C3, reactive with 3-O-sulfate of both motifs. To target different 3-O-sulfate motifs specifically, characterization of the interaction between HS4C3 and the 3-O-sulfated HS is required. Here we characterize this interaction between HS4C3 and heparin, using the 'protect & label' methodology. Lysine and arginine residues not binding to heparin were chemically modified with respectively N-hydroxysuccinimide (NHS) acetate and phenylglyoxal, whereas interacting residues were labeled with NHS-biotin and hydroxy-phenylglyoxal. After digestion, labeled residues were identified using mass spectrometry. Results indicate that most labeled arginines and lysines were concentrated in and surrounding the complementarity determining region 3 (CDR3) of the antibody, but were also present in other regions. Using 3D models of heparin (1HPN) and HS4C3, we predict that the arginine residue preceding the CDR3 of the heavy chain is essential for binding to the 3-O-sulfate epitope, while arginines and lysines of the CDR3 bind surrounding sulfate groups. Labeled residues located in other regions provided a binding site for flanking saccharides. Comparison of these data with those obtained for antibodies that recognize different HS epitopes (e.g. LKiv69 and HS3A8) will give us insight in specific mutations required to change the reactivity of the HS4C3 antibody towards specific 3-O-sulfate containing motifs. Antibodies targeting different 3-O-sulfation patterns will be used to investigate the function of 3-O-sulfation in (patho)physiological processes.

P69 (Session B)

Porous Titanium Fiber Mesh with Tailored Elasticity and its Effect on Stromal Cells

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Porous titanium fiber mesh (TFM) is considered a suitable scaffold material for bone reconstruction. Also, TFM can be used to cover the surface of bone-anchored devices, i.e. orthopedic or dental implants. The titanium fiber size has an effect of the modulus of elasticity as well as porosity of the titanium mesh, which can influence the behavior of bone forming cells. Therefore, the aim of this study was to vary TFM composition, in order to achieve different Young's modulus, and to assess the effects of such variation on the behavior of bone marrow-derived stromal cells (BMSCs). With that purpose, nine types of TFM (porosities 60-87%; fiber size 22-50 μm), were examined for their mechanical properties as well as their effect on the proliferation and differentiation of rat bone marrow-derived stromal cells (rBMSCs) up to 21 days. Dynamic Mechanical Analysis revealed that the Young's moduli of TFM were lower than of solid titanium and decreased with larger fiber sizes. The Young's modulus could effectively be tailored by altering fiber properties, which altered the pore simultaneously. For the 22 and 35 μm size fiber meshes with the highest porosity, the Modulus of Elasticity closely matched the value found in literature for cortical bone. Finally, all tested TFM types supported the growth and differentiation of rBMSCs. We concluded that TFM material has been proven cytocompatible. Further preclinical studies are needed to assess which TFM type is most suitable as clinical use for bone ingrowth and bone regeneration.

P70 (Session B)

Circadian proximal tubule function, as different as night and day

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The proximal tubule is the major resorptive segment of the nephron in the kidney and handles the reabsorption of the majority of all filtered water, sodium, glucose and proteins. Also, this kidney segment plays an important role in regulating the acid-base balance. The enormous nutrient resorptive capacity of the proximal tubule is mainly driven by the ATP-dependent transporter Na/K-ATPase. For this reason, the proximal tubule is densely packed with mitochondria to provide sufficient ATP.Proximal tubule activity is regulated by hormones including endothelin, noradrenalin, and angiotensin-II, which are released in a circadian fashion. Blood pressure and renal blood flow, filtration, secretion and reabsorption follow a circadian rhythm and increase shortly before and during the active phase. In line with this, proximal tubules show extensive mitophagy in the inactive phase when blood pressure and reabsorption are low, and it is therefore likely that proximal tubule function also follows a circadian pattern.Taking the above into consideration, it can be hypothesized that proximal tubule reabsorption together with the mitochondrial function, are decreased in the inactive phase. The generation of the circadian proximal tubule data provides more insight into circadian proximal tubule function, drug transporters expression, the reabsorption of many nutrients and the changes in metabolic pathways.To study the circadian rhythm of the proximal tubule, convoluted and straight proximal tubules were microdissected from mouse kidneys every four hours and the RNA was extracted and sequenced. The sequences were mapped to a mouse reference genome and the differentially expressed genes were identified using the DESeq2 package. Pathway analysis was done using the DAVID Tool and showed that pathways involved in oxidative phosphorylation, metabolism and cell cycle progression are significantly changed between the active and inactive phase. This dataset will further unravel the physiological functions of the proximal tubule in the circadian rhythm.

P71 (Session B)

PIWIs on action: Characterization of PIWI proteins and associated small RNAs in the vector mosquito Aedes aegypti

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The PIWI-interacting RNA (piRNA) pathway is composed of small RNAs ranging from 23-30nt that are bound by PIWI-clade Argonaute proteins. This small RNA-based defense system has been extensively studied in the germline of Drosophila melanogaster where it has been shown to maintain the genome integrity by controlling the expression of transposons. Recently, however, it has been shown that the piRNA pathway has additional functions than repressing transposable elements. Our group has shown that Aedes aegypti, a vector mosquito which transmits arthropod-borne (arbo-) viruses also have an active piRNA pathway. In addition to the transposons, Ae.aegypti piRNA pathway produces de-novo piRNAs from the viral RNA of arboviruses. These observations led to the question if the piRNA pathway in Ae.aegypti has additional functions as contributing to the antiviral immunity against arboviruses. Moreover, transcriptome analysis of Ae.aegypti revealed that PIWI gene family encoding PIWI proteins has expanded to 7 members (Piwi2-7 and Ago3). Of these, 4 are also somatically expressed in mosquitos (Piwi4-6 and Ago3) in contrast to flies where the PIWI expression is germline-specific. Although our group has shown that distinct sets of PIWI proteins are needed to produce piRNAs from different sources (transposon, viral sequences), the individual functions of these PIWI proteins remain to be investigated. In this study, we examined the expression and (sub-)cellular localization of PIWI proteins in Ae.aegypti and in Aag2 cells derived from them. We showed that PIWI proteins are expressed in Aag2 cells, and in the soma as well as the germline of Ae.aegypti. To provide clues for functions of somatic PIWI proteins, we characterized the PIWI-bound small-RNA populations in Aag2 cells. To that end, we performed RNA-Immunoprecipitation (RIP) followed by small-RNA deep-sequencing. We analyzed the repertoire of small RNA populations deriving from endogenous and exogenous sources both in uninfected and arbovirus-infected Aag2 cells.

P72 (Session B)

Gene co-expression analysis identifies gene clusters associated with isotropic and polarized growth in Aspergillus fumigatus conidia

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Aspergillus fumigatus is a saprophytic fungus that extensively produces conidia. These microscopic asexually reproductive structures are small enough to reach the lungs. Germination of conidia followed by hyphal growth inside human lungs is a key step in the establishment of infection in immunocompromised patients. Until now, transcriptome and proteome studies in A. fumigatus were only performed on breaking of dormancy and early germination. In this study, we used RNA-Seq and focused on the later stages of germination in A. fumigatus. These stages are characterized by two distinct morphological phases. The first morphological change is swelling of the cell, referred to as isotropic growth. The second, polarized growth is characterized by the formation of a germ tube. These morphological changes are probably induced by a selective set of genes. Therefore, RNA-Seq was used to analyze the transcriptome of dormant and germinating A. fumigatus conidia.Therefore, we constructed a co-expression network, identifying genes with similar expression patterns. These expression patterns may be associated to the distinctive morphological phases seen in conidial germination. Construction of a gene co-expression network revealed four gene clusters (modules) correlated with a growth phase (dormant, isotropic growth, polarized growth). Transcripts levels of genes encoding for secondary metabolites were high in dormant conidia. During isotropic growth, transcript levels of genes involved in cell wall modifications increased. Two modules encoding for growth, cell cycle and DNA processing were associated with polarized growth. In addition, the co-expression network was used to identify highly connected intermodular hub genes. These genes may have a pivotal role in the respective module and could therefore be compelling therapeutic targets.Generally, cell wall remodeling is an important process during isotropic and polarized growth, characterized by an increase of transcripts coding for hyphal growth, cell cycle and DNA processing when polarized growth is initiated.

P73 (Session B)

Mapping RNA-binding regions in protens in mammalian brain tissue

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From transcription to degradation, mRNA is always bound by RNA-binding proteins (RBPs). RBPs regulate the events in the lifecycle of mRNA, including capping, splicing, alternative splicing, editing, transport, translation and its repression, degradation, etc. Through these events, RBPs regulate gene expression by diversifying the transcriptome and affecting when and where mRNA is translated or degraded. The importance of proper post-transcriptional regulation of gene expression is underscored by many studies that have linked several RBPs to various diseases. Most notably RBPs have been linked to various neurological and neurodegenerative diseases. In this study we have applied an optimized pCLAP workflow directly to brain tissue. pCLAP combines UV-crosslinking of RNA and protein, poly(A)-enrichment and high resolution mass spectrometry for the global identification of RNA-binding regions in vivo, with high reproducibility and specificity. We have identified 526 RBPs and the regions in them that bind RNA directly from mouse brain tissue, many of which have not been annotated as RNA-binding previously.

P74 (Session B)

Pharmacologically stimulating nitric oxide-soluble Guanylate Cyclase Signaling to prevent podocyte injury

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Background: The effects of nitric oxide (NO) on podocytes are not known. We hypothesize that NO production by glomerular endothelial cells (GEnC) acts on podocytes as a protective paracrine factor in the glomerulus, thereby preventing podocyte injury. We propose a mechanism in which NO-mediated soluble guanylyl cyclase (sGC) activation results in enhanced cGMP synthesis and reduced expression/activity of the Ca2+-permeable Transient Receptor Potential Channel 6 (TRPC6), thereby inhibiting deleterious podocyte signalling processes. Several market approved drugs for non-renal disorders act on sGC. We aim to investigate glomerular NO-sGC signalling and the potential of repurposing sGC activators to prevent podocyte injury. Methods: In vitro experiments were performed using conditionally immortalized GEnC and podocytes. NO production was visualized using the NO sensitive dye DAF-FM diacetate. Podocyte injury was induced with 0.25µg/mL adriamycin for 24hrs, with or without co-exposure of NO-donor SNAP (200µM) or sGC activators Cinaciguat (2µM) and Riociguat (20µM) or the sGC inhibitor ODQ (200µM). Results: Two forms of nitric oxide synthases (NOS; i.e. iNOS and eNOS) were expressed by GEnC and podocytes, whereas both cell types produced NO. GEnC particularly produced NO under (physiological) flow conditions. Interestingly, neuronal NOS (nNOS) was solely expressed by podocytes when injury was induced. All sGC subunits were expressed by podocytes. Stimulation of sGC via either SNAP or Riociguat elevated cGMP production in podocytes, which could be blocked using ODQ. Importantly, SNAP, Cinaciguat and Riociguat all reduced adriamycin-induced TRPC6 overexpression in podocytes. Finally, Riociguat, Cinaciguat and SNAP all reduced Adriamycin-induced podocyte death. Conclusion: Our data supports the hypothesis of a paracrine NOS-NO-sGC axis between GEnC and podocytes. Moreover, sGC stimulation via SNAP or through repurposing drugs that activate sGC exert a protective effect on podocytes. Glomerular NO production might therefore play an important role in preserving the integrity of the glomerular filtration barrier.

P75 (Session B)

B3GLCT-catalyzed O-fucose glucosylation is not required for secretion of TSP1 from retinal pigment epithelial cells

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Background: Variants in the B3GLCT gene have been found to be protective for age-related macular degeneration (AMD) in a genome-wide association study. B3GLCT is coding for beta1-3 glucosyltransferase, which catalyzes the second step of glycosylation on thrombospondin type I repeats (TSR), forming Glc-beta1-3Fuc-O. This modification has been reported to play a role in protein secretion via an endoplasmatic reticulum quality control mechanism, although the terminal glucose is thought to be essential for secretion of only a subset of TSR-containing proteins. Since nothing is known yet about a possible protective mechanism in AMD via B3GLCT, we aimed to further explore this. Methods: We generated B3GLCT knockout (KO) hTERT RPE1 cells using CRISPR/cas9 genome editing, and investigated whether this KO causes secretion defects of TSR-containing proteins highly expressed in the RPE. For this purpose, we evaluated gene expression of TSR-containing proteins by qPCR and subsequently we tested the presence of thrombospondin I (TSP1) and connective tissue growth factor (CTGF) in cell lysates and conditioned medium/Opti-MEM by Western blot. Results: Gene expression analysis showed that 3 of the in total 60 TSR-containing proteins are highly expressed in hTERT RPE1 cells, which are TSP1, CTGF and cysteine rich angiogenesis inducer 61 (CYR61). CTGF and CYR61 had similar RNA levels in WT and KO cells, whereas TSP1 expression was slightly increased in the KO. On protein level, CTGF secretion was similar from WT and KO cells and TSP1 was slightly more abundant in conditioned medium from KO cells, corresponding to RNA levels. Conclusion: KO of B3GLCT does not result in secretion defects of TSP1 and CTGF from RPE cells. Our future studies will investigate whether B3GLCT-attached glucose is required for secretion of other proteins from RPE, or whether it has additional functional roles, which could potentially be linked to AMD pathogenesis.

P76 (Session B)

Electrostimulation of the Carotid Sinus Nerve in Mice attenuates Inflammation via Glucocorticoid Receptor on Myeloid immune cells

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Since the discovery of the inflammatory reflex, it is well established that nerves have a role in regulating immunity and this role has been expanded in recent years. The carotid body (CB), through its innervating nerve the carotid sinus nerve (CSN), is a paranganglia that detects and modulates multiple physiological stimuli and potentially the immune system. It was our hypothesis that electrical stimulation of the CSN would attenuate inflammation and protect against LPS-induced endotoxemia in mice. This was investigated via isolation of the CSN and electrode implantation, prior to electrical stimulation. Blood was collected for serum analysis by assay 90 minutes after LPS intraperitoneal injection. CSN electrostimulation attenuated LPS-induced inflammatory cytokines - TNF, IL-1β and IL-6 - independently of the vagus nerve and the know mechanism of the inflammatory reflex. However, electrostimulation of the CSN increased corticosterone. Preventing the increased production of corticosterone via bilateral adrenal gland removal or blocking its receptor - glucocorticoid receptor - reversed the attenuation of inflammation demonstrated by CSN electrostimulation. Furthermore, the effect of CSN stimulation was abolished in LysmCre GRLoxP mice (no glucocorticoid receptor on myeloid immune cells). These results were replicated in conscious mice and survival to a lethal dose of LPS was further investigated. Conscious stimulation of the CSN protected mice against LPS-induced endotoxemic shock compared to sham controls. Ultimately, CSN stimulation attenuates inflammation and protects against LPS-induced endotoxemia via an increase in corticosterone which acts on the glucocorticoid receptor of myeloid immune cells. This potential electroceutical could be adapted to treat typical immune-mediated inflammatory disorders or perhaps be uniquely adapted to patients requiring long-term administration of glucocorticoids, such as an organ transplant recipient.

P77 (Session B)

The effect of metformin on human non-small cell lung carcinoma cells: the role of mitochondria

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Human non-small cell lung carcinoma (NSCLC) represents 85% of all lung cancer cases, and has high metastasis potential and frequent post-surgical relapse. Unfortunately, available chemotherapeutic options aren't satisfactory in improving overall survival and patients quality of life. One of the approaches in discovering new anticancer agents is repurposing the common drugs as adjuvant or neo-adjuvant therapeutics. Metformin, first-line choice antidiabetic in type II diabetes, has been investigated in different clinical trials as an addition to standard chemotherapeutic regiments, including NSCLC treatment. The rationale behind metformin usage in cancer therapy is the in vitro data suggesting that metformin inhibits mitochondrial respiratory chain complex I, impairs energy balance and redox state, inducing apoptotic cell death. Still, most studies investigated metformin's mechanism of action using concentrations up to a 1000-fold higher than those measured in the bloodstream of patients receiving high-doses of metformin. The aim of our study was to investigate the effects of micromolar metformin concentrations on human NSCLC in vitro. We assessed the influence of metformin on cell viability and mitochondrial function - reactive oxygen species production, mitochondrial mass, and mitochondrial respiration. The obtained results suggested that low doses of metformin for up to 5 days exhibit no influence on NSCLC cells survival in vitro. Metformin didn't change the potential of mitochondrial membrane in short- or long-term exposure, but did increase the production of superoxide anion after 5 days. Surprisingly, mitochondrial mass was increased in NSCLC cells after 5 days of treatment, together with the increase in overall oxygen consumption without complex I respiratory chain inhibition. Since the data on using micromolar concentrations of metformin in NSCLC are scarce, our results imply that further research to understand anticancer mechanisms of metformin is needed, and in particular the research using cell culture models that can be better translated to human conditions.

P78 (Session B)

The role of Notch signaling in Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma(HNSCC) defines a group of solid tumors originating from the mucosa of the upper aerodigestive tract, pharynx, larynx, mouth and nasal cavity. It is the sixth most common cancer in the world, with 600 000 new cases reported every year, with a metastatic evolution and poor prognosis. HNSCC heterogeneity and complexity reflects in a multistep progression, involving crosstalk of several molecular pathways. The Notch pathway is associated with major events supporting cancerogenic evolution: being crucial in regulating cell proliferation, maintenance of undifferentiation, angiogenesis and preservation of a pro-oncogenic microenvironment. Notch signaling is highly conserved and encompasses four transmembrane receptors in mammals, Notch1-4. Notch positive cells can therefore receive signals from neighboring cells expressing Notch ligands Delta-like ligands 1,3,4 and ligands Jagged1,2. The Notch signaling pathway is central in tumor development and plays a dual role acting as both oncogene and tumor suppressor. However, it remains to be elucidated how and in which stages, the Notch pathway is involved in the pathogenesis of oral squamous cell carcinoma, especially in the most affected tissue of the oral cavity, the tongue. In order to investigate the role of Notch signaling in the development and progression of oral cancer, a conditional knock-out mouse, lacking the Notch ligand Jagged1 is used as well as the 4-NQO model, representing a chemically inducible cancer mouse model. Exploiting these models, we investigate the role of Notch signaling in cancer formation and progression by performing qRT-PCR, western blot and immunohistochemical approaches.

P79 (Session B)

Clinical practice variation and overtreatment risk in women with abnormal cervical cytology in The Netherlands; two-step versus see-and-treat approach

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Objective: To determine overtreatment rates in the two-step versus see-and-treat approach in women referred for colposcopy because of abnormal cervical cytology and to evaluate clinical practice variation in The Netherlands. Methods: A retrospective cohort study including 36,581 women with a biopsy or treatment result of the cervix in 2016-2017 with preceding abnormal cytology obtained from the Dutch Pathology Registry. Odds ratios of overtreatment, were determined for both strategies in relation to age, HPV-status and referral cytology. Results: 10,713 women (29,3%) received the two-step method, 6,851 women (18.7%) underwent the see-and-treat approach, and 19,017 women (52.0%) received conservative management with cytologic follow-up or another type of treatment. Despite the existence of a national guideline, there is a wide practice variation between the two management options in The Netherlands. 4,119 women (23.5%) were overtreated, with older women, HPV negative women and women with low-grade cytology being more likely to be overtreated. Women receiving see-and-treat were associated with a higher overtreatment rate than women receiving the two-step method (OR 1.79; 95%CI 1.62-1.98). Especially in women with low-grade cytology, see-and-treat management was associated with overtreatment (OR 3.34; 95%CI 2.92-3.82). However, in women with high-grade cytology see-and-treat management was inversely associated with overtreatment (OR 0.68; 95%CI 0.58-0.81). Conclusion: Women receiving the two-step method are less likely to be overtreated compared to the see-and-treat approach. See-and-treat is only justified in women with high-grade cytology, which is in concordance with the (inter)national and guideline(s). There is a wide practice variation between the two management options in The Netherlands and gynecologists should adhere the guidelines more to prevent overtreatment.

P80 (Session B)

Characterization of tumour-infiltrating iNKT (invariant Natural Killer T) cells in colorectal cancer (CRC)

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Immune cell infiltration of colorectal tumors can positively or negatively influence the disease outcome. iNKT cells, a subset of lipid-specific T lymphocytes can mediate anti tumour responses by secreting IFNg and cytotoxic molecules. Nonetheless, evidences indicate that the tumour microenvironment, including immune cells, the gut microbiota and transformed epithelial cells, skew the phenotype and function of tumour infiltrating lymphocytes, including iNKT cells, from inflammatory to pro-tumorigenic. Here we aimed at evaluating the crosstalk between tumour infiltrating iNKT cells and the tumour microenvironment. Paired surgical specimens (peritumoral area-tumour lesion) from 50 CRC patients at different stages of tumorigenesis were analysed by multidimensional cytofluorimetry, with a12 markers panel. Already at stage T1 tumour-infiltrating iNKT cells were associated with a pro tumorigenic phenotype, characterised by elevated IL17 production and inhibitory molecules expression. At later stages the presence of iNKT into the tumour diminished and increased in the peritumoral area, maintaining the pro-tumorigenic phenotype. Our study shows therefore that already at early stages of tumorigenesis the tumour microenvironment can affect the cytotoxic potential of iNKT cells, skewing their phenotype toward a pro-tumorigenic one.

P81 (Session B)

Therapeutic metformin concentrations cause an increase, not an inhibition, of mitochondrial respiration in mouse muscle cells

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Metformin is an oral antihyperglycemic drug widely used in treatment of type 2 diabetes. It inhibits gluconeogenesis and increases insulin sensitivity in peripheral tissues, including muscles. One of the proposed molecular mechanisms by which metformin exerts these effects is inhibition of complex I of the mitochondrial respiratory chain. Still, different metformin concentrations and biological models used in studies lead to inconsistent conclusions about the precise mechanism and properties of this inhibition. Metformin concentrations used in most in vitro studies (1-10 mM) are significantly higher than plasma concentrations found in patients taking metformin (10-60 μM), and used in vivo could cause toxic effects.The aim of this study was to compare the effects of different concentrations of metformin on cell viability and mitochondrial respiration of C2C12 mouse myoblasts.C2C12 cells were treated with a wide range of metformin concentrations for 24 h for measuring cell viability using acid phosphatase and crystal violet viability assays. For measuring mitochondrial respiration by high-resolution respirometry (O2k Oroboros Oxygraph) 50 μM and 5 mM metformin was used for 24 h.Metformin treatment caused a slight, dose-dependent decrease in C2C12 cell viability, in concentrations higher than 200 μM. Respirometry analysis showed that 50 μM metformin treatment caused an increase in mitochondrial respiration of intact cells, and an increase in respiration using complex I and complex II-linked substrates. In contrast, 5 mM metformin treatment caused a decrease in intact cell respiration, and respiration using complex I-linked substrates, while complex II respiration was unaltered.We conclude that micromolar concentrations of metformin do not cause inhibition of complex I activity in C2C12 cells, but an increase in total mitochondrial respiration. This pilot study confirms the need to further explore if the therapeutic, glucose-lowering effect of metformin can indeed be attributed to complex I inhibition.

P82 (Session B)

Targeting colorectal cancer through the tumor microenvironment

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Colorectal cancer (CRC) kills around 700,000 people worldwide every year. The majority of these deaths are the result of metastatic dissemination to foreign organs. In the absence of prevalent mutations associated with metastatic dissemination, we have recently found that TGF-beta signaling in the tumor microenvironment (TME) operates as a major mechanism of immune evasion during metastasis formation. Data from our lab shows that elevated TGF-beta levels impose T cell exclusion, a phenomenon associated with poor outcome in CRC and other tumor types, and blocks anti-tumor Th1 effector phenotype. As part of the characterization of the TME remodeling in response to TGF-beta, this project will analyze in detail the strategies used by tumor cells to evade the immune system. The main focus will be on how T-cells recognize antigens in tumor cells and how TGF-beta remodeling impinges this process.

P83 (Session B)

S. aureus prevalence and the effect of repeated mupirocin nasal ointment on eradication and catheter related infections in patients on home parenteral nutrition.

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RATIONALE: catheter related Staphylococcus aureus infections frequently lead to long-lasting hospitalization and catheter loss in patients on home parenteral nutrition (HPN). While in other groups S. aureus eradication is a proven effective infection prevention measure, studies that explore the effect of mupirocin in HPN patients are lacking. This study investigates the efficacy of chronic nasal mupirocin use on S. aureus eradication and prevention of catheter related infections (CRIs) in HPN patients. METHODS: we collected data from the clinical records of our tertiary HPN referral center. Between 2012 and 2017 HPN patients were screened to assess their S. aureus carrier state. In case of carriage, the patient was instructed to apply mupirocin nasal ointment during 5 consecutive days of each month. The control group comprised the same group's historical infection data. Primary outcome was the percentage of successful S. aureus eradication and secondary outcomes were effect on incidence of CRIs, time to central venous catheter (CVC) change and development of mupirocin resistance. Event rates were analyzed using Poisson regression. RESULTS: S. aureus eradication with mupirocin was successful in 66% (69/104) of the patients. In successfully eradicated patients, CRIs decreased by 40% (p=0.015). This was mostly due to a decrease in catheter related bloodstream infection rates (0.63 vs. 0.32 per 1000 CVC days; p=0.007). A strong reduction of 50% in the overall incidence of CRIs caused by S. aureus was seen as well, though this was not significant (p=0.055). The amount of cvc changes decreased and time to first cvc change increased after the commencement of mupirocin. Diminished mupirocin susceptibility was observed in 4 patients. CONCLUSION: mupirocin ointment prophylaxis seems an effective measure to obtain S. aureus eradication and is associated with a significant decrease in the incidence of CRIs. However, awareness for the development of mupirocin prophylaxis is necessary.

P85 (Session B)

MSK1-mediated metastasis-stroma interaction in breast cancer dormancy

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Many patients that suffer from estrogen receptor positive breast cancer (ER+ BCa) have been found to undergo late relapse after decades from tumor resection. Disseminated cells that colonized distant organs can survive during years without forming overt metastasis, a process that is called dormancy. When tumor mass dormancy occurs, there is a balance between proliferation and apoptosis, keeping an equilibrium with the host microenvironment. In our lab, through an in vivo loss-of-function genome-wide shRNA screening in dormant bone metastatic cells we identified Mitogen and stress-activated kinase 1 (MSK1) as a player in the latent state. MSK1 is a nuclear serine/threonine kinase downstream stress pathways such as p38 and ERK. Depletion of MSK1 reduces cellular differentiation, increasing the bone homing capacity and uptake of BCa metastatic cells. We showed that MSK1 is mediating the expression of luminal genes through chromatin remodeling. This kinase phosphorylates Histone 3 at Serine 10 and 28 of the surrounding histones of different luminal genes, controlling the differentiation phenotype of the cells. Unraveling novel mechanisms downstream MSK1 is key to understand how this molecule could be mediating the interaction between the tumor cells and the stroma, including the immune response, in order to keep a dormant state. Therefore, MSK1 could be a potential biomarker to increase accuracy to select breast cancer patients for therapy.

P87 (Session B)

Characterization of the HIV-1 latent reservoir in early treated individuals

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The main barrier to cure HIV-1 infection in patients is the latent reservoir, a pool of latently infected CD4+ T cells containing replication-competent but transcriptionally silent provirus. The latent reservoir is established early during infection and persists even during suppressive antiretroviral treatment (ART). The latent reservoir is maintained through the longevity of latently, HIV-1 infected resting T cells, potential clonal expansion of those cells, and possibly, due to low-level virus replication followed by infection of new target cells, however, the mechanisms of persistence are not completely understood. The persistence of the latent reservoir through these mechanisms will be studied in patients who started ART in less than 180 days after primary HIV-1 infection. Viral diversity is low at this time point enabling the investigation of virus evolution, which is a marker for ongoing replication. Near full-length HIV-1 genome PCR and next generation sequencing will be applied to longitudinal patients' blood samples to assess virus evolution in latently HIV-1 infected T cells and potential emergence of drug-resistant viruses. Together with HIV-1 reservoir size estimates, viral load data and other clinical parameters, this study will allow us to estimate the impact of low level replication on the maintenance of the latent reservoir in early treated individuals.

P88 (Session B)

Dissecting the role of YAP/TAZ activity during intestinal regeneration

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The adult intestinal epithelium is continuously renewing, and constant production of new epithelial cells is driven by intestinal stem cells (ISCs). Remarkably, upon damage, the intestinal epithelium has the capability to repair itself in a process that is characterized by rapid expansion and differentiation of ISCs into mature epithelial cells. Previous published work of our group has shown that this repairing process is trigger by changes in extracellular matrix composition that ultimatively lead to increased YAP/TAZ transcriptional activity and a transient reprogramming of the epithelium into a foetal-like state. Here I will present our curent data exploiting in vivo colitis models and organoid culture system that recapitule the repairing process to: i) dissect the signalling pathways through which YAP/TAZ is activated; ii) define the transcriptional and epigenetic consequences of YAP/TAZ activation; and iii) establish the effect of enforcing YAP/TAZ activity during intestinal regeneration in vivo. We envisage this knowledge may inform new therapeutic approaches, particularly for patients with inflammatory bowel diseases, where the intestinal epithelium undergoes constant damage.

P89 (Session B)

Targeting non-canonical p38 MAPK signaling

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The p38a MAPK signaling pathway plays an important role in several human pathologies, including inflammatory and cardiovascular diseases as well as cancer. Thus, inhibiting this pathway may represent a promising therapeutic approach. Whereas p38a is normally activated by upstream dedicated kinases, there is evidence that p38a can be also activated through a non-canonical mechanism, and this activation has been associated to cardiomyocyte death.Over the last two decades, many p38a inhibitors that function as ATP competitors have been developed by the pharmaceutical industry. However, these compounds have shown disappointing results in clinical trials. As an alternative to the current inhibitors, we have identified novel compounds that selectively inhibit p38a by modulating the non-canonical activation pathway. These molecules could affect only a subset of p38a functions, thus resulting in a more specific inhibition.This project aims at improving the potency of these compounds and assess their efficacy in models of p38a-associated diseases. We have progressed this drug discovery program to the lead optimization phase, and the results indicate that our compounds are highly selective towards p38a and are active in cells. Moreover, preliminary results using cultured murine cardiomyocytes suggest that these compounds reduce cell death induced by simulated ischemia-reperfusion injury. We are currently performing ADME-Tox assays to determine the drug-like properties of our compounds, to be considered in parallel to their inhibitory potency.

P90 (Session B)

Unraveling the role of immune checkpoint pathways in immune escape after allogeneic stem cells transplantation.

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Allogeneic stem cell transplantation (alloSCT) has a curative potential for patients suffering from hematological malignancies by, amongst others, inducing donor T cell responses against minor histocompatibility antigens or tumor-associated antigens expressed by the recipient's malignant cells. However, tumor evasion mechanisms, such as the induction of co-inhibitory and inhibition of co-stimulatory molecules on T cells, can lead to impaired T cell functionality and patient relapse. Previously, relapse of alloSCT recipients was associated with high co-expression of PD1, TIGIT and KLRG1 on minor-specific T cells. Yet, more extensive studies are needed to delineate the role of other co-signaling molecules and T cell phenotype on relapse post-alloSCT. In this study, we established two 18-color flow cytometry panels to comprehensively determine T cell differentiation and checkpoint profiles in patients with hematological malignancies upon alloSCT. Additionally, an 8-color combinatorial encoding panel was designed to simultaneously screen patients for up to 28 peptide-specific T cell responses. This extensive T cell characterization in patients revealed dominance of effector memory T cells and hardly any naïve T cells compared to healthy controls. Moreover, both for CD4+ and CD8+ T cells of patients, a higher percentage of cells co-expressed =2 co-inhibitory molecules, while no co-stimulatory molecules were expressed by 50% of patient-T cells. Notably, the patients had cytomegalovirus reactivation post-alloSCT which served as proof-of-principle for our explorative study. When focusing on the virus-specific T cells, they were characterized by an effector memory phenotype and high expression of co-inhibitory markers PD1, TIGIT, BTLA and senescence-associated marker CD57. Overall, the panels designed can be exploited for studying the tumor-specific T cells to boost T cell responses in alloSCT and additional T cell-based (immuno)therapies.

P91 (Session B)

PD-L1 siRNA-mediated silencing in acute myeloid leukemia enhances anti-leukemic T cell reactivity

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Immunotherapeutic approaches, including donor stem cell transplantation, have demonstrated curative potential in high-risk myeloid malignancies. However, despite induction of tumor-reactive immune responses, many patients eventually progress or relapse. By exploiting suppressive mechanisms, including inhibitory signalling via checkpoint molecules, tumor cells can dampen tumor-reactive T cell responses. Importantly, systemic immune checkpoint blocking antibodies, targeting e.g. the PD-1/PD-L1 signaling pathway, have demonstrated impressive clinical results in cancer patients. Yet, the negative consequence of systemically releasing the immune brake is the induction of (severe) toxicity in healthy tissues.The aim of this study was to locally interfere with inhibitory PD-1/PD-L1 signaling in the acute myeloid leukemia (AML) micro-environment by PD-L1 siRNA delivery. Notably, using siRNA/SAINT-RED transfection technology, we efficiently silenced PD-L1 in AML cell lines as well as in patient' AML cells. Next, the functional effect of the PD-L1 silencing in AML on leukemia-reactive T cell activity was investigated in co-culture assays. We observed increased activation of WT1 TCR mRNA transfected PD-1+ Jurkat cells upon co-culture with PD-L1 silenced WT1+ AML. Moreover, we showed significantly enhanced activation, degranulation and IFN-? production of minor histocompatibility antigen (MiHA)-specific CD8+ cytotoxic T cells in response to PD-L1 silenced MiHA+ AML cells. Importantly, PD-L1 silencing was equally effective as PD-1 antibody blockade in boosting tumor-reactive T cell function.Together, these results demonstrate that PD-L1 silencing using RNAi technology enhances the immune-susceptibility of AML cells, facilitating improved recognition by tumor-reactive CD8+ T cells. This provides rationale for development of targeted siRNA delivery technology to selectively interfere with immune escape mechanisms in the local myeloid tumor micro-environment. Hereby, the risk of eliciting severe toxicity can be limited as compared to systemic checkpoint interference. In combination with adoptive T cell transfer, this strategy could be very appealing to improve outcome in patients with high-risk myeloid malignancies.

P92 (Session B)

iPlacenta- First European consortium specialising in placental health: Pre-eclampsia and extracellular vesicles

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Placenta is the least studied organ in the body resulting in pregnancy complication being unmet with clinical need. iPlacenta is an inter-disciplinary European training network project which aims to improve modelling of the placenta and maternal cardiovascular system. The network combines the expertise of clinicians and basic scientists with that of mathematicians, physicists and engineers. Pre-eclampsia affects between 5-8% of pregnant women resulting in mortality of 76,000 mothers and 500,000 babies annually. The babies and mothers which survive preeclampsia suffer with serious life-long cardiovascular and neurological complications. However, the aetiology of the disease is still unknown. Majority of literature points at the placenta as the origin of preeclampsia. Extracellular vesicles such as exosomes, microvesicles and apoptotic bodies are emerging as important players for intracellular communications and are implicated in normal physiology and pathophysiology of various diseases. Extracellular vesicles encapsulate various bioactive molecules which aid in cell-cell communication. During pregnancy, placental-derived extracellular vesicles have been identified in maternal blood and amniotic fluid therefore being implicated in cell-to-cell communication between the placenta, and peripheral blood immune cells. Several factors diffuse from the placenta through the placental membranes into amniotic fluid. We hypothesize that placental-derived EVs released in amniotic fluid may possess angio-modulating properties that could be relevant in placental angiogenesis and that these characteristics may be altered in pre-eclampsia.

P93 (Session B)

Posaconazole is a novel inhibitor for alphavirus viral entry

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Chikungunya virus (CHIKV), a mosquito-borne alphavirus, causes millions of infection globally. Posaconazole (PCZ) is an antifungal drug, which we and others have previously found to inhibit replication of a number of viruses, including dengue virus, a member of the Flaviviridae family. In this study, we analyzed the antiviral activity of PCZ against alphaviruses. We found that PCZ potently inhibits a number of alphaviruses, including Semliki forest virus (SFV), Sindbis virus (SINV) and CHIKV with half maximal effective concentration (EC50) of 2.3 µM, 4.0 µM and 0.8 µM, respectively. Time-of-addition assays indicated that PCZ treatment before and at the time of SFV infection showed potent inhibition, whereas addition of PCZ at later time points post infection showed minor to no inhibition, suggesting inhibition at an early stage of the replication cycle. In accordance, PCZ treatment of a temperature sensitive mutant of SFV that is capable of cell entry and translation, but not RNA replication, resulted in an almost 90% reduction in luciferase activity. To confirm these findings, PCZ resistant mutant virus were generated and we identified mutations in E1 (V148A) and E2 (H255R) viral glycoproteins, of which the E2 mutation confers partial resistance to PCZ when introduced into wild-type SFV. To see whether PCZ alters clathrin-mediated endocytosis, we analyzed the uptake of fluorescence-tagged transferrin and found that PCZ reduced transferrin uptake by 50% compared to DMSO-treated cells. Together, these results establish PCZ as a novel inhibitor of alphaviruses and identify viral entry as its target.

P94 (Session B)

Effectiveness of Somatostatin Analogues in Patients with hereditary hemorrhagic telangiectasia and symptomatic gastrointestinal bleeding, the SAIPAN-trial: a multicenter, randomized, open-label, parallel-group, superiority trial.

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Rationale: Hereditary Hemorrhagic Telangiectasia (HHT), also referred to as Rendu-Osler- Weber syndrome (ROW), is an autosomal dominant hereditary disease which affects 1/5- 8000 individuals. It is characterized by arteriovenous malformations (AVMs) and telangiectasias in multiple organs, including the gastrointestinal tract. Patients can be transfusion dependent due to severe gastrointestinal bleeding from those telangiectasias. Endoscopic treatment with Argon Plasma Coagulation (APC) is not as effective due to the recurrent character of the telangiectasias. Based on literature in patients with non-HHT AVMs and telangiectasias, we hypothesize that short-acting octreotide 0,1mg twice a day for 26 weeks (on top of standard care) will lead to a reduction in the number of blood and/or iron transfusions compared to standard care alone in HHT patients with GI bleeding due to AVMs. Furthermore, we hypothesize an improvement in global health outcomes and a reduction in epistaxis severity. Objective: To determine if Somatostatin Analogues (SSA) are effective in decreasing transfusion requirements and improving quality of life while being cost-effective.Study design: A phase III, randomized, open-label, parallel-group, superiority, multicenter trial. Study population: Patients with HHT with GI bleeding and transfusion dependency (i.e. at least 4 blood units and/or intravenous (IV) iron in the previous 26 weeks). Intervention: Short-acting octreotide subcutaneously 0.1mg twice daily for a period of 26 weeks, on top of standard of care. Main study parameters/endpoints: The primary endpoint is the difference between the octreotide and observational arm in patients with a clinical relevant successful decrease in number of IV iron and blood transfusions. Clinical relevant successful decrease is defined as a decrease of 50% or more in the number of IV iron and/or blood transfusions. Secondary endpoints are: quality of life, level of fatigue, epistaxis severity, number of endoscopic APC treatments, and cost-effectiveness.

P95 (Session B)

Combination therapy of gemcitabine and CD34+ progenitor-derived NK cells results in superior ovarian cancer killing

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Ovarian cancer (OC) is the most lethal gynecological malignancy. To improve survival, novel therapeutic strategies are urgently needed. As ovarian tumors often downregulate MHC class I and increase expression of activating ligands, they are prone to Natural Killer (NK) cell-mediated immunity. Previously, we demonstrated that umbilical cord blood-derived CD34+ hematopoietic progenitor cell (HPC)-NK cells are highly capable of killing OC monolayers and spheroids. Furthermore, intraperitoneal infusion of HPC-NK cells in human OC-bearing immunodeficient mice significantly limits tumor progression and improves survival. However, HPC-NK cell treated mice do not remain tumor-free, indicating that further improvement of HPC-NK cell therapy is needed. Combining HPC-NK cells with the chemotherapeutic drug gemcitabine could be an attractive treatment strategy for ovarian cancer patients, since gemcitabine is used in second-line treatment. Importantly, gemcitabine increases expression of NKG2D ligands in various cancers, thereby providing activating signals to NK cells. The goal of this study was to investigate the cytotoxic, phenotypical and functional effects of gemcitabine on HPC-NK and OC cells. First, we established that 2.5 nM gemcitabine induced 50-60% of OC cell death after 2 days, while HPC-NK cells were relatively spared (24% death). Notably, this did not negatively impact HPC-NK cell degranulation nor production of IFN?, perforin and granzyme B. Importantly, we demonstrated that HPC-NK cells combined with 2.5 nM gemcitabine kill 80% of OC cells after 2 days, compared to 60% killing by gemcitabine or HPC-NK cells alone. Moreover, 10 nM gemcitabine increased expression of NK cell activating ligands and apoptosis receptors on OC cells. Finally, gemcitabine decreased tumor growth in human OC-bearing immunodeficient mice. In conclusion, HPC-NK cell therapy combined with gemcitabine treatment could be a promising approach to treat OC. Our next step is to combine gemcitabine with HPC-NK cells in vivo in order to investigate if HPC-NK cells further decrease tumor growth.

P96 (Session B)

Clinical auditing as an instrument of improving care: The Dutch Gynaecological Oncology Audit (DGOA)

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Introduction: The Dutch Gynaecological Oncology Audit (DGOA) was initiated at the end of 2013 to serve as a nationwide registration where all patients are registered for ovarian, vulvar, endometrial and cervical cancer. The aim of this study is to present the first results of clinical auditing from the DGOA for all the four types of gynaecological oncological malignancies. Methods: The DGOA is facilitated by the Dutch Institute of Clinical Auditing and run by its own scientific committee existing of several disciplinary members. Items are collected through a web-based registration based on a set of predefined quality indicators, mainly on ovarian cancer. The results are weekly updated and the benchmarked information is given back to the user. Data verification was done in 2016 where the accuracy and completeness of the data was checked. Results: Between the 2014 and 2018, 6535 patients with ovarian cancer, 1503 patients with vulvar cancer, 7009 patients with endometrial cancer and 2706 patients with cervical cancer were registered. Case ascertainment was nearly 100% in 2016. Results of some quality indicators are on waiting time and completeness of staging. The percentage of patients waiting less than 28 days to start with therapy decreased over time from 57.3% in 2014 to 40.9 in 2018 ($p<0.001$). Patients with a complete staging procedure remained similar over the years, as well as 30 day in and out hospital mortality and complicated course. Conclusion: At the start of registering the main goal was to focus on ovarian cancer resulting in a main focus of quality indicators for ovarian cancer. As the audit matures, the other gynaecological malignancies will get more involved. The nationwide audit shows valuable data which surely can improve quality of care

P97 (Session B)

Single nucleotide polymorphisms in EGFR gene in NSCLC patients

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Lung cancer is a leading cause of mortality, taking pandemic proportions worldwide and Non-small-cell lung cancer (NSCLC) is the most frequent lung cancer type. Epidermal growth factor receptor (EGFR) was used as a potent biomarker for the development and implementation of NSCLC target therapy. Still, it was noticed that not all of the NSCLC patients responded equally to therapy, so it was proposed that polymorphisms might be one of potential cause of these differences affecting EGFR gene regulation. It was shown that single nucleotide polymorphisms (SNPs), namely -216G>T (rs712829) and -191C>A (rs712830) located in promoter region and 181946C>T (D994D) (rs2293347) in exon 25, could regulate activity of EGFR. This study was part of my PhD and one of purposes was to represent frequency of EGFR polymorphisms in population from Republic of Serbia and to identify potential risk factor for developing lung cancer phenotype. Tobacco smoking is cause of premature death and Republic of Serbia takes high place in annual mortality in Europe from lung cancer. We have genotyped those SNPs and to our knowledge that was the first time to show their genotype frequencies for NSCLC patients in Republic of Serbia. For this genotipisation procedure a sensitive, specific and optimized method was demanded. We used polymerase chain reaction-restriction length polymorphism (PCR-RFLP), that allows wide modifications, but its‘ optimisation might be time and cost consuming. All of obstacles were overcomed and we have succesfully optimized and performed PCR-RFLP, followed by direct sequencing, indeed. Based on this study, environmental factor like tobacco consumption and genetic susceptibility are potent risks for NSCLC patients in Republic of Serbia. Namely we have shown that GG genotype of EGFR polymorphisms -216G/T, was a risk factor for smokers to develop non small call lung cancer, so T allele might have protective role. Acknowledgement: The study was financially supported by the Ministry of Science, Republic of Serbia, 175056.

P98 (Session B)

Motored PLGA drug carriers for periodontal treatment

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Local drug delivery systems have recently been developed for multiple diseases that have the requirements of site-specific actions, prolonged delivery periods, and decreased drug dosage to reduce undesirable side effects. The challenge for such systems is to achieve directional and precise delivery in inaccessible narrow lesions, such as periodontal pockets or root canals in deeper portions of the dentinal tubules. The major strategy to tackle this challenge is fabricating a smart tracking delivery system. Here, we report drug-loaded biodegradable micromotors showing self-propelled directional movement along a hydrogen peroxide concentration gradient produced by phorbol esters-stimulated macrophages. The drug-loaded PLGA Janus micromotors were prepared by electrospraying and post-functionalized with catalase via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide coupling. Doxycycline, a common drug for the treatment of periodontal disease was selected as a model drug, and the release study by high-performance liquid chromatography showed that both the post-functionalization step and the presence of hydrogen peroxide had no negative influence on drug release profiles. The movement behavior in the presence of hydrogen peroxide was confirmed by nanoparticle tracking analysis. An in vitro model was designed and confirmed the response efficiency and directional control of the micromotors towards phorbol esters-stimulated macrophages.

P101 (Session C)

Assessment of intrahepatic transplantation of islet of Langerhans grafts using dynamic exendin PET imaging

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Aim/Introduction: Patients with complicated type 1 diabetes (T1D) and unstable glycemic control can receive islet grafts via intrahepatic transplantation as treatment. This procedure results in an improved glycemic control and quality of life. Graft function will however deteriorate over time due to various factors. A tool to assess transplantation success and monitor islet survival and functionality would be of great clinical value. We applied dynamic PET imaging with the beta cell-specific tracer 68Ga-labeled exendin-4 to study the presence of transplanted islets in T1D patients. Methods: Dynamic PET scans were acquired after intravenous injection of 68Ga-NODAGA-exendin-4 of 5 T1D patients who previously underwent intrahepatic islet transplantation (Tx-group). Graft function in these patients was biochemically proven prior to imaging with a mixed-meal tolerance test (MMTT). In addition, 3 control patients that awaited islet transplantation were imaged. Thresholding was applied to identify areas with high hepatic tracer uptake. Kinetic modeling was then used to measure tracer uptake by determining the distribution volume. Islet function was expressed as the area under the curve of the measured c-peptide in the MMTT. Islet function was compared to the PET signal obtained from the analysis of the dynamic PET data. Results: Transplanted patients had a significantly higher c-peptide production during the MMTT (22.6 vs. 151.8 nmol.min/L, $p<0.05$). The distribution volume of the PET tracer obtained through kinetic modeling was significantly higher in the Tx-group ($p=0.036$), indicating an increased retention of the tracer in the liver i.e. the presence of intrahepatic islets. There was no significant correlation found in the Tx-group between Vt and IEQ, neither between Vt and c-peptide production. Conclusion: The data of this explorative study indicate that dynamic PET imaging using 68Ga-labeled exendin-4 is a highly promising tool to assess intrahepatic transplantation of pancreatic islet grafts in T1D patients.

P102 (Session C)

Dysregulated microRNA expression in circulating plasma cells in multiple myeloma

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MicroRNAs (miRNAs) are short non-coding RNA molecules that are involved in many physiological and pathological processes. Multiple myeloma (MM) is the second most common hematological malignancy of plasma cells (PCs). These cells are dependent on the BM microenvironment. However, a subclone of these cells can escape from the bone marrow (BM) either infiltrating soft tissues (extramedullary disease, EM) or escaping to peripheral blood (PB) (as so-called circulating plasma cells, cPCs). In both cases, loss of BM dependence is a negative prognostic marker for MM patients. If more than 20% of cPCs are found in PB, the disease is reclassified as plasma cell leukemia (PCL).The importance of miRNA in the pathogenesis of MM has been demonstrated by several studies. Thus, we hypothesize that miRNA dysregulation is involved in the BM escape of PCs.Using next generation sequencing (NGS), 36 BM PCs from MM patients, 9 BM PCs from EM patients and 17 cPCs samples were analyzed (from MM and PCL patients).NGS analysis showed 2335 different miRNAs that were present in analyzed samples; 578 miRNAs were in at least 30 samples and had more than 1 read per million, were included in subsequent analysis. Out of these miRNA, there are 5 miRNAs ($p<0.000001$) that are significantly dysregulated between cPCs and BM PCs from EM patients. Furthermore, there are 7 miRNAs ($p<0.0000000001$) significantly dysregulated in cPCs in comparison to BM PCs from MM patients.The aim of this work was to analyze different expression of miRNA between BM PCs samples of EM and MM patients compared to cPCs.Using NGS, we showed that they are differentially expressed miRNA between MM BM PCs and EM BM PCs and cPCsThis work was supported by AZV 17-29343A and AZV 18-003-00203.

P104 (Session C)

Understanding Magnesium regulation by FAM111A

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Background: Kenny-Caffey syndrome type 2 (KCS2) is a condition that affects growth, skeletal structure and electrolyte balance. Distinct electrolyte imbalance observed includes primary hypomagnesaemia with secondary hypocalcaemia and hypoparathyroidism. Mutations in FAM111A are responsible for KCS 2. FAM111A has been reported to have antiviral activity and play a role in DNA replication, however it is still unclear how this protein affects electrolyte transport. The aim of this project is to describe the functions of FAM111A and its role in electrolyte homeostasis. Methods: Preliminary data from our group using mass spectroscopy identified STAT1a as a binding partner to FAM111A. STAT1A has been reported to increase the activity of the calcium sensing receptor (CaSR). We are currently validating this interaction by performing co-immunoprecipitation and investigating the effects of FAM111A on STAT1 transcriptional activity. RNA sequencing performed on kidney tissues of wildtype and FAM111A knockout mice will identify genes regulated by FAM111A involved in electrolyte handling. We will validate these genes and elucidate how FAM111A regulates their functions by performing further experiments to understand how FAM111A regulates electrolyte transport. Conclusion: Understanding the molecular mechanisms by which FAM111A affects transport of electrolytes (Mg2+, Ca2+ and phosphate) will improve current knowledge of the protein which is currently sparse. This study will help to improve the management of patients with KCS 2 by identifying potential treatment targets.

P105 (Session C)

Identifying cancer driver transcripts in ERG+/PTEN- RWPE-1 PCa onset in vitro model

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Prostate cancer (PCa) progression is largely dependent on epigenetic mechanisms, including concurrent global DNA hypomethylation and gene promoter hypermethylation, which are specific features of epithelial-to-mesenchymal transition (EMT) and in line with this, we have demonstrated that DNMT3A is essential for PCa progression and EMT activation. Moreover, TMPRSS2/ERG gene fusion was recently discovered to be a frequent event in PCa and together with the concomitant presence of PI3K signaling alterations, such as PTEN inhibition, results in the development of pre-neoplastic lesions and invasive carcinomas.We aim to explore these mechanisms identifying new differentially expressed and methylated (DE-DM) cancer driver genes, including alternative gene isoforms and splice variants, which expression is deregulated by the combination of ERG and PTEN modulation.To this end, we exploited an in vitro model set up by prof. Lunardi based on the immortalized epithelial prostate cell line RWPE-1, genetically engineered with a doxycycline inducible vector to mimic TRMPSS2/ERG over-expression and siRNA silenced to obtain PTEN down-regulation, reproducing the molecular events leading to PCa onset. Preliminary results confirmed that doxycycline treatment nicely induces ERG over-expression, while PTEN down-regulation inversely correlates with p-AKT. The combined ERG/PTEN deregulation in these cells induced up-regulation of EMT markers/factors (VIM and EZH2) and down-regulation of epithelial markers (CDH1 and GRHL2), indicating an initial EMT phenotype switch.To identify new DE-DM PCa driver transcripts in our cell model, we will siRNA silence the de novo DNA methyltransferases (DNMT3A/B) before ERG/PTEN deregulation and combine RNA-seq to DNA-methyl-seq through a bioinformatics approach. To final confirm their driving role, we will analyze the resulting phenotypes of silencing/over-expression of coding and non-coding RNAs prior to ERG/PTEN modulation.

P106 (Session C)

Identification of p38a-regulated phosphorylation networks in breast cancer

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Signaling pathways based on mitogen-activated protein kinases (MAPKs) play a prominent role in the process of integration of information, which allow cells to interpret environmental clues and elaborate on the appropriate responses. Upon activation, p38a can phosphorylate a variety of substrates both in the cytoplasm and in the nucleus, including many transcription factors. The set of p38a substrates that are phosphorylated in response to different stimuli is likely to contribute to the particular cellular response. To better understand the function of MAPK14 and the underlying molecular mechanisms, our lab has generated inducible p38 depletion cell line derived from PyMT tumors which can be induced by 4-OHT treatment. Phospho-proteomic screenings were used to investigate the quantitative changes of the cellular phosphor-proteome between p38 WT and KO cells. This analysis revealed that out of 6726 phosphorylation sites identified, 265 and 442 were significantly downregulated and upregulated, respectively, on interfering with p38a signaling. At the level of total protein expression, out of 2916 proteins identified, 115 and 177 were significantly downregulated and upregulated, respectively, on interfering with p38a signaling. Functional annotations of proteomic data showed that phosphoproteins affected by p38a deletion were more involved in cell cycle regulation and cell adhesion.

P107 (Session C)

Comprehensive genomic profiling of gallbladder cancer in a Western population identifies potentially actionable therapeutic targets

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Gallbladder cancer (GBC) is rare in Western countries and carries a dismal prognosis because of its late diagnosis, aggressive behavior and absence of effective treatment options. Only a minority of patients is eligible for surgical resection at time of diagnosis, offering the only chance of cure. There are no targeted therapies available for GBC. We identified patients with primary GBC between 2000 and 2019 in the Netherlands using PALGA, the national pathology database, and the Netherlands Cancer Registry. Formalin-fixed paraffin-embedded (FFPE) tissue material, pathological data and clinical data of >800 patients were collected. Clinical data will be combined with histopathological review, immunohistochemistry and molecular analyses. Genomic profiling was performed for 26 cases as pilot using the Illumina TruSight Oncology-500 DNA assay, which simultaneously detects small variants (SNV/INDEL) and copy number amplifications in 523 genes, tumor-mutational burden (TMB) and microsatellite instability (MSI). Year of diagnosis ranged from 2014 to 2019 and tumor load varied from 30% to 80%. Median exon coverage ranged from 179 to 653 counts (one sample was excluded). Four cases had a TMB of >10 mutations/Mb (range 10.3-161.1); the highest mutational load was observed in a case carrying a pathogenic variant in POLE, presumably causing this high TMB. MDM2 was amplified in 4 cases (15.4%), with co-amplification of FRS2 and CDK4 in 100% and 50% of cases, respectively. One case (3.4%) showed amplification of KRAS and the tumor of another case had two histologically distinct components of which only one harbored a MET amplification, highlighting intra-tumor heterogeneity of GBC. Pathogenic variants were observed in amongst others TP53 (50.0%), KRAS (19.2%), ERBB2 (p.S310F, 3.4%) and IDH1 (p.R132C, 3.4%), the latter two with potential therapeutic options. In conclusion, genomic profiling of GBC highlights extensive molecular heterogeneity and identifies potentially actionable therapeutic targets in a subset of patients.

P108 (Session C)

Mechanisms of dendritic cell cross-presentation induced by saponin-based adjuvants

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Introduction: Current adjuvants induce robust antibody responses, but are weak in priming CD8+ killer cells (cell-mediated immunity), which is crucial for effective host defense against viruses, intracellular parasites and cancer. Saponin-based adjuvants (SBAs) are promising new adjuvants that stand out as they not only enforce CD4+ T cell-mediated immunity and antibody responses, but also induce an unprecedented level of antigen cross-presentation by dendritic cells (DC) and subsequent CD8+ T cell activation. The importance of adjuvants enhancing DC cross-presentation has been reviewed recently1. SBAs are now being applied in human vaccines, and several clinical trials have proven safety and efficacy. How SBAs act to induce antigen cross-presentation has remained obscure. The Adema lab has uncovered that SBAs ability to boost cross-presentation depends on the induction of so-called lipid bodies2. Objective: The aim is to unravel the mechanisms behind the induction of lipid bodies and DC cross-presentation by SBAs. Understanding the pathways involved in SBA induced cross-presentation and immune activation should ultimately lead to the development of vaccines with improved efficiency and safety. Methods: RNA sequencing has been performed on SBA responding and non-responding DC populations. Differentially expressed genes involved in SBA stimulation will be studied using a combination of immunological (flow cytometry, functional assays), molecular (RT-qPCR) and cell biological (microscopy) techniques. Publications: 1. Huis in 't Veld LGM*, Ho NI*,Raaijmakers TK and Adema GJ. Adjuvants Enhancing Cross-Presentation by Dendritic Cells: The Key to More Effective Vaccines? Front. Immunol. 9:2874, 2018.2. Den Brok MH, Büll C, Wassink M, De Graaf AM, Wagenaars JA, Minderman M, Thakur M, Amigorena S, Rijke EO, Schrier CC, Adema GJ. Saponin-based adjuvants induce cross-presentation in dendritic cells by intracellular lipid body formation. Nat Commun. 7:13324, 2016.

P109 (Session C)

FASTKD Family Proteins: Exploring Non-canonical Mitochondrial RNA Processing

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Mitochondria are often referred to as eukaryotic cellular powerhouses. The human mitochondrial genome encodes only 37 genes, all of which are required for ATP production through oxidative phosphorylation. Hence, the accumulation of mutations in mitochondrial DNA (mtDNA) as well as in nuclear DNA genes encoding mitochondrial gene expression machinery causes mitochondria-related failures, which lead to incurable neuromuscular disorders known as mitochondrial diseases. Mitochondrial transcription generally initiates at two distinct promoters on the circular mtDNA, one on each strand, giving two genome-length polycistronic transcripts. This highlights the importance of post-transcriptional regulation of mitochondrial gene expression. The Fas-activated serine/threonine kinase domain (FASTKD) family proteins, a putative RNA binding protein family, are emerging key mitochondrial post-transcriptional regulators. While they all have same domain structure with three uncharacterised domains, FAST1, FAST2, and RAP domain, each protein has distinct functions ranging from RNA processing to translation, and their molecular mechanisms still remain to be elucidated. To uncover the molecular mechanism, we purified and crystalized recombinant FASTKD4 protein as a representative of the FASTKD family. Interestingly, the crystal structure revealed that the RAP domain resembles the PD-(D/E)-XK nuclease superfamily. Given that it has been reported that FASTKD proteins are likely involved in non-canonical mtRNA processing, we hypothesize that they might have a ribonuclease activity which is required for this atypical mtRNA processing whose molecular mechanism is unclear. To test this hypothesis, we are attempting to verify the RNase activity of purified recombinant FASTKD4 protein in vitro, and to characterise the catalytic sites. Importantly, as the RAP domain is an ancestral domain which is present in many different organisms from archaes to plants, the characterisation of this domain may not only reveal the mode of action of FASTKD proteins, but also shed light on a new key RNA regulation conserved during evolution.

P110 (Session C)

Study of prevalence and sequence characteristics of antibodies with cofactor-mediated polyreactivity

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Heme, an intracellular cofactor, serves as a prosthetic group for many enzymes and proteins that are involved in electron and gas transport, and redox reactions. Diseases accompanied by tissue damage and hemolysis result in the release of large quantities of heme into circulation, where it can interact with plasma proteins, including antibodies. It has been shown that in healthy immune repertoire there is a fraction (about 20%) of immunoglobulins whose interaction with heme results in the appearance of polyreactivity, reactivity toward various structurally unrelated antigens. However, physiological relevance and molecular mechanisms of heme-induced alteration of antibody specificities are not understood. In order to elucidate the origin and prevalence of heme-sensitive antibodies in normal immune repertoires, we performed cloning and expression of >250 recombinant Abs from naive and memory B cell subpopulations isolated from healthy donors. This set of antibodies was screened for binding to heme and subsequent gain of polyreactivity towards self- and pathogen-derived proteins. Bioinformatics analyses of gene sequences of heme-binding Abs revealed that they possess longer CDR3 region of heavy chain and a higher number of aromatic amino acid residues, namely tyrosine, compared to non-sensitive antibodies. Indeed, the aromatic macrocyclic structure of heme provides a potential for many non-covalent contacts with aromatic amino acids. Two immunoglobulins that are identified to be the strongest heme-binders were selected for further studies. The importance of heavy chain for their activity was confirmed by investigating chimeric antibodies obtained by replacing heavy or light chain of sensitive immunoglobulin by that of the non-sensitive one. Single point mutations were introduced into CDR3H by replacing individual aromatic and positively charged amino acids with alanine. In this way we were able to identify and assess the importance of amino acids implicated in the interaction with heme and gain a first insight into molecular mechanisms involved.

P111 (Session C)

How endosomal recycling can regulate cell migration and cytokinesis

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The correct targeting of endocytosed proteins and lipids is key to normal cell function. Once at the early endosome, cargoes are selected by different mechanisms to follow a certain route that will determine their destination within the cell. Many of those cargoes will be recycled back to the plasma membrane in a process that is finely tuned and that will impact essential cellular processes in higher eukaryotes. In our lab we have identified a protein called Kazrin that modulates endosomal trafficking by promoting a long recycling route, through the recycling endosome, over a short recycling route. We use Transferrin as a model cargo to study recycling and have observed that Kazrin KO cells are not able to accumulate Transferrin at the recycling endosome and have a faster recycling rate, which indicates a disruption in the long recycling pathway. Consequently, we have checked the implication of Kazrin in processes involving the recycling endosome route. Indeed, Kazrin KO cells have a slower and a more directional migration, a process known to depend on the modulation of integrin recycling. Another recycling endosome-dependent cell function that we have shown to involve Kazrin is cytokinesis. Interestingly, Kazrin directly interacts with complexes involved in early sorting. In particular, we have found interactions with the clathrin-coating machinery, the actin polymerization machinery, and phosphoinositide conversion regulators. We thus propose a model in which Kazrin promotes trafficking to the recycling endosome through the regulation of the actin cytoskeleton, the formation of clathrin-coated intermediates and the regulation of phosphoinositide metabolism. This mechanism could be necessary for correct cell migration and cytokinesis, two processes of outstanding biomedical importance.

P112 (Session C)

ERG promotes the methylation reprogramming in prostate cancer onset

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ETS-related gene (ERG) is one of the E-26 transformation-specific family of transcription factors. Tomlins et al. demonstrated that 50% of PCa is characterized by the formation of the TMPRSS2 androgen-dependent promoter and ERG gene fusion that promotes ERG overexpression and thus contributes to an androgen-independence development. The ERG overexpression phenotype was studied using a model based on a normal immortalized human prostate epithelial cell line, RWPE-1, engineered to allow the inducible expression of ERG by doxycycline treatment. Induced migration was assessed by the wound healing assay, while transcriptional and translational levels were defined using qPCR and Western Blotting.ERG overexpression in normal epithelial cell line promotes a time-dependent migration. This early gain of function aligns with what we firstly confirmed, in fact ERG overexpression assesses an initial EMT phenotype switch, promoting the upregulation of mesenchymal markers and factors such as vimentin, CDH2, and ZEB1, while on the other hand a slight reduction of CDH1 and Slug expression. Interestingly, our analyses showed that ERG upregulation also affects DNA methyltransferases expression. The maintenance and de novo methyltransferases (DNMT1 and DNMT3B, respectively) decrease both at a transcriptional a nd translational level, while, surprisingly, DNMT3A expression levels increase after ERG induction. In this scenario, we hypothesized that ERG overexpression promotes a global DNA methylation reprogramming to support prostate cancer progression. Besides, as we have previously demonstrated (Pistore et al., 2017), DNMT3A is able to bind the CDH1 promoter and thus contribute to the mesenchymal phenotype that we observed.ERG overexpression contributes to PCa progression promoting an EMT acquisition. This process could be supported by a switch in DNMTs expression that allows DNMT3A to reprogram DNA methylation levels and to support mesenchymal phenotype. To deepen our hypothesis, we will carry out the silencing of DNMT3A before and after ERG induction to intercept DE and DM genes.

P113 (Session C)

Ultrastructure and viscosity affect solute diffusion in the mitochondrial matrix

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Changes in mitochondrial matrix shape and volume alter the kinetics of biochemical reactions in the matrix (Lizana et al., 2008), in particular when the involved substrates and enzymes are mobile. Since many human diseases display alterations in mitochondrial shape, volume and (internal) structure (Bulthuis et al., 2018), it is highly relevant to quantitatively understand how these morphological changes are coupled to mitochondrial function. To probe this system, we quantified the diffusion constant of fluorescent proteins (FPs) by combining sub-mitochondrial FRAP (fluorescence recovery after photobleaching) experiments with random-walk modeling. This demonstrated that matrix-protruding folds in the MIM ('cristae') substantially hinder protein diffusion (Dieteren et al., 2011).Here we aimed to determine whether FP diffusion in the mitochondrial matrix is size-limited, and thereby if cristae can act as a 'molecular sieve'. This is important since diffusing protein complexes of a relatively large size (>100 kDa) have been detected in the matrix.We created stable HeLa cell lines that express matrix-targeted GFP-based concatemers of increasing size. The mobility of these FPs was quantified using FRAP and fluorescence loss in photobleaching. Cells were treated with chloramphenicol (CAP), an inhibitor of mitochondrial protein synthesis, which is known to reduce the number of cristae. Cristae morphology was visualized using electron microscopy and quantified.FP mobility decreases with increasing size and molecular weight. However, all FPs were fully mobile. CAP treatment is associated with reduced cristae number and FP mobility, strongly suggesting that it increases the viscosity of the matrix fluid. We are currently performing quantitative random-walk modelling to determine absolute solute-dependent diffusion constants and matrix viscosity values.

P114 (Session C)

Functional diversification of hybridoma produced antibodies by CRISPR/HDR genomic engineering

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Hybridoma technology is instrumental for the development of novel antibody therapeutics and diagnostics. Recent preclinical and clinical studies highlight the importance of antibody isotype for therapeutic efficacy. However, since the sequence encoding the constant domains is fixed, tuning antibody function in hybridomas has been restricted. Here, we demonstrate a versatile CRISPR/HDR platform to rapidly engineer the constant immunoglobulin domains to obtain recombinant hybridomas which secrete antibodies in the preferred format, species and isotype. Using this platform, we obtained recombinant hybridomas secreting Fab' fragments, isotype switched chimeric antibodies, and Fc-silent mutants. These antibody products are stable, retain their antigen specificity, and display their intrinsic Fc-effector functions in vitro and in vivo. Furthermore, we can site-specifically attach cargo to these antibody products via chemo-enzymatic modification. We believe this versatile platform facilitates antibody engineering for the entire scientific community, empowering preclinical antibody research.

P115 (Session C)

The influence of membrane organisation on therapeutic target PD-L1

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Checkpoint inhibition therapy that targets PD-1 and PD-L1 has shown remarkable potential, and currently, the selection of patients is made by checking the expressions of checkpoints, such as PD-L1 on tumour cells. Nevertheless, the expression does not guarantee anti-PD-L1 treatment effectiveness, and there is a need to find new mechanisms that would predict positive treatment outcomes. An overlooked factor is the membrane organisation of PD-L1, which has never been studied before. Membrane organisation is essential for various cellular processes such as signalling, adhesion, migration and protein functionality. Organised membrane clusters of PD-L1 will serve as a hotspot for checkpoint inhibitors to bind, whereas a more dispersed PD-L1 pattern will create an overall spread checkpoint inhibitor load. Therefore, different PD-L1 membrane organisations can change the efficacy of anti-PD-L1 therapy. In this study, we investigated which mechanisms mediate PD-L1 membrane distribution on melanoma cells, focussing on the actin cytoskeleton, cholesterol-mediated membrane organisation and membrane-organising proteins called tetraspanins. By disrupting each of these levels of membrane organisation, we looked at how the PD-L1 cluster distribution changes using flow cytometry and confocal microscopy. Blocking the actin network displayed no alterations in the size of the PD-L1 clusters. However, other results suggest that actin can have a potential role in stabilising the PD-L1 clusters to a specific location on the membrane. After depleting the cholesterol from the membrane, PD-L1 clusters became smaller, showing that the treatment disrupted the organisation. This indicates that cholesterol plays an essential role in keeping PD-L1 clustered. Furthermore, we identified that tetraspanin CD9 colocalises with PD-L1 and although preliminary, the relationship between CD9 and PD-L1 could be of great interest for further research. Taken together, this study provides new fundamental insights into the mechanisms of PD-L1 membrane organisation.

P116 (Session C)

Elucidating the role of DNA polymerase theta in DNA replication stress response

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DNA polymerase-theta (Polθ) is a low fidelity class A family DNA polymerase comprising a helicase-like domain on the N-terminal and a DNA polymerase domain on the C-terminal. Adjoining the helicase-like and polymerase domain is a large central region with no predicted function. Polθ has been implicated to play a role in translesion DNA synthesis, base excision and alternative end-joining repair pathway. Retrospective clinical studies showed Polθ overexpression in breast and ovarian cancer patients is correlated with high tumor grade and poor response to chemotherapeutic drugs. Thus, multiple pharma companies are now developing potential Polθ inhibitors. However, the underlying molecular mechanism for Polθ overexpression and its association with adverse clinical outcomes in various tumors is unclear. In this study, we aim to bridge the gap between clinics and pharma groups by dissecting the molecular mechanism of action of Polθ. In order to establish a toolbox to answer these questions, we cloned and purified the full length and different domains of Xenopus laevis Polθ and generated antibody against it. Both, the purified full-length Polθ, and the polymerase domain alone were able to polymerize short DNA oligos in vitro. Our preliminary findings showed enrichment of Polθ upon aphidicolin treatment at stalled/collapsed replication forks. Immunoprecipitation of Polθ from Xenopus egg extract followed by western blot analysis further displayed the interaction between Polθ and other DNA damage response proteins. Characterization of these interactions using mass spectrometry and electron microscopy based experiments is ongoing. Advanced understanding of Polθ function and of its interacting partners will help to target breast and ovarian cancers effectively by offering combinatorial therapy alongside routine chemotherapeutic drugs.

P117 (Session C)

In vitro and in vivo models to study immune cell function in PMM2-CDG

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PMM2-CDG is a congenital disorder of glycosylation (CDG) and is caused by mutations in the gene encoding the enzyme phosphomannomutase 2, which is essential in GDP-mannose synthesis. PMM2-CDG patients display a wide variety of symptoms affecting multiple tissues. Several studies suggest immune system dysfunction in CDG patients and report recurrent infections and poor vaccine response. The molecular mechanism behind these immunological symptoms is currently unknown. Cell and animal models are required due to limited access to patient material. We aim to develop models to study immune cell function in PMM2-CDG. The GlcNAc phosphotransferase inhibitor, tunicamycin, was used to inhibit the first step in N-glycan synthesis. Four days of treatment in THP-1 cells, a human monocytic cell line, resulted in 50-80 % reduction of membrane glycosylation, slight increase of ER stress but only mildly affected cell viability. Uptake of fluorescent labelled zymosan particles was reduced in tunicamycin treated cells as observed with flow cytometry and microscopy. Interestingly, glycosylation impaired cells were still able to bind the zymosan particles.Ongoing efforts are focussed on the development of a morpholino-based PMM2-CDG zebrafish model to study phagocytosis and migration of immune cells in a living organism. Studying important immune cell functions in these models will provide further insight into the mechanisms of immune system dysfunction in PMM2-CDG.

P118 (Session C)

Elucidating the structure and molecular mechanism of Yarrowia lipolytica complex I

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The currency that drives all energy conversions in biological entities is ATP, which, in aerobic cells is predominantly generated by the oxidative phosphorylation pathway occurring in the inner membrane of mitochondria. During this process, the transfer of electrons between the complexes leads to subsequent generation of membrane potential which further drives the synthesis of ATP. Complex I, a 1 MDa multiprotein complex, is the largest and the most elusive of them all. In mammalian cells ˜90% of reducing equivalents are extracted from food sources as NADH and are fed into the respiratory chain via complex I (proton-pumping NADH:ubiquinone oxidoreductase). Hence, it is not surprising that complex I dysfunction has been implicated in numerous human pathologies, including inherited mitochondrial diseases, M. Parkinson, M. Alzheimer, ischemia/reperfusion injuries and biological ageing. Recent X-ray and cryo EM models have brought about a much-needed insight into the structural aspects of complex I. With this detailed insight into the structural aspects of Complex I, we proposed an integrated mechanistic model for catalysis and regulation of complex I. Using this hypothesis; we will elucidate the still enigmatic molecular mechanism of complex I and its poorly understood regulation by the so-called active/deactive (A/D) transition. Specifically, we will work out in detail the 'mechanics' of the conformational changes by which the charge induced reorganization of the ubiquinone binding site leads to the generation of the power stroke transmitted into the membrane arm to drive proton pumping. To this end, we will use an advanced complexomics approach and mutagenesis based structure/function analysis. Finally, we aim at understanding; the role of accessory subunits that envelop the core of complex I subunit; and how the mechanism of energy conversion of complex I is controlled by the A/D transition at the molecular level.

P119 (Session C)

Kidney Organoids form a leak-tight and polarized distal convoluted tubule-like Monolayer

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Introduction: The kidneys are essential for maintaining electrolyte homeostasis. Transport processes in the tubular system regulate electrolyte reabsorption and secretion. Defects in these transport systems, either acquired or genetic, can lead to electrolyte wasting. While various disease-causing genes have been identified, understanding of disease mechanisms is still limited. Models like kidney organoids that can be expanded directly from patient material are emerging. However, detailed knowledge on organoid function is necessary before disease modelling can be performed. Our project aims to adapt renal organoid culture to obtain functional distal convoluted tubule (DCT) epithelium. Methods: Kidney organoids were grown from primary epithelial cells of human kidney tissues (Schutgens et al., Nat. Biotechnol. 2019). They were seeded on Transwell filters, expanded to form epithelial monolayers and differentiated towards the DCT. Transepithelial resistance (TEER) was measured using Millicell-ERS-2. Expression of epithelial and DCT-specific markers was assessed in the monolayers, using immunofluorescence and quantitative reverse transcription PCR (RT-qPCR). Finally, apical-to-basolateral magnesium transport was analyzed by measuring transport of the stable isotope 25Mg with inductively coupled plasma mass spectrometry (ICP-MS). Results: The TEER increased during differentiation (1,500 ohm*cm2 day 1 to 2,400 ohm*cm2 day 4), and immunofluorescence showed expression of the lateral marker zonula occludens 1 (ZO-1) and basolateral marker Na+/K+-ATPase, suggestive of tight polarized epithelium. This is in line with previous results from carboxydichlorofluorescein diacetate (CDFDA) and inulin-FITC permeability assays. RT-qPCR of differentiated organoid monolayers demonstrated expression of DCT markers (i.e. SLC12A3, TRPV5). Moreover, organoid monolayers exhibit cellular uptake and apical-to-basolateral transport of 25Mg of respectively 5.8% and 3.7 atomic% (empty filter corrected). Conclusion: Our results demonstrate that kidney organoids form a leak-tight and polarized epithelium that expresses DCT markers and exhibits facilitated magnesium transport, a hallmark feature of the DCT. Further studies will investigate transport of other electrolytes to prepare for future disease modelling.

P120 (Session C)

Make more mitochondria: can mitogenesis attenuate hypertension?

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Introduction: Mitogenesis refers to the growth and replication of mitochondria. Recent studies suggest that drugs promoting mitogenesis often lower blood pressure, possibly providing an explanation for the link between mitochondria and hypertension.The kidney is one of the most mitochondria-rich organs in the body, and plays a major role in blood pressure regulation. We hypothesize that promoting mitogenesis would decrease sodium reabsorption to attenuate hypertension. More specifically, we suspect a role for the thiazide-sensitive sodium-chloride costransporter (NCC) in the distal convoluted tubule. Objective: To investigate the effect of altered mitogenesis on renal sodium transport and further characterize the link between mitochondrial dysfunction and hypertension in patients. Methods: Recombinant TFAM, gemfibrozil, rotenone and other drugs were used to alter mitogenesis in a DCT cell model. To measure the effect of treatment on mitochondrial mass, a citrate synthase activity assay was established in our lab and immunoblot for mitochondrial proteins. We employed a live cell-imaging technique to functionally assess NCC activity after the same treatment. In short, cells are transfected with NCC and a double fluorophore construct to ratiometrically measure iodide influx through NCC. Lastly, a retrospective study to assess the prevalence of hypertension among different mitochondrial disease patients at the Radboudumc (˜300 patients) was initiated. Results: The tested drugs did not lead to a substantial increase or decrease in citrate synthase activity or expression of mitochondrial proteins. Rotenone did not significantly influence NCC activity, although a large interassay variation was observed. Results on the retrospective study are pending. Discussion: The tested drugs did not significantly alter mitochondrial mass in our cell model. We currently aim to knock down PPARGC1A with shRNAs as an alternative. Moreover, we plan to study sodium handling by a mouse model of genetically impaired mitogenesis and find markers of impaired mitogenesis in patients with essential hypertension.

P121 (Session C)

A regulatory network of a host-derived piRNA and lncRNA acts during embryonic development of Aedes mosquitoes

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Introduction: In animals, PIWI-interacting RNAs (piRNAs) protect the germline by silencing transposons. Interestingly, Aedes mosquitoes have an expanded PIWI gene family, whose expression is extended to somatic tissues, hinting towards undiscovered roles of piRNAs in RNA-mediated gene silencing. Hence, our goal is to uncover piRNA-dependent regulatory networks in Aedes mosquitoes. Methods: PIWI protein immunoprecipitation from Aedes aegypti cells followed by sequencing of the associated small RNAs identified abundantly expressed piRNAs, among which Sid7, which associates with both Piwi4 and Piwi5. RNA sequencing on cells treated with anti-sense oligonucleotides that block Sid7-mediated gene silencing, was applied to reveal its target genes. Results: RNA sequencing revealed a limited set of Sid7 targets, among which the long non-coding RNA (lncRNA) AAEL027353. This target was confirmed by qPCR and dual luciferase assays, both upon Piwi4 and Piwi5 knockdown and treatment with Sid7 anti-sense oligonucleotides. Interestingly, small RNA sequencing and Northern Blot analyses revealed that Sid7-mediated cleavage of AAEL027353 results in the production of piRNAs from the lncRNA transcript, the most abundant of which associates to another PIWI protein, Ago3. We hypothesize that these lncRNA-derived piRNAs can engage in further gene regulatory networks. Strikingly, we discovered that Sid7 expression increased during the first hours of embryonic development, which coincided with a decreased expression of AAEL027353. Moreover, the Aedes albopictus genome appeared to have a similar Sid7 target site, suggesting that Sid7 has important functions that are maintained during evolution. Conclusion: These findings show that piRNAs are part of regulatory circuits, that are active during embryonic development. Targeting of lncRNAs can induce the formation of secondary piRNA/PIWI complexes, thereby expanding the regulatory network. The sudden increase in expression of Sid7 during development and the conservation of the lncRNA target site in Aedes albopictus, hints towards important roles in embryos of Aedes mosquitoes.

P122 (Session C)

An integrated Bioinformatics Approach reveals Pinkbar as a novel transcriptional Target of HNF1ß

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Hepatocyte nuclear factor 1 homeobox β (HNF1β) is an essential transcription factor in kidney development and function. Mutations or deletions in HNF1β cause a heterogenous phenotype including renal cysts and/or malformation, maturity-onset diabetes of the young,liver malfunction and hypomagnesemia. Cyst development has been repeatedly associated with ciliary and cell polarity defects. Here, we aim to identify new HNF1β targets that can explain the unknown mechanism of renal cyst formation in HNF1β patients.A bioinformatic approach has been applied on all available large datasets including ChiP-seq, RNA-seq and gene expression array studies to identify novel transcriptional targets of HNF1β. Differential expression analysis revealed 22 genes that were differentially expressed (fold change > 1.5) in at least three out of 8 datasets or two out of three kidney specific datasets. We pursued our analysis with the 5 most promising genes based on function and kidney expression levels and validated the candidates by qPCR analysis. Planar Intestinal- And Kidney-Specific BAR-Domain Protein(Pinkbar) showed 50% decreased expression in mouse inner medullary collecting duct-3 cells (mIMCD3) expressing dominant-negative HNF1β compared to WT cells. A luciferase reporter assay demonstrated that HNF1β activates the Pinkbar promotor. Pinkbar expression was detected in epithelial cells of the mouse kidney by immunohistochemistry. In line with other BAR-domain proteins we showed that Pinkbar is associated with the cytoskeleton in mIMCD3 cells using a cytoskeleton extraction assay. Moreover, knockdown of Pinkbar in a mIMCD3 3D spheroid model resulted in a significant 20% decrease in size of spheroids.Here, we identified a target of HNF1β, Pinkbar, that may have a role in regulating the cytoskeleton and cell polarity. This study demonstrates that an integrated analysis of multiple datasets is a promising approach for identifying novel disease targets.

P123 (Session C)

Subtyping of Immune Cells Present in the Skin and Blood after Acute Burn Injury

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Burn injury is characterized by excessive immune responses which can persist up to months and can lead to hypertrophic scarring and organ damage. A better understanding of the immune pathology is needed to improve treatment of burn injury. We hypothesize that acute phase immune cells remain proinflammatory in burns, thereby creating a chronic inflammation leading to delayed healing and scarring.Burn wound tissue (eschar) and blood from burn patients admitted to the Red Cross Hospital (Beverwijk) was used for flow cytometric analysis. Immunohistochemistry was performed using the multiplex Vectra imaging system.We developed a protocol to isolate leukocytes from eschar by enzymatic and mechanical dissociation. Preliminary analysis of blood from patients with severe burns showed a 5.9 fold increase in total leukocytes for at least three weeks. Interestingly, there seem to be some changes within the T cell population. Analyses of eschar revealed high numbers of CD10+ neutrophils, macrophages and T cells up to weeks post burn.The type of immune cells in early burn samples seems to remain skewed to an acute phase instead of a recovery state, which hampers wound healing. We will continue to explore the relation with clinical outcomes of wound healing and scar formation.

P124 (Session C)

PP2A at the Crosstalk between Tumor Cell Metabolism and the DNA damage response

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Tumor plasticity in response to metabolic, replicative and environmental stress is a topic of great interest given the current need to improve present therapies against cancer. Protein Phosphatase 2A (PP2A) is the major serine/threonine phosphatase in eukaryotic cells and it couples several processes, among which: I) metabolism, II) DNA damage response, III) nucleus mechanics. Since PP2A has tumor suppressor properties, we recently demonstrated that PP2A over-activation (through direct activation or inhibition of its suppressors) in combination with metabolic stress, led to enhanced anti-tumor effects. Metformin (a drug widely used for types II diabetes) induced the inhibition of Cancerous Inhibitor of PP2A (CIP2A). The combination of metformin with low glucose levels (in vitro) or intermittent fasting (in vivo) led to synergistic anti-neoplastic effects mediated by PP2A. Since PP2A is involved in the DNA-damage response (DDR), our goal is to over-activate PP2A to counteract cell-cycle arrest and trigger apoptosis in the presence of DNA-damaging agents.

P125 (Session C)

Intestinal absorption of natural ACE inhibitory peptides using a novel ex-vivo intestinal perfusion method (EIPM)

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The natural dipeptide isoleucine-tryptophan (IW) is a potent inhibitor of the angiotensin-converting-enzyme (ACE), which is, beside others, responsible for blood pressure control. Therefore, ACE-inhibitors are the first line strategy to treat hypertension. The in-vivo inhibition of ACE by IW is known. However, the absorption of IW is not clarified. Thus, the aim of our study was to investigate intestinal absorption of IW with a novel ex-vivo intestinal perfusion method (EIPM), which could also be used to determine absorption of substances.The EIPM is designed as an intestinal flow-through setup optimized to simulate physiologic conditions. An intestinal segment was continuously perfused with PSS and conveys substances from the input to the outflow end. The intestinal segment is submerged in a buffer bath to preserve the tissue physiology. The intestinal outflow refers to substances flowing through the intestinal lumen. The absorbed content refers to substances that get absorbed through the intestinal epithelium into the surrounding buffer bath. Before IW was analyzed, caffeine and erythrocytes were tested as positive and negative controls. In the outflow 81 $\pm$ 4% of caffeine given was quantified and 14 $\pm$ 2% was absorbed (mass balance 95 $\pm$ 6%). In contrast, 99 $\pm$ 16% of erythrocytes was recovered from the outflow and as expected nothing was absorbed. No IW was detected in the outflow or in the absorbed content. However tryptophan was quantified. This leads to the suggestion that IW was rapidly degraded and tryptophan was released as a product of the degradation.In conclusion, EIPM is a novel approach that can be used to examine the intestinal absorption of substances. The ACE-inhibitory peptide IW was not absorbed intact. This was due to the rapid degradation. This leads us to the suggestion that the absorption of IW is not through the intestines and this will help for further development of natural ACE inhibitors and its application.

P126 (Session C)

Identification of an antagonistic aptamer to target CD73 in an immunosuppressive tumor microenvironment

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CD73 or 5' ecto nucleotidase is an endonucleolytic enzyme that converts AMP into the immunosuppressive nucleoside Adenosine. CD73 is often over-expressed in a variety of tumors and in their stroma, where the accumulation of adenosine creates an immunosuppressive tumor milieu. Adenosine impairs optimal T cell differentiation, activation and effector functions, and promotes the activity of MDSCs and T Regs. Additionally, CD73 functions as an adhesion molecule that promotes metastatic dissemination. Thus, the blockade of CD73 may exhibit an anti-tumor effect by rescuing an immunosuppressive tumor microenvironment, and by suppressing metastasis.Herein, we identify and characterize candidate aptamers - single stranded RNA oligonucleotide species- that can bind to CD73 on murine mammary carcinoma cells, and can inhibit CD73 enzymatic activity in vitro. Several candidate aptamers against CD73 were selected after 6 rounds of HT SELEX (High Throughput Systematic Evolution of Ligands by EXponential enrichment), an iterative selection process that enriches the best binders against a target of interest from a library of random oligonucleotides species. Candidate aptamers are currently being studied to characterize their ability to rescue the T cell proliferation suppression in vitro, and pilot studies to assess in vivo anti-tumor activity of CD73 aptamers are being planned.

P127 (Session C)

Tumor immunosuppression by myeloid p38a signalling

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Melanoma is one of the most aggressive types of skin cancer and this is due to its high capacity to metastasize and become resistant to therapies. The current therapeutic strategy for metastatic melanoma consists in the use of immune checkpoint inhibitors (ICI), and also targeted therapies against BRAF and MEK. However, resistance to ICI is recurrent, and this points at the possible existence of an alternative immunosuppressive player. In that sense, recent data from our lab shows that the inactivation of the kinase p38a specifically in myeloid cells decreases the tumor burden in different models of lung metastasis. Preliminary analysis suggests that the effect might be mediated by the polarization towards an anti-tumoral immune response, indicating a potential key role of myeloid p38a in the establishment of tumor-associated immunosuppressive mechanisms. In this study, we propose to elucidate the role of myeloid p38a signaling in primary melanoma and lung metastasis. We hypothesize that myeloid p38a constitutes a potential target for the re-programing of the immune system in a tumor setting. The inhibition of p38a could be therapeutically useful, perhaps in combination with immunotherapies to strengthen their effect.

P128 (Session C)

The role of cell-cell adhesions in collective tumour cell invasion

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Breast cancer metastasis accounts for a substantial amount of deaths in woman all over the world. Among breast carcinomas, Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC) are the most common types. ILC is often characterized by loss of cell-cell junction molecule E-cadherin, resulting in the loss of adherens junctions that are important for collective migration. However, despite lacking adherens junctions, ILC cells mostly migrate collectively instead of as single cells, for reasons that remain poorly understood. It is known that confinement of the tumour by the extracellular matrix (ECM) influences migration mode, but how confinement impacts cell-cell adhesions in adherens junction-negative tumours is not yet known. Here, we assess the presence of alternative adhesions by knocking down junction molecules that potentially influence migration mode, by using a p120-catenin KO cell line in which adherens junctions are destabilized. Furthermore, we use the biomimetic polyisocyanopeptide (PIC) hydrogels to investigate the impact of pre-confining spheroids before migration. Lastly, we developed a method that enables us to stain spheroid cores with immunofluorescence. Our results identify several candidates that may be involved in the formation of alternative adhesions. In addition, no effect of pre-confinement was found on interphase migration in p120-positive spheroids, and we successfully stained spheroid cores with immunofluorescence. In conclusion, we found potential target molecules important for alternative adhesions and we developed a method that enables us to investigate the effect of confinement on junction localization and intensity within tumour spheroids.

P129 (Session C)

An Organotypic Model of Melanoma Disease

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Despite the efforts that have been done to address melanoma metastatic process and to discover targetable elements that drive metastasis formation, we still lack a detailed understanding of how melanoma cells interact with the microenvironment in the primary tumor and in the metastasis. A role for activate cancer associated fibroblast and endothelial cells in promoting melanoma aggressiveness has been extensively demonstrated, as well as their involvement in the alteration of antitumoral immune response. Indeed, even if melanoma is considered an immunogenic tumor, many immunomodulatory mechanisms mediate its escape from the immune system surveillance during the neoplastic process. Comprehensive knowledge of the controversial relationship between cancer cells, stroma and the immune infiltrate are required for the development of new, increasingly effective therapies. To investigate how perturbations of the tumor microenvironment (TME) influence primary tumour and metastasis formation, we propose an highly plastic 3D organotypic model of melanoma malignancy, able to mimic patients' tumor microenvironment, both in terms of cellular composition and environmental stimuli. We took advantage of human melanoma primary cultures and patient-derived xenografts previously established in our lab to reconstitute the tumor tissue with the addition of stromal component, such as fibroblasts and endothelial cells, and immune system components. We have generated 3D organoid cultures embedded in matrices, recapitulating TME diversity. These organoids will be extensively characterized and maintained in long term cultures, assessing loss of viability of the various cellular components. Primary and metastatic cells will be tested for their capacity to respond to external stimuli within the organoids. This model will allow us not only to dissect the influence of TME on primary and metastatic melanoma cells, but also to test new drugs in a system where all cell populations are modelled.

P130 (Session C)

Self-healing dental composites: Influence of microcapsule size on self-healing efficiency

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Introduction: Fracture is one of the main reasons for dental restorative composite failure. Therefore, self-healing dental composites based on microcapsules encapsulated self-healing liquids have attracted great research interests. The progression of microcracks in dental composites will lead the eruption of the microcapsules, and the self-healing liquid will be released, filled the microcracks and polymerized with the initiators in the composite matrix. In this way, self-healing composites are able to repair microcracks automatically and will prevent or postpone the fracture from the early stage. The most common processing method for microcapsules is in situ polymerization, which has been applied to prepare poly(urea-formaldehyde) (PUF) microcapsules. However, self-healing efficiency with different microcapsule size showed different results. In self-healing system the size of the microcapsules plays an important role in the self-healing mechanism. The aim of this study is to explore how different size of microcapsules affects the material mechanical properties and efficiency of self-healing dental composites. Moreover, this study will draw some inspiration to optimize the microcapsule size for self-healing dental composites.

P131 (Session C)

Uterine immune dynamics assessed from pre-pregnancy endometrium to delivery

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Introduction: Upon initiation of pregnancy, the uterine immune environment has to tolerate the semi-allogeneic fetus. Correctly functioning maternal immune cells are vital for adequate trophoblast invasion and placenta formation. Impaired induction or function of any counterpart in this tug-of-war at the fetal-maternal interface can be detrimental for mother and child, leading to pregnancy complications such as preeclampsia (PE). While in the US and Western Europe PE affects ˜5% of pregnancies, incidence is about three times higher in developing countries, and 10-25% of these PE cases lead to maternal death. We assessed when and to what extent changes in placental immune signature are induced. Here, we examined >30 different lymphocyte (sub)populations and their dynamics during gestation. Methods: Extensive flowcytometry phenotyping was performed on lymphocytes isolated from uterine mucosa throughout gestation. Pre-pregnancy endometrial lymphocytes, lymphocytes from placenta at different time points and peripheral blood mononuclear cells (non-pregnant and throughout gestation) were processed. Results: Supervised and unsupervised flow cytometry clustering strategies confirmed that NK cells were enriched in endometrium up until the 2nd trimester of pregnancy, while the percentage of T cells increased after >17wk gestation. Various subpopulations revealed gestation-dependent shifts. While previous studies based on term placentae only link B cell presence to prematurity, we observed a well-defined B cell population in 1st and 2nd trimester placentae of healthy pregnancy. Especially CD24HiCD38Hi B cells were increased in 2nd trimester. IL-10 production by placental B cells was increased compared to PBMC indicating that the prominent B cell subpopulations have regulatory capacities. Conclusion: Understanding of uterine immunity is key to develop better early detection and treatment options for pregnancy complications like PE. Contrary to a common believe that placental B cells are almost absent or only present in pathological conditions, our results highlight possible functional implications in early to mid gestation.

P132 (Session C)

Multi-drug antibiotic chemotherapy: More may not always be better

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Non-tuberculous mycobacteria are environmental, opportunistic pathogens that can cause deadly pulmonary disease. Mycobacterium avium (M. avium) is the most common causative strain. The current recommended treatment regimen against M. avium is a combination of at least three drugs (rifampicin - ethambutol- azithromycin) for at least 1.5 years, to which in severe cases, up to two additional drugs (amikacin - clofazimine) are added, forming a 5 drug regimen. Despite the high drug load, cure rates range only from 50 to 70%. Preclinical evidence for this regimen is largely absent. This treatment is not only associated with severe side-effects, it is also unknown if these drugs interact synergistically or not.To assess if the standard regimen has synergistic drug-drug interactions in vitro, and to possibly identify a more synergistic multi-drug regimen, we performed time-kill kinetics experiments of the single drugs as well as all two- three- four- and five- drug combinations, monitoring the bacterial load of M. avium over the course of 28 days. Using Bliss-independence calculations, based on maximum drug effect size, we assessed if the recommended drug regimen, and all clinically feasible combinations in between, are synergistic or not. We also investigated minocycline as an experimental compound as a prospective substitute for especially toxic drugs in this regimen.Based on the bacterial load in the time-kill kinetics experiments, the addition of another drug to the multidrug regimen does not always lead to increased killing, especially when 3 or more drugs are already used. We find that two-drug interactions between amikacin and other drugs tend to be antagonistic, while combinations with clarithromycin tend to be very synergistic relative to Bliss independence. These data emphasize the need for evidence-based regimen design to formulate a more effective treatment regimen against M. avium pulmonary disease.

P133 (Session C)

Podocytes count! Treatment response in patients with primary nephrotic syndrome

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Primary nephrotic syndrome (PNS) includes a spectrum of kidney diseases that are characterized by massive proteinuria and severe edema. Clinical course of untreated patients with PNS is highly variable. Some patients enter proteinuria remission with symptomatic treatment, but many others experience a relapsing and progressive disease course that will ultimately lead to end-stage renal disease. Complete proteinuria remission is a strong predictor of long-term preserved renal function, and partial remission is associated with improved renal survival.Accumulating evidence suggests that podocytes are the primary target of injury in the pathogenesis of PNS. Podocytes are terminally differentiated cells of the kidney glomerulus that wrap around capillaries. According to the podocyte depletion hypothesis, significant loss of podocytes leads to glomerular scarring (glomerulosclerosis). Progressive podocyte depletion and glomerulosclerosis will ultimately result in end-stage renal disease. Second, while the mechanisms underlying glucocorticoid response have traditionally been linked to immunosuppressive effects, recent studies have demonstrated that glucocorticoids act directly on glomerular cells. In experimental models of glomerular injury, glucocorticoid receptor signaling (GR) in podocytes and parietal epithelial cells (PEC) mediated direct protective effects of glucocorticoids.Therefore, we hypothesize that proteinuria outcome of PNS depends on podocyte depletion and GR expression in glomerular epithelial cells. The aim of this project is to evaluate the extent of podocyte depletion and podocyte/PEC GR expression as predictors of treatment response. To accomplish this aim we will address the following key objectives:I. Set up and optimize novel methods to measure:a) Glomerular podocyte density, glomerular volume, and glomerular podocyte number in kidney biopsy sections;b) Podocyte and PEC-specific GR expression in kidney biopsy sections;c) Podocyte-specific mRNA concentrations in urine of patients with PNS.

P134 (Session C)

Development of iPSCs-derived kidney organoid monolayers as a model to evaluate electrolyte transport

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Introduction: Maintenance of the electrolyte balance by the kidney is essential for regulating key physiological processes. The kidney contains ˜1 million nephrons that are responsible for blood filtration and electrolyte reabsorption and secretion. The lack of reliable models reproducing the complex in vivo situation represents a limit to study molecular mechanisms of electrolyte disorders. The past decade has witnessed an increasing interest in kidney organoids, which are multicellular self-organizing 'mini kidneys'. While they represent a promising model to study renal (patho)physiology, little is known about their electrolyte transport properties. Objective: We aim to study transport properties in induced pluripotent stem cells (iPSCs)-derived kidney organoids, by dissociating and growing them into epithelial monolayers. Methods: Nephron segmentation in kidney organoids was assessed at different stages of development using quantitative real-time PCR (RT-qPCR). Additionally, organoid dissociation into single cells was optimized using enzymatic and mechanical approaches. The cells were seeded on polyester and polycarbonate membrane transwells and cultured in APEL II and Basal Renal Epithelial Cell Growth Medium. Furthermore, organoid cryo-sections were immunostained using lectins Peanut Agglutinin (PNA) and Wheat Germ Agglutinin (WGA). Results: RT-qPCR analysis demonstrated the expression of several segment-specific markers, with an increased expression of genes associated with tubule maturity over time. Moreover, we showed that all dissociation protocols resulted in single cell suspensions with similar cell viability and ability to grow on polyester and polycarbonate membrane transwells. Immunostaining of kidney organoid sections demonstrated localization of the lectin PNA, and not WGA, at the distal convoluted tubule (DCT). Conclusion: iPSC-derived kidney organoids contain various nephron segments and can be dissociated into viable single cells. We identified PNA as potential lectin marker for the DCT. This can ultimately be used for fluorescence-activated sorting of DCT cells from dissociated kidney organoids, in order to obtain epithelial monolayers suitable for electrolyte transport analysis.

P135 (Session C)

Anti-glycan antibodies in colorectal cancer diagnosis

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Due to the asymptomatic progression of colorectal cancer (CRC) on early stages, the disease is detected too late to be successfully treated, so the problem of determining CRC in the initial phases with minimally invasive methods is currently extremely urgent. One of the methods for tumor detection is the determination of serological molecular markers. It is known that the glycosylation pattern of the cells changes during malignant transformation. In such events, tumor-associated glycans can be recognized by anti-glycan antibodies, which can be detected in the circulation in the early stages of cancer.We developed a hydrogel microarray-based test system for simultaneous determination IgG and IgM antibodies to 51 glycans in serum samples of 44 CRC patients with stages I-III of the disease and 53 healthy donors. ROC Analysis, logistic regression, Kruskal-Wallis test, and calculation of 95% CI were carried out using MedCalc 14.8.1 software. The differences were assumed to be significant at $p<0.05$.We discovered that the level of IgG antibodies to glycan 3'O-su-Lea allows identifying CRC patients with a sensitivity of 70% and specificity of 63%. The percentage of correctly predicted cases of CRC using a combination of two markers, IgM antibodies against 3'sialyl-TF and 3'O-su-Lea, was 75%. Along with that, the level of IgM antibodies to glycan 3'O-su-Lea was significantly different in patients with regional metastases compared to patients without them. The levels of antibodies of the M or G isotypes significantly differed patients with different tumor localizations and different tumor grades.As a result, the levels of IgM and IgG antibodies to the glycans in different patient populations were simultaneously determined, and statistically significant markers and their combinations were found to diagnose the disease and to possible control the therapy.

P136 (Session C)

Systemic effects of CIN tumor development – The ultimate evidence that size does not matter

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Genomic instability is a striking feature found in human cancers that results in multiple problems: from structural changes at the sub-chromosomal level until the loss or gain of entire chromosomes (Clemente-Ruiz et al; 2014; Dekanty et al.; 2013) . Chromosomal instability (CIN) is a form of genomic instability found in 80% of solid tumors and associated with poor treatment response in multiple cancer types, such as lung, breast and colon cancer.In a CIN scenario, the JNK stress-responsive signaling pathway plays a major role in promoting the removal of aneuploid cells and in generating compensatory proliferation by the induction of mitogenic molecules. Nonetheless, JNK becomes pro-tumorigenic when aneuploid cells are not successfully removed by apoptosis, and the chronic expression of the mitogenic molecules eventually leads to tumor growth and tissue invasiveness (Clemente-Ruiz et al; 2014; Dekanty et al.; 2013; Giam et al; 2012). JNK signaling also drives the expression of systemic signaling molecules. These molecules are produced by the growing tumor and can travel the host organism producing multiple alterations in different tissues.Using the Drosophila melanogaster fly model, we can thoroughly analyze these long-range signaling molecules and provide information about their systemic impact and their main targets. Specifically, we can elucidate the key players responsible for the alterations in developmental timing generated by the absence of the major steroid hormone produced in the brain that coordinates developmental transitions. Moreover, we can also describe the metabolic state and inflammation processes that are happening to the tumor host, providing evidence about the multiple mechanisms that might be affected during CIN tumor development. Our study aims to explain how the inter-talks between tumor and host happen, since understanding such interaction might influence the outcome of certain treatments or the re-incidence of the disease.

P137 (Session C)

Hematopoietic Progenitor Cell-Derived BDCA3+ Myeloid Dendritic Cells Efficiently Cross-Present Tumor Antigens and Boost Tumor-Reactive T and NK Cell Responses

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Allogeneic stem cell transplantation (alloSCT) can be curative for hematological cancer patients. The therapeutic graft-versus-tumor (GVT) effect is mediated by donor-derived T and NK cells. However, relapse remains the major cause of treatment failure, illustrating the need for adjuvant immunotherapy to boost anti-tumor immunity. In this respect, vaccination with naturally occurring dendritic cell (DC) subsets is highly attractive, harnessing their distinct and unique biological functions. An interesting DC subset is the BDCA3 myeloid DC (mDC), because of the superior capacity to cross-present tumor antigens. We recently developed a 14-day culture protocol to generate high numbers of BDCA3 mDCs from hematopoietic progenitor cells (HPCs) of alloSCT donors. The aim of this study was to investigate whether HPC-BDCA3 mDCs possess true BDCA3 mDC features and can prime and boost tumor-reactive T and NK cells. We reproducibly obtain up to 7.5x106 HPC-BDCA3 mDCs from 1x106 HPCs. Compared with their blood counterparts, HPC-BDCA3 mDCs highly expressed the BDCA3mDC restricted transcription factor BATF3, upon TLR stimulation potently upregulated co-stimulatory molecules CD80, CD83, CD86 and secreted pro-inflammatory cytokines IL-12 and TNF-a. Furthermore, they migrated efficiently towards lymph node homing chemokine CCL21, prerequisite for proper anti-tumor T cell activation. Furthermore, these mature HPC-BDCA3 mDCs induced alloreactive T cell expansion and IFN-? secretion. Moreover, HPC-BDCA3 mDCs efficiently cross-presented HA-1 long peptide resulting in profound activation of HA-1 specific T cells. Moreover, they boosted the expansion of patient-derived HA-1 specific T cells. Additionally, HPC-BDCA3 mDCs enhanced cytotoxic properties of tumor-reactive NK cells illustrated by upregulation of CD69 and TRAIL and efficient killing of THP-1 AML cells. Together, these data demonstrate that HPC-BDCA3 mDCs are highly capable of harnessing both innate and adaptive anti-tumor immunity. This makes them highly attractive means to further boost GVT immunity and induce long lasting tumor control.

P138 (Session C)

Cationic liposomes: a flexible vaccine delivery system for physicochemically diverse antigenic peptides

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Purpose: Personalized peptide-based cancer vaccines will be composed of multiple patient specific synthetic long peptides (SLPs) which may have various physicochemical properties. To formulate such SLPs, a flexible vaccine delivery system is required. We studied whether cationic liposomes are suitable for this purpose. Methods: Fifteen SIINFEKL T-cell epitope-containing SLPs, widely differing in hydrophobicity and isoelectric point, were separately loaded in cationic liposomes via the dehydration-rehydration method. Particle size and polydispersity index (PDI) were measured via dynamic light scattering (DLS), and zeta potential with laser Doppler electrophoresis. Peptide loading was fluorescently determined and the immunogenicity of the formulated peptides was assessed in co-cultures of dendritic cells (DCs) and CD8+ T-cells in vitro. Results: All SLPs were loaded in cationic liposomes by using three different loading method variants, depending on the SLP characteristics. The fifteen liposomal formulations had a comparable size (< 200 nm), PDI (< 0.3) and zeta potential (22 - 30 mV). Cationic liposomes efficiently delivered the SLPs to DCs that subsequently activated SIINFEKL-specific CD8+ T-cells, indicating retained immunological activity of the SLPs. Conclusion: Cationic liposomes can accommodate a wide range of different SLPs and are therefore a potential delivery platform for personalized cancer vaccines.

P139 (Session C)

Patients' and healthcare professionals' experiences of a joint Morbidity and Mortality meeting.

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Background: Patient participation in healthcare is seen as a way to better meet patient's needs, preferences and values and so improve the quality of care. However, it is also a challenge of today's healthcare, particularly at surgical departments. There, Morbidity and Mortality meetings (M&MM) need quality improvement to better share learning points in the organisation and achieve practice change. This might be possible by patient involvement. A gynaecological oncology department invited patients to join their own M&MM and evaluated which factors and conditions, and similarities and differences, from both patients' and healthcare professionals' perspectives, were important to ensure a safe environment and contribute to the goals of the M&MM. Methods: Qualitative research was performed with semi-structured, in-depth, interviews including patients and professionals who attended M&MMs in the period of 2016 - 2018. Two interview guides were developed, focusing on their experiences and practical elements of the meeting. The interviews were transcribed, coded and analysed by thematic content analysis. Results: Eight patients and seventeen healthcare professionals (nurses, registrars, consultants) participated in the study. The most significant themes in the domain of interpersonal dynamics, were trust and safety for patients, notions of quality for healthcare professionals, the patient-doctor relationship, language, openness of communication and learning for both patients and healthcare professionals. All participants suggested to maintain the current practical design of the meeting, especially the support from the case manager prior, during and after the meeting and to keep inviting all involved healthcare professionals. Conclusion: Both patients and healthcare professionals valued the M&MM with patient participation. Its new design can be organised within a safe environment when conditions such as a stable patient-doctor relationship are adhered. It also provided more opportunities to learn from the meeting in which healthcare professionals felt more comfortable over time.

P141 (Session C)

A rapid DNA-test for early detection of bloodstream infections in intestinal failure patients

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Objective: To compare the diagnostic accuracy of the droplet digital Rapid Sepsis Test (ddRST) with the mostly used molecular test (SeptiFast) for rapid detection of bloodstream infections in intestinal failure (IF) patients. Blood culture results combined with clinical data will be used as gold standard. Background: IF patients depend on life-long home parenteral nutrition, a complex treatment that centers on management of their central venous catheter to prevent the most daunting complication: catheter-related bloodstream infection. Use of molecular techniques holds promise to improve and speed up bloodstream infection diagnostics as compared to the traditional culturing of pathogens, since several assays have become available for rapid detection of pathogens in whole blood. The SeptiFast has a good specificity (85%), but moderate sensitivity (65%). The latter severely limits its use in a clinical setting. The ddRST is a novel culture-independent molecular test that has been developed to improve sensitivity of pathogen detection in whole-blood. Methods: This study concerns a prospective two-year single-blind cohort study in which 125 IF patients presenting to the Radboudumc with a suspected diagnosis of bloodstream infection will be included. First, blood cultures will be collected according to current standard care procedures. Next, 10 mL blood samples will be collected (no additional punctures needed) and split for ddRST and SeptiFast analyses. Two blinded research analysts will analyze the samples. After adequate treatment, patients will be followed for three months. Primary outcome is the difference in sensitivity between ddRST and SeptiFast to detect pathogens in blood (identical to results from the gold standard). Secondary outcomes include test characteristics (e.g. specificity), diagnostic accuracy of ddRST and SeptiFast in patients who received antibiotics within 3 and 7 days before presentation, and for detecting Gram-positive/Gram-negative bacteria and fungi. Anticipated results: We anticipate that ddRST has a higher sensitivity when compared to SeptiFast.

P142 (Session C)

The Significance of Tumour Deposits in Rectal Cancer Following neo adjuvant therapy: A systematic review and meta-analysis

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Background: tumour deposits (TD) are a poor prognostic markerin colorectal cancer but their significance following neoadjuvantchemoradiotherapy is less certain as this group of patients is excludedin most studies. Post-treatment TD might even be a sign of tumourresponse. No previous reviews have assessed outcomes in this group. Materials and Methods: A systematic review and meta-analysis wasundertaken according to PRISMA guidelines in order to determine therelevance of post-treatment TD. Inclusion criteria were studies assessing TD in patients who had undergone preoperative treatment with radiotherapyand/or chemotherapy and reporting prevalence and survival outcomes. Studies that did not include histological revision of cases were excluded. Results: Eight studies and 1283 patients were included in the review. Prevalence of TD varied from 11.8%-44.2% (mean 23.7%), similar tountreated patients. Presence of TD after chemoradiotherapy was associatedwith invasion depth, lymph node involvement, perineural invasion andsynchronous metastases. The pooled hazard ratio for 5 year adversedisease-free survival was 2.3 (95% CI 1.8-2.9) and 2.5 (95% CI 1.9-3.3) for overall survival. One study showed a survival benefit with adjuvanttherapy in the TD positive group. Conclusions: In analogy with untreated patients, the presence of TD inpatients with rectal cancer after neoadjuvant treatment is associated with advanced disease and a poor outcome.

P143 (Session C)

Joint complaints in IBD: a single-center, prospective observationallongitudinal cohort study

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Rationale: Articular involvement is the most common extra-intestinal manifestation in inflammatory bowel diseases including M. Crohn and ulcerative colitis and could be inflammatory (axial or peripheral arthritis) or non-inflammatory. Joint complaints have a major impact on quality of life. However, there is no standardized care or therapy for joint complaints in IBD-patients. Especially joint pain without inflammation is a common problem in IBD patients. Insights in pathogenesis, prevalence and therapy-luxated arthritis and non-inflammatory joint pain are warranted. Aim: To identify predictors of inflammatory and non-inflammatory joint complaints in IBD-patientsTo determine the impact of articular involvement in IBD on the quality of life and patients' experiences on the quality of care.To gain insights in pathomechanisms and therapy-specific impact on the development of arthritis and arthralgia in IBD. Study design: A single-center, prospective observational longitudinal cohort study. Study population: All IBD-patients of 16 years or older will be approached during outpatient visitMethods: As a part of standard care, all IBD-patients visiting the outpatient clinic will be asked for the presence of joint complaints. Every participant will be asked to fill out 3 questionnaires via IBDream. Blood samples will be stored for later analysis, in line with the agreement in the protocol Biobank IBDream. If there is articular involvement, these complaints will further be explored giving optimal standard care. Differences in quality of life between IBD-patients with and without joint complaints will be compared together with possible predictors/ risk factors for developing joint complaints. The follow-up time will be 1 year.

P144 (Session C)

Role of quiescence in Acute Myeloid Leukemia growth

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Acute myeloid leukemia (AML) is an aggressive hematological disease characterized by a malignant proliferation of hematopoietic myeloid progenitor cells. AML is the most common acute leukemia and its prognosis is poor. One of the major causes of therapy failure and leukemia relapse is the genomic and biological heterogeneity of the tumor. The genomic complexity depends on the presence of multiple subclones within the leukemic population, while at biological level AML is hierarchical organized with leukemia stem cells (LSCs) at the apex. LSCs are a rare cell population able to initiate and sustain the tumor growth and share many features with hematopoietic stem cells (HSCs), including self-renewal capacity and quiescence. Traditional therapies, which target only actively cycling cells, have limited effects on LSCs, mainly due to their quiescent state. A series of data obtained in our group suggested that the ability to enforce quiescence in HSCs is a common feature of different leukemia-initiating oncogenes (NPMc+, PML/RARa, AML1/ETO, MLL/AF9). Therefore, deeper characterization of the molecular mechanisms underlying oncogene-induced quiescence will shed light on key pathways in AML development, maintenance and response to therapies. To examine the relevance of 100 quiescence-related genes in AML, we exploited RNA interference technology to perform an in vivo screening. Among the genes found depleted during the screening, there were Socs2, Stat1, Sytl4. Silencing of those genes in AML blasts was sufficient to decrease in vitro self-renewal and delay leukemia growth in vivo. Moreover, preliminary data shown that the quiescence state is not confined to LSCs, but the whole leukemic blasts population progressively enters in a quiescent state required for AML growth. We are currently evaluating, through single cell RNAseq analysis, the molecular mechanisms underlying this peculiar behavior to find therapeutic interventions interfering with the establishment of the quiescence phenotype and eventually with AML growth.

P145 (Session C)

A poly-neoantigen DNA vaccine synergizes with PD-1 blockade to induce T cell-mediated tumor control

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Neoantigen vaccines require a flexible system which allows incorporation of multiple heterogenous sequences and can be readily produced in a patient-tailored fashion. Several synthetic peptide- and mRNA-based formulations have been explored to achieve this. Here, we developed and tested a DNA carrier that contains multiple independent neo-epitope coding sequences in one formulation. DNA represents an ideal platform for neoantigen vaccination as production and its sequence can be easily manipulated. The construct is composed of five mini-genes encoding only for the amino acid area harboring T cell epitopes. First, the vector was tested for its ability to generate the expected epitopes and induce functional T cell responses to the reporter ovalbumin CTL and T helper epitopes in mice. Next, CD8 T cell responses were detected to three neo-epitopes of the mouse colorectal tumor MC38. Finally, we evaluated the effectiveness of this vector as anti-tumor vaccine. The vector was functional as a prophylactic vaccine for B16 melanoma expressing ovalbumin. Furthermore, therapeutic vaccination successfully contributed to MC38 tumor control and cure of 25% of the mice when combined with anti-PD1. These data demonstrate the potential of DNA vaccination to target multiple neoepitopes in a single formulation and highlight the cooperation between vaccine-based and checkpoint blockade immunotherapies for successful eradication of established tumors.

P146 (Session C)

Unraveling the potential of muscle stem cells for a therapy in myotonic dystrophy

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We are exploring the possibility of a cell-based therapy to combat muscular dystrophy in patients with myotonic dystrophy type 1. Accordingly, we have successfully isolated a distinct class of myogenic progenitor cells, called pericytes, from skeletal muscle of patients and transgenic mice with the disease.Myotonic dystrophy is caused by an expanded (CTG•CAG)n repeat in the DMPK/DM1-AS gene pair. Transcription of the repeat produces expanded repeat-containing transcripts that deplete RNA-binding proteins and produce toxic homopolymeric proteins. Ultimately, expression of these pathogenic gene products results in a severe myopathy, characterized by muscle weakness and wasting.Muscle biopsies from mice and patients with myotonic dystrophy were cultured under conditions to promote outgrowth of pericytes, the stem cells wrapped around blood vessels. Efficient isolation of ALP+/CD31- pericytes was confirmed by RT-PCR analysis and immunocytochemistry. No differences in the number of pericytes, nor in the proliferation capacity between cells from patients and controls were found. Expression of pathogenic expanded DMPK RNA was visible as typical FISH-detectable foci in cell nuclei. Interestingly, despite the presence of disease biomarkers, patient pericytes maintained myogenic potential and differentiated efficiently into multinucleated myotubes in vitro.We will now work towards autologous and possibly CRISPR/Cas-corrected pericytes that can systemically be applied, renew the progenitor cell pool and fuse with existing myotubes to form regenerating fibers, hereby relieving muscle symptoms in patients with this devastating disease.

P147 (Session C)

Superior effectiveness outcomes for ustekinumab- compared to vedolizumab- treated Crohn's disease patients with prior failure to anti-TNF: a comparative effectiveness study from the Dutch ICC Registry

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Background and aim: The anti-adhesion antibody vedolizumab and the interleukin 12/23 inhibitor ustekinumab can both be considered for the treatment of Crohn's disease (CD) when anti-TNF treatments fail. However, head-to-head trials are not available and methodological differences limit the comparison of the registration studies. We therefore aimed to compare vedolizumab and ustekinumab for CD patients who failed anti-TNF treatment using a quasi-experimental design in a prospective registry specifically designed for comparative studies. Methods: CD patients who failed anti-TNF treatment and started vedolizumab or ustekinumab in standard care as second-line biological were identified in the observational ICC Registry. Corticosteroid-free clinical remission (Harvey Bradshaw Index ≤ 4), biochemical remission (CRP ≤ 5 mg/L, fecal calprotectin ≤ 250 µg/g), combined endpoint remission (corticosteroid-free clinical and biochemical remission), and safety were compared after 52 weeks of treatment. To adjust for confounding and selection bias, we performed two types of analyses: multiple logistic regression and propensity score matching. Results: 128 vedolizumab- and 85 ustekinumab-treated patients fulfilled the study criteria. Ustekinumab-treated patients were more likely to achieve corticosteroid-free clinical remission (OR: 2.56, 95% CI: 1.35-4.87, p=0.004), biochemical remission (OR: 2.22, 95% CI: 1.04-4.74, p=0.040), and combined endpoint remission (OR: 2.58, 95% CI: 1.15-5.78, p=0.022), while the safety outcomes (infections: OR: 1.26, 95% CI: 0.63-2.54, p=0.517, adverse events: OR: 1.33, 95% CI: 0.62-2.81, p=0.464, hospitalizations: OR: 0.67, 95% CI: 0.32-1.39, p=0.282) were comparable. Conclusion: The effectiveness of ustekinumab was superior to vedolizumab while the safety outcomes were comparable after 52 weeks of treatment in this quasi-experimental study of CD patients with prior failure to anti-TNF treatment.

P148 (Session C)

Highly sulfated chondroitin Sulfate as a new Class of Biomarker for ovarian Cancer with Potential for early Diagnostics

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Background: Epithelial ovarian cancer is the fifth leading cause of female cancer death worldwide. Due to the absence of clear symptoms at an early stage of the disease and the lack of adequate screening methods, over 70% of ovarian cancer patients are diagnosed with advanced stages associated with a poor 5-year survival. Clearly, there is an urgent need for new biomarkers for ovarian cancer. Traditionally, the search for specific biomarkers has focused on the tumor cell itself. In this study we will focus on the tumor's microenvironment, the Extracellular-matrix (ECM). The ECM is a highly organized three-dimensional structure which maintains tissue integrity and is actively involved in many physiological and pathological processes. Recent observations indicate that one specific type of polysaccharides, a highly sulfated chondroitin sulfate (CSE), is specifically upregulated in malignant ovarian tumors. In this study focus will be on CSE and its use a biomarker for ovarian cancer. Study approach and Methods: Primary ovarian tumors and metastases will be evaluated for the presence and localization of CSE. Specimens (embedded in paraffin) will be obtained. Four subtypes of ovarian cancer will be examined; serous, endometrioid, clear cell and mucinous type. Benign ovarian tumors and normal ovaries will be included as controls. CSE will be analyzed using phage display-derived antibodies and which specifically recognize CSE motives (antibodies GD3G7). Immunostainings will be semi-quantitatively scored on basis of the proportion of the tumor stained. We recently found that overexpression of CSE is already present at an early stage. This, combined with the notion that CSE is present in the blood and/or urine, opens the possibility to evaluate if CSE is present in serum/urine of patients with (early) ovarian cancer and comparisons between the semi-quantification of CSE in tumors and the levels of CSE in serum/urine will be made.

P149 (Session C)

A Colorectal Carcinoma in 3D: Merging Knife-Edge Scanning Microscopy and Deep Learning

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Background & objectives: A three-dimensional visualization of a human carcinoma could provide invaluable diagnostic information and redefine how we perceive and analyze cancer invasion. As deep learning begins automating the diagnostic workflow and cutting-edge microscopy provides unprecedented ways of visualizing tissue, combining these methodologies could provide novel insight into malignant tumors and other pathologic entities. By combining Knife-Edge Scanning Microscopy with convolutional neural networks, we set out to visualize an entire three-dimensional colorectal carcinoma segmented into specific tissue classifications. Methods: A Knife-Edge Scanning Microscope (KESM), developed by Strateos (San Francisco, CA, USA), was used to digitize a whole-mount, H&E stained, formalin-fixed paraffin-embedded human tissue specimen obtained from the Radboudumc (Nijmegen, Netherlands). Sparse manual annotations of 5 tissue types (tumor, stroma, muscle, healthy glands, background) were provided using KESM data to train a convolutional neural network developed by the Computational Pathology Group (Radboudumc) for semantic segmentation of the colorectal carcinoma tissue. A three-dimensional visualization was generated using 3Scan's proprietary visualization pipeline. Results: The convolutional neural network was used to process roughly 1200 slices of KESM data. The stitched and rendered segmentation maps demonstrate the formalin-fixed paraffin-embedded carcinoma of approximately 5 millimeters in depth. As shown in the figure, the tumor invasive margin can be seen advancing into the surrounding tumor stroma. Conclusion: Based on our findings, we were capable of training a segmentation model on the 3D KESM data to create an accurate representation of an entire formalin-fixed paraffin-embedded colorectal carcinoma tissue block segmented into five tissue classifications. Going forward, this can have much broader implications on the research and understanding of invasive tumors.

P150 (Session C)

The putative role of the Gypsy68 gag protein in piRNA pathway inhibition

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The PIWI interacting (pi)RNA pathway is an important small RNA pathway involved in the silencing of transposons in the germline of animals. Aedes aegypti, the mosquito transmitting viruses such as dengue and Zika virus, express the PIWI proteins Piwi4-6 and Ago3 not only in the germline, but also in somatic cells. Here, piRNAs do not only repress transposons, but are also involved in antiviral defense. Immunoprecipitation of somatically expressed PIWI-proteins in A. aegypti cells followed by mass spectrometry, revealed that peptides from the gag fragment of the gypsy68 transposon are strongly and significantly over-represented in Piwi4-pulldown. Therefore we hypothesized that this transposon might inhibit the piRNA pathway to alleviate its own repression. In this study we aimed to investigate this putative inhibitory effect of the gypsy68 gag protein on the piRNA pathway in mosquitos. To this aim, we overexpressed various parts of the gypsy68 gag region and performed knockdown experiments targeting transposon genes. Subsequently, we assessed the effect on the piRNA pathway by measuring viral piRNA biogenesis, the expression of endogenous piRNA targets and the activity of a piRNA repression reporter system. Although overexpression and knockdown of the transposon were successful, neither of these had any significant or consistent effect on piRNA biogenesis, expression of endogenous targets or reporter activity. Therefore, we have found no evidence that the gypsy68 gag protein modulates the activity of the piRNA pathway.

P151 (Session D)

Evaluation of defective Splicing in Patients with Mutations in Pax6 Gene

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The PAX6 gene belongs to a family of genes that play a critical role during oculogenesis and other developmental processes. Mutations in PAX6 have been found to cause aniridia, which is an absence of the coloured part of the eye (the iris). Patients with aniridia spectrum may experience severe visual impairment or even total blindness.We have performed direct sequencing of PAX6 gene (NM_000280) in two families with aniridia. In silico analysis was used to predict the effect of identified rare variants on splicing pre-mRNA by Human Splicing Finder, NNSPLICE, NetGene2 and MaxEntScan. Pathogenicity of splicing variants was further experimentally confirmed by exon-trapping assay using pET01 vector in HEK293T human cell lines. Direct sequencing was also used for segregation analysis within the families.Two intronic variants located in canonical splice sites were identified; c.1032+1G>A and c.1183+1G>T. Both variants were evaluated by prediction tools as pathogenic for loss of splice site and subjected to the exon-trapping assay that further showed skipping of exon 11 and 12, respectively. Mutations segregated with disease in other affected family members and were not present in two unaffected first-degree relatives that approved pathogenicity of detected variants.Loss of visual functions in aniridia is often very severe, therefore establishing molecular diagnosis highly impacts patient management, enabling prenatal and preimplantation diagnostics. The functional analysis of defective splicing can be challenging in case of genes, which are expressed in poorly amenable tissues. Exon-trapping assay represents effective tool for analysis splicing abnormalities this group of genes. Supported by SVV 260367/2017 and GAUK 250713/82318-2018

P152 (Session D)

Tracing the evolution of two transmissible cancers in Tasmanian devils

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Transmissible cancers are clonal lineages that spread through populations via contagious cancer cells. Such epidemics have only ever been observed to affect two mammals: dogs and Tasmanian devils (Sarcophilus harrisii). While a single canine transmissible venereal tumour (CTVT) has survived for several thousand years, two distinct clones known as devil facial tumour 1 and 2 (DFT1, DFT2) have only emerged within the past two decades. As a consequence of these aggressive infectious cancers and their rapid spread across Tasmania, world's largest carnivorous marsupials have been undergoing substantial population declines. Using whole genome sequencing, drug screening and cytogenetics, we have recently provided a first genetic and functional comparison of these two transmissible Tasmanian devil cancer lineages. Our results suggest that both DFT1 and DFT2 emerged from similar Schwann cell related progenitors in two - hitherto - genetically unremarkable devils, e.g. analyses of the sex chromosomes show that DFT1 arose in a female devil, whereas DFT2 first arose in a male. By deep sequencing of whole genomes from tumour cohorts of DFT1 and DFT2 infected devils, we are now starting to shed light on the evolutionary trajectories of both lineages. Their somatic phylogenies allow for a detailed longitudinal comparison of inter and intra-specific diversification, mutation rates, clonal selection and immunological adaptation events. Additionally, we integrate long nanopore DNA sequencing reads (up to 1.85 Mbp) to improve the existing Tasmanian devil reference and to resolve two transmissible cancer genomes at unprecedented detail.

P153 (Session D)

Characterisation of PDE6D RAB28 protein network and its connection with glucose metabolism

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Objective: The light sensing outer segment of photoreceptors (PRs) requires renewal every ten days due to its high photoactivity, especially of the cones during daytime vision. This turnover of biosynthetic compounds (membranes, proteins) as well as a lot of energydemands a high . Therefore, PRs are highly dependent on converting glucose into metabolites by aerobic glycolysis. Furthermore, a refined proteostasis network (PN) is crucial for PR viability. The PN and glucose metabolism are both regulated by several intimately intertwined pathways, but details of these pathways in PRs remain largely unknown. Recently, it has been shown that the GTPase RAB28 is involved in GLUT4 trafficking in muscle cells and adipose tissue. Interestingly, RAB28 is the first RAB associated with inherited retinal blindness (autosomal recessive cone-rod dystrophy, CORD18). Furthermore, it has been shown that PDE6D, a protein facilitating intracellular trafficking of prenylated proteins in PRs, interacts with RAB28. Here, we investigated the protein interactome of PDE6D and RAB28 to identify their association with glucose metabolism. Methods: We used tandem affinity purification (TAP) of the proteins of interest, which were transiently expressed in HEK293T cells, to co-purify their associated interactomes. The co- precipitated proteins were subsequently analyzed by mass spectrometry (MS). Results: Our preliminary results show the identification of all the known interactors of PDE6D, including ARL3, INPP5E, RPGR and RAB28. Interestingly, we also found a group of newly identified interactors of PDE6D that belong to the transducin family, which are essential for phototransduction. Moreover, several interactors of the Rab28 TAP are involved in energy/glucose metabolism. This connection to the energy/glucose metabolism is confirmed by our interaction screen of GLUT4 identifying several cilia-gold-standard proteins and proteins associated to ciliopathies, including RAB28. Conclusion: Our results show a preliminary association between PR/cilia specific proteins and glucose metabolism. Unraveling PR/cilium specific networks that associate with glucose metabolism might reveal targets for broadly applicable treatments of inherited retinal diseases.

P155 (Session D)

Whole exome sequencing reveals MNS1 and DNAH9 as novel laterality defect genes in humans

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Introduction: Laterality defects are rare developmental disorders, occurring isolated or as part of more complex syndromes. Underlying cause is most frequently dysfunction of motile cilia causing Primary Ciliary Dyskinesia (PCD), and more rarely, non-motile cilia defects or non-ciliary causes. In addition to randomization of the left right body axis, PCD is characterized by frequent respiratory infections and infertility. Mutations in known genes explain approximately 50%-70% of all cases. Objective: To study the molecular mechanism underlying the laterality disorders.Methods: We used Whole Exome Sequencing (WES) to delineate the underlying molecular cause in 42 mainly consanguineous families with laterality defects. Results: We identified causative variants in approximately 30% of all cases with majority of mutations detected in genes previously associated with PCD and more rarely or isolated laterality defects. We further identified homozygous Null mutations in two genes not previously associated with human disease, MNS1 and DNAH9, both in cases with situs inversus but little or no respiratory symptoms. Gene matcher database revealed two additional families with biallelic DNAH9 mutation and immunofluorescence analysis revealed complete absence of DNAH9 from respiratory cilia of one affected while DNAH9 localises to the distal half of cilia in controls. Further, we found direct interaction of DNAH9 with the ODA docking complex protein CCDC114 using Y2H screening as well as interaction with DNAH5 and DNAI2 using co-Immunoprecipitation. Conclusion: WES is effective for gene discovery in laterality defect patients and we propose MNS1- and DNAH9 as novel causative genes with DNAH9 representing an ODA type 2 heavy chain protein in human respiratory cells.

P156 (Session D)

From a variant of unknown significance to pathogenic: reclassification of a large novel duplication in BRCA2 by high-throughput sequencing

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Background: Germline mutations in BRCA1/2 significantly contribute to hereditary breast and/or ovarian cancer. Here, we report a novel BRCA2 duplication of exons 22 to 24 in a female patient with bilateral breast cancer at age 35 and 44. The duplicated region was initially detected by gene panel sequencing and multiplex ligation-dependent probe amplification. However, the location and orientation of the duplicated region was unknown. Therefore, it was initially classified as a variant of unknown significance. Methods: The spatial-directional characterization of the BRCA2 duplication was achieved by targeted enrichment of the whole genomic BRCA2 locus including exons and introns and subsequent high-throughput sequencing. Subsequently, bioinformatics tools and a breakpoint spanning PCR were used for identification of location and orientation of the duplication. Results: The duplicated region was arranged in tandem and direct orientation (Chr13(GRCh37):g.32951579_32960394dup; NM_000059.3 c.8754+651_9256+6112dup p.(Ala3088Phefs*3)). It is predicted to result in a frameshift and a premature stop codon likely triggering nonsense-mediated mRNA decay. Consequently, it is regarded as pathogenic. Conclusion: This case study demonstrates that a comprehensive characterization of a structural variant by breakpoint assessment is crucial for its correct classification. Therefore, sequencing strategies including non-coding regions might be necessary to identify cancer predispositions in affected families.

P157 (Session D)

The identification of pathogenic variants in BRCA1/2 negative, high risk, hereditary breast and/or ovarian cancer patients

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Introduction: In the majority of hereditary breast and /or ovarian cancer (HBOC) patients the genetic predisposition is unknown. Currently extensive research focusses on the identification of pathogenic variants causative for the development of the disease. Materials and Methods: NGS-based multiple gene panel resequencing in combination with a high resolution CGH-array was used to identify genetic risk factors for HBOC in 237 high risk patients who were previously tested negative for pathogenic BRCA1/2 variants. Results: We identified 32 pathogenic variants in 14 different genes (ATM, BLM, BRCA1, CDH1, CHEK2, FANCG, FANCM, FH, HRAS, PALB2, PMS2, PTEN, RAD51C and NBN) in 30 patients (12.7%). Two pathogenic BRCA1 variants that were previously undetected due to less comprehensive and sensitive methods were found. Five pathogenic variants are novel, three of which occur in genes yet unrelated to hereditary breast and/or ovarian cancer (FANCG, FH and HRAS). In our cohort we discovered a remarkably high frequency of truncating variants in FANCM (2.1%), which has recently been suggested as a susceptibility gene for hereditary breast cancer. Two patients of our cohort carried two different pathogenic variants each and 10 other patients in whom a pathogenic variant was confirmed also harbored a variant of unknown significance in a breast and ovarian cancer susceptibility gene. Conclusions: With our screening strategy, we were able to identify pathogenic variants predisposing for tumor formation in 12.3% of BRCA1/2-negative breast and/or ovarian cancer patients. Grant: Claudia von Schilling Stiftung to B.S. and D.S.

P158 (Session D)

Building a synthetic cell: transcription in capsules

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The mystery of how the first cell emerged on Earth has fascinated scientists since the 19th century. One of the ways to unveil this mystery is to build a cell from scratch. The advent of genetic engineering and microfluidics provided researchers with a powerful tool to test this method. But the cell consists of multiple parts, so where should the researchers start from? Well, the membrane of the cell is the most important component since it tells apart the insides of the cell from the surrounding environment. In this internship project, I employed two charged polymers, chitosan and alginate, to make a spherical capsule that resembles the cell membrane. To show that the capsules are able to sustain biochemical reactions such as DNA transcription, I encapsulated DNA and enzymes that are required for transcription in living cells. The capsules were incubated in a solution with nutrients such as nucleotides and magnesium ions. The DNA encoded a fluorogenic RNA that allowed me to keep track of transcription. All things considered, the capsules showed a characteristic increase in fluorescence over time. This demonstrates the ability of the capsules to support DNA transcription. The next step will be to test the translation of protein inside the capsules and study the communication between the capsule-based synthetic cells. I believe that the study of such artificial cells will not only be beneficial for fundamental research but find applications in 3D-printed tissues and biosensors.

P159 (Session D)

ARL15 regulates CNNM2-dependent Mg2+ transport by modulating its N-linked glycosylation

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Background: A large Genome-wide association study identified that ARL15, a small GTP-binding protein, is associated with urinary Mg2+ excretion. Within the kidney, ARL15 is highly expressed in the thick ascending limb (TAL) and distal convoluted tubule (DCT), where Mg2+ reabsorption is tightly regulated. However, the exact function of ARL15 and the mechanism by which ARL15 regulates renal Mg2+ handling are still unknown. Method: To identify protein-interaction between ARL15 and Cyclin M (CNNM) proteins, proximity-dependent biotin identification (BioID) and co-immunoprecipitation were performed. Immunohistochemistry were used to investigate co-localization in mouse kidney and human embryonic kidney (HEK293) cells. Furthermore, cell surface biotinylation and 25Mg2+ uptake assays were used to assess cell surface expression of CNNM2 and Mg2+ transport activity. The glycosylation pattern of CNNMs was determined by far lectin Western blot and glycosidase assays. Results: We identified members of the CNNM family as direct interaction partners of ARL15 by BioID. Immunoprecipitation with truncated CNNM2 proteins indicated that ARL15 interacts with CNNM2 at its carboxyl-(C)-terminal conserved CBS domain. CNNM2 and ARL15 co-localize in the basolateral membrane of DCT. Interestingly, overexpression of ARL15 in HEK293 cells showed subcellular localization in the Golgi-apparatus and resulted in an increased N-glycosylation of CNNM proteins. This ARL15-mediated glycosylation was Mg2+-sensitive and encompassed hybrid and complex glycosylation. The functional consequences of ARL15-dependent glycosylation were examined by 25Mg2+ uptake experiments. ARL15 increased 25Mg2+ uptake via CNNM2 by increasing its cell surface expression. Conclusion: ARL15 increases CNNM2 plasma membrane expression by regulating its N-glycosylation pattern. Altogether, our results establish ARL15 as novel regulatory mechanism of Mg2+ transport within the DCT.

P160 (Session D)

The potential of organoids as a model system for physiology

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Introduction: Intestinal organoids are self-renewing, 3D epithelial cultures that can be generated by crypt isolation. Recent studies have shown that organoids can be also used to generate 2D monolayer cultures. Therewith it is possible to study ion transport and regulatory function of transporter proteins not only segment- and epithelium-specific, but also in different stages of differentiation. To exploit this potential it is of high importance to assess ion transporter expression profiles and cell type compositions of the organoid cultures. Materials and Methods: Different culture conditions were used to study the effect on organoid growth and ion transporter expression. The 3D organoid culture was used to grow primary intestinal cells in a 2D monolayer on permeable supports. Results: This study characterized the ion transporter profile dependent on different growth conditions in 3D and 2D organoids. A protocol for monolayer growth generated of the 3D cultures was established. Conclusion: The data shows the potential of an intestinal organoid culture to study the role of transporter proteins as regulators and in ion transport. It highlights the importance of standardization and characterization to make it to a robust model system.

P161 (Session D)

Molecular basis of myosin VI alternative splicing in cellular transformation

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Alternative splicing is a finely regulated process that plays a role in cancer development, with molecular mechanisms largely unknown. Our lab recently characterized an alternatively-spliced exon cassette, called large insert, present in the actin motor protein myosin VI. Notably, the inclusion or the skipping of the large insert causes different structural conformations of myosin VI that in turn determines specific interactomes of the two isoforms, long and short. As a consequence, the two isoforms are involved in different biological functions, clathrin-mediated endocytosis (long) and cell migration (short). This finding is relevant to cancer where myosin VI alternative splicing is deregulated and exon skipping dictates addiction to myosin VI short for tumour cell migration. The aim of this project is to understand how the isoform choice is regulated. Our setup includes biochemical, mass spectrometry and cellular biological assays using A549 and Caco-2 as cellular models.We have found that these epithelial cell lines express both isoforms and modify their expression according to cell culture conditions. In sparse conditions, they express predominantly short isoform but they switch to an almost exclusive expression of the long upon reaching confluency and a polarized epithelial architecture. The same switch is evident in 3D matrigel culture of Caco-2 cysts. We are taking advantage of these model systems to identify both the trans-acting regulators and the signalling pathways that trigger the isoforms switch. Our results suggest the involvement of the splicing regulators RbFOX2 and SRSF2 in myosin VI alternative splicing.A long-term goal of the project will be to shed light on the alternative splicing reprogramming during tumorigenesis looking for genes sharing a common regulation.

P162 (Session D)

Activation and inactivation properties of the TRPV family and their structural origin

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The transient receptor potential vanilloid (TRPV) family consists of six cation channels that play a role in processes ranging from nociception to calcium homeostasis. The family can be divided into two functionally divergent groups, TRPV1-4 and TRPV5-6. TRPV5-6 are constitutively open at physiological membrane voltages, whereas TRPV1-4 are activated by stimuli such as vanilloids. Their architectural resemblance, as observed in recently elucidated structural models, calls these distinct channel properties into question. Combining experimental and structural analyses of TRPV1 and TRPV5, we therefore aimed to provide a better understanding of the functional characteristics of the two groups. This study specifically challenged the vanilloid-insensitivity of TRPV5 and studied calmodulin (CaM)-dependent inactivation of TRPV1. A combination of radioactive calcium uptake assays and Fura-2-mediated calcium imaging demonstrated that vanilloids are unable to alter TRPV5 activation. Structural analysis explained these findings by revealing that TRPV5 is not tailored for vanilloid binding. Additionally, a homology model combined with sequence analysis suggested that complex formation between CaM and TRPV1 could be similar as observed for TRPV5. Immunoprecipitation assays and Fluorescence lifetime imaging of Förster resonance energy transfer (FLIM-FRET) demonstrated a calcium-dependency for the interaction between CaM and TRPV1, which was comparable to, but weaker than the calcium-dependency of TRPV5-CaM binding. These results suggest different modes of activation, but a related inactivation mechanism for TRPV1 and TRPV5. This knowledge, as well as the structural insight provided in this study, has broadened our understanding of TRPV biology and can ultimately be employed in the development of novel TRPV-targeting drugs.

P163 (Session D)

A highly conserved satellite repeat-derived piRNA regulates gene expression in early embryonic development in Aedes aegypti mosquitoes

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Aedes aegypti mosquitoes are important disease vectors, and understanding their development is key to develop vector control strategies.PIWI-interacting (pi)RNAs serve as guardians against endogenous parasitic elements like transposons in the germline of flies and most other metazoans. The pathway of mosquitoes, however, differs significantly from flies in several aspects, and it has most likely acquired additional functions beyond transposon control. We identified an ultra-conserved satellite repeat locus that gives rise to two highly abundant and extremely conserved piRNAs in Ae. aegypti. Interestingly, at least one of the piRNAs is able to strongly regulate gene expression in trans in cells and early embryos.Expression of this piRNA commences in the zygote few hours after egg laying. Blocking this single piRNA leads to strong deregulation of a broad range of genes, resulting in developmental arrest. Moreover, target sites were enriched among maternal transcripts that are degraded during maternal-to-zygotic transition, a key developmental process that coincides with the start of expression of the piRNA, suggesting that it evolved as a zygotic mechanism to eliminate parts of the maternal mRNA pool during development.Our results reveal a novel mechanism in which a zygotic piRNA is mediating maternal mRNA decay during embryonic development. These findings highlight the regulatory potential of PIWI-interacting RNAs, and contribute to the understanding of the development of this important disease vector.

P164 (Session D)

DACT1 is a novel intracellular signalling regulators of cartilage progenitor cell populations.

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Cartilage has long be considered a non-regenerative tissue because it is formed of terminally differentiated cells. The superficial zone of articular cartilage has however been shown to host a population of progenitors. Cells in this zone are the first to be lost in connection with aging, suggesting their role in tissue homeostasis and repair. Isolated chondrocytes from this zone display features common to mesenchymal stromal cells (MSCs). MSCs have been studied for their ability to undergo chondrogenic differentiation and drive in vivo regeneration of cartilage damage. Chondrocyte progenitors are involved in cartilage repair and, like MSCs, are increasingly identified as target for joint repair and regenerative therapy. Dishevelled Binding Antagonist of Beta-Catenin (Dact) are important modulator of both Wnt and TGFβ pathways. These pathways are key to MSC and chondrocyte function but the role of DACT proteins iremains elusive in these cells.We investigated the presence of DACT1 and DACT2 in human cartilage. We observed that both proteins are present in chondrocytes throughout the osteoarthritic tissue. In undamaged cartilage, DACT1 and DACT2 are localised in the articular surface. In mouse embryos (E.15.5), DACT2 is expressed in mesenchymal cells that will give rise to the articular joint. We subsequently found that DACT1 and DACT2 are expressed in human MSCs. DACT1 knockdown in both chondrocyte and MSCs causes the cells to undergo apoptosis. Transcriptomics study on DACT1 knockdown MSCs identified DACT1 to act on of both ubiquitination and phosphorylation, regulating the Wnt canonical pathway.We describe for the first time the presence and biological relevance of DACT1 and DACT2 in chondroprogenitors. DACT1 is involved in MSCs survival and is downregulated in OA. Further studies on DACT1 could not only help elucidate mechanisms involved in OA, but also uncover the relevance of cartilage progenitors loss in the development of cartilage degeneration.

P165 (Session D)

A simple and fast assay based on fluorescent liposomes for quantitative DNA detection.

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Endogenous nucleic acids, present in biological fluids, have seen a growing interest as markers for different pathologies. Circulating tumor DNAs, deriving from neoplastic cells, DNAs and RNAs released by microvesicles, inflammatory cells or pathogen microorganisms, could give rise to new non-invasive diagnostic and therapeutic techniques.In our lab, we developed and characterized a fast and quantitative assay able to detect specific ssDNA sequences with a concentration limit of 1 pM.The proof of concept is based on the possibility to bind different ssDNA strands using complementary oligonucleotides, exploiting the target ssDNA as a linker between these two artificial strands.The first oligonucleotide is biotinylated at its 3' end, interacting with Streptavidin coupled magnetic beads while the other oligonucleotide is coupled to a cholesterol molecule, intercalating inside the phospholipidic membrane of a liposome loaded with a 50 mM Carboxyfluorescein Hepes solution. These nanoparticles have a 0.1 femtomolar limit of detection .The presence of the target nucleic acid is therefore mandatory for the correct formation of a construct where few ssDNAs produce a strong and detectable fluorescence signal. The studies performed so far reveal the correct behaviour of the tool in Hepes and PBS buffer solution, with a detection limit of 1 pM of target ssDNA concentration, while studies performed in human Serum also show the correct assembling of the probe in less than 1 hour, but with a lower detection limit of 1nM [ssDNA].NMR experiments shows the presence of the cholesterol tagged oligonucleotide inside the liposome membrane, and experiments performed with dsDNA suggest a correct function of the assay.This liposome signal amplification strategy could not only be used for the detection of DNA, but for many other different nucleic acids difficult to be quantified at low concentrations by common diagnostic protocols, such as microRNAs or Viral nucleic acids.

P166 (Session D)

Molecular analysis of the replication stress response at human telomeric repeats

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Accumulation of short, dysfunctional telomeres contributes to ageing and tumor suppression, but can also induce genome instability that fuels tumorigenesis. Apart from the gradual telomere shortening in the absence of telomerase, dysfunctional telomeres can arise as a consequence of telomere replication failures. Telomeric repeats behave like replication fragile sites, although the molecular nature of the replication problems at telomeres have not been elucidated. Studies in yeast using two-dimensional agarose gel electrophoresis (2D-gels) revealed frequent replication fork pausing at telomeric repeats. The same analysis cannot be performed in mammalian cells, due to the size and length heterogeneity of telomeres. To overcome this limitation, we have introduced telomeric repeats with different lengths and orientations, in an SV40-based plasmid that replicates autonomously in human cells. We are using this system, in combination with 2D-gels and Electron Microscopy analysis to study replication intermediates at telomeric repeats in human cells. Our preliminary results suggest that replication forks transiently pause at telomeric repeats although the pausing is not prolonged and does not prevent the replication of the plasmid in human cells. We are now following replication of the telomeric repeats after the conditional deletion of candidate genes, known to play important roles in telomere replication, like the shelterin component TRF1 and the BLM helicase. Our preliminary results will be discussed.

P167 (Session D)

Electron Microscopy analysis of mammalian telomere structure

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Telomeres are made of tandem TTAGGG repeats that extend for several kilobases and prevent the recognition of chromosome ends by the DSB response, thereby permitting the maintenance of linear chromosomes. Telomeres are notoriously difficult to replicate and behave like replication fragile sites. Their repetitive nature, the tendency to form secondary structures (like G4 DNA) and ongoing transcription (TERRA), are thought to interfere with telomere replication, but the molecular nature of telomere replication problems has not been elucidated. We have developed a new procedure for the purification of mammalian telomeric repeats and are using it to study telomere structure and telomere replication intermediates in electron microscopy. Using this approach, we were able to visualize telomere features (i.e. t-loops) and replication intermediates (i.e. replication forks). We are currently working on conditions that enrich for telomere replication intermediates that are quite rare in our preparations. We will use this approach to analyse the structural transitions that occur during telomere replication in different genetic backgrounds.In our initial analysis in mouse cells, we observed that molecules containing internal loops were 2-3-fold more frequent in telomere-enriched samples compared to non-enriched genomic DNA. We extended our analysis in ALT cells and found that molecules with internal loops represent the vast majority of telomeric structures that accumulate in U2OS (ALT) cells. We can induce the formation of these structures in vitro by damaging telomeric repeats, showing that internal loops can form spontaneously at damaged telomeres.We propose that internal loops are formed by strand exchange events at sites of telomeric nicks and gaps, and resemble intramolecular Holliday Junctions. They could be substrate for HJ resolvases and therefore might represent a common intermediate of extrachromosomal telomeric circle formation and telomere deletion, in response to damage.

P168 (Session D)

Downstream process considerations for industrial polymeric submicron particle manufacturing

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The development of parenteral submicron particles for clinical and commercial use often faces the challenges of successful process scaling in a sterile or aseptic environment complying with good manufacturing practices. The path from lab-scale submicron particle formulations to the final drug approval is often covered with pitfalls due to the complexity of manufacturing nanomedicines.Often, specific downstream processing is necessary to recover materials in the desired form and purity. In early stage development, submicron particle purification is usually performed by lab-scale centrifugation and decantation. However, the reproducibility of the results obtained by this process may be challenging. Moreover, in many cases it requires manual handling. Up scaling to larger volumes makes it even more labor intensive and time consuming. In this study, tangential flow filtration (TFF) was used as an alternative purification method. Here, the fluid is circulated along the surface of a membrane; no additional manual steps are required during the process.The physical instability of particles in aqueous suspension can in general cause aggregation, flocculation, hydrolysis of particle-forming materials, and extraction of drugs out of the particles. Submicron particles are commonly dried via lyophilization to achieve long-term stability. Besides optimizing the lyophilization cycle, the optimal cryoprotectant formulation is critical in keeping the particles intact.The objective of this study was 1) to evaluate TFF's effectiveness in removing impurities, and 2) to develop a suitable lyophilization formulation using trehalose as cryoprotectant.

P169 (Session D)

Exploring the Role of the loop TMH1-2 of Subunit ND3 in the molecular Mechanism and Regulation of human mitochondrial Complex I

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In aerobic organisms, mitochondria are considered as the energy plants of cell and cellular respiration is accomplished by mitochondrial respiratory chain comprising four large, multi-subunit complexes. Complex I (NADH:ubiquinone oxidoreductase), the largest membrane-bound enzyme of respiratory chain, powers ATP synthesis in mammalian mitochondria by using the reducing potential of NADH to drive protons across the inner membrane. Complex I consisting of a peripheral arm protruding into the mitochondrial matrix, where the electron transport takes place, and a membrane-embedded arm, where proton translocation takes place. However, the catalytic mechanism of coupling the redox reaction to the vectorial proton pumping still remains enigmatic. Furthermore, mammalian complex I exists as a mixture of interconvertible active (A) and de-activate (D) forms. A particular cysteine (Cys 39 in bovine and human) which is located in the mitochodrially-encoded subunit ND3 is only accessible in the D-form; thus, it has been used to test the fraction of complex I in the deactive state. In fact, a number of hereditary, mitochondrial, and degenerative diseases have been described in which complex I is involved such as Leigh syndrome, which is a progressive neurodegenerative disorder with onset usually in infancy or early childhood. Three mutations in crucial positions of the TMH1-2 loop of subunit ND3 have been described in patients with Leigh-syndrome, but the most common variant found in patients is the m.10191T>C mutation, which leads to the substitution of the highly conserved serine-45 in subunit ND3 to proline. It has been suggested that this mutation may impair the function of complex I by affecting the A/D transition. Therefore, we will study the function of complex I and its A/D transition using human cybrid cell lines carrying this particular mutation. Our aim is to elucidate the molecular mechanism and regulation of mitochondrial complex I by the cysteine-switch controlled A/D transition.

P170 (Session D)

Global profiling of protein-DNA and protein-nucleosome binding affinities using quantitative mass spectrometry

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Gene expression is influenced by binding of transcription factors to regulatory regions, which in turn is influenced by chromatin accessibility, underlying DNA motifs and DNA secondary structures. Studying protein-DNA interactions using mass spectrometry-based interaction proteomics can provide insights into these regulatory mechanisms. Current workflows are mainly semi-quantitative, so they provide information about the specificity of an interaction but not its affinity. Typical affinity quantitation methods are applied to single bait-prey interactions and require recombinant proteins. We developed a method that utilizes a series of DNA titrations and affinity purifications from crude nuclear extracts followed by 10-plex TMT labeling and mass spectrometry analysis to determine the apparent dissociation constant (KdApp) of dozens or hundreds protein-DNA interactions in a single experiment. These experiments are also compatible with high-throughput screenings because affinity purifications are performed on a 96-well filter plate system. Benchmarking with the SP/KLF motif showed that we can reproducibly determine the KdApp for various protein-DNA interactions. Furthermore, we screened six common DNA motifs for the KdApp of binding proteins and we also determined protein-binding affinities for two single-stranded G-quadruplex (G4)-forming sequences. The later provides new insights into the potential G4-interactome. Lastly, we demonstrated that our workflow is also compatible to determine binding affinities between proteins and nucleoprotein-complexes. For that, we used mono-nucleosomes, di-nucleosomes and modified di-nucleosomes and quantified the specific protein-nucleosome interactome. Taken together, our method can be utilized to measure affinities of protein-DNA and protein-nucleosome interactions, thereby providing novel insights into transcriptional regulation in health and disease.

P171 (Session D)

Combined sialic acid and histone deacetylase (HDAC) inhibitor treatment up-regulates the neuroblastoma antigen GD2

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Neuroblastoma cells highly express the disialoganglioside GD2, a tumor-associated carbohydrate antigen, which is only sparsely expressed on healthy tissue. GD2 is a primary target for the development of immunotherapy for neuroblastoma. Immunotherapy with monoclonal anti-GD2 antibodies has proven safety and efficacy in clinical trials and is included in the standard treatment for children with high-risk neuroblastoma. Strategies to modulate GD2 expression in neuroblastoma could further improve anti-GD2-targeted immunotherapy. Here, we report that the cellular sialylation pathway, as well as epigenetic reprogramming, strongly modulates GD2 expression in human and mouse neuroblastoma cell lines. Recognition of GD2 by the 14G2a antibody is sialic acid-dependent and was blocked with the fluorinated sialic acid mimetic Ac53FaxNeu5Ac. Interestingly, sialic acid supplementation using a cell-permeable sialic acid analogue (Ac5Neu5Ac) boosted GD2 expression without or with minor alterations in overall cell surface sialylation. Furthermore, sialic acid supplementation with Ac5Neu5Ac combined with various histone deacetylase (HDAC) inhibitors, including vorinostat, enhanced GD2 expression in neuroblastoma cells beyond their individual effects. Mechanistic studies revealed that Ac5Neu5Ac supplementation increased intracellular CMP-Neu5Ac concentrations, thereby providing higher substrate levels for sialyltransferases. Furthermore, HDAC inhibitor treatment increased mRNA expression of the sialyltransferases GM3 synthase (ST3GAL5) and GD3 synthase (ST8SIA1), both of which are involved in GD2 biosynthesis. Our findings reveal that sialic acid analogues and HDAC inhibitors enhance GD2 expression and could potentially be employed to boost anti-GD2 targeted immunotherapy in neuroblastoma patients.

P172 (Session D)

Trained autoimmunity as a driver in the pathogenesis of Systemic Lupus Erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibodies against chromatin. Elevated levels of circulatory chromatin are detected in SLE patients, which may be a result of aberrancies in apoptosis or neutrophil extracellular trap (NET) formation or insufficient clearance of apoptotic material or NETs. Recently, we showed that SLE-derived PBMCs appeared far more sensitive to apoptotic microparticles (MPs) than those from controls, for which there is no clear explanation yet. In fact, recently the concept of trained immunity was described, meaning that innate immune cells can develop an unspecific memory. Therefore, we hypothesized that sources of nuclear antigens in SLE, including MPs and NETs, can train PBMCs, thereby induce trained autoimmunity. Methods: Monocytes from SLE patients or healthy donors were let to rest for five days and then the cells were stimulated for 24 hours with different TLR agonists (Pam3CSK4, LPS:B5). After the stimulation, IL-6 and TNF-a levels were measured. Healthy monocytes were trained with 10% healthy or SLE plasmas or with different stimuli for 24 hours. After five days rest, the cells were restimulated with TLR agonists and IL-6, TNF-a levels were measured. Results: Plasma samples from SLE patients induced innate immune training in healthy monocytes as measured as elevated levels of IL-6 and TNF-a production after second stimulation. The training inducing capacity of SLE plasmas correlated with the amount of an apoptosis induced histone modification (KM-2: H4K8, 12, 16Ac) in their microparticles. The plasma training capacity also correlated with cytokine response of ex vivo stimulated SLE PBMCs. In vitro-produced NETs induced monocyte training dose dependently. MPs, on the other hand, induced training at a lesser extent. Conclusion: Trained autoimmunity play an important role in the pathogenesis of SLE.

P173 (Session D)

Overcoming immunosuppression in the tumor microenvironment with CD11b-CpG antibody conjugates

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Introduction: Dendritic cells have the capacity to elicit an immune response against cancer cells. However, tumors generate an immunosuppressive tumor microenvironment that inhibits immune effector cells and promotes tumor growth. We want to reverse this immunosuppression by targeting immunosuppressive cells such as myeloid regulatory cells (MRCs) with antibodies conjugated to the immune modulator CpG. Methods: Antibody-CpG (TLR9 agonist) conjugates were prepared by crosslinking amine groups of the antibody to sulfhydryl moieties on CpG-thiol using SMCC as a linker. The conjugates were characterized by protein gel analysis. The binding and activation potential of the conjugates was studied using murine bone marrow-derived dendritic cells (BMDCs) that, like MRCs, express CD11b. Flow cytometry and confocal microscopy was used to detect binding and internalization of CD11b-CpG conjugates. Activation of BMDCs was confirmed with flow cytometry and ELISA. Finally, the biodistribution of the conjugates as well as activation of CD11b+ target cells in vivo was assessed. Results: CD11b-CpG and corresponding isotype-CpG conjugates were prepared with on average 3 molecules of CpG conjugated per antibody. The CD11b-CpG conjugates, but not the isotype-CpG conjugates, specifically bound to BMDCs. After binding, the conjugates were efficiently internalized by BMDCs, resulting in their activation as measured by the upregulation of the co-stimulatory molecules CD86, CD80 and CD40. The level of activation by the CD11b antibodies was comparable to stimulation with an equivalent amount of free CpG. Activation with CD11b-CpG also resulted in the production of the pro-inflammatory interleukin 6. Biodistribution experiments showed that 2 hours after i.v. injection into C57bl/6 mice, the conjugate could be detected on CD11b+ cells in the blood, spleen and lymph nodes. Moreover, i.v. injection of CD11b-CpG conjugates resulted in robust activation of CD11b+ cells after 24 hours. This shows that CD11b antibodies are potent vehicles to specifically deliver CpG to myeloid cells in vivo.

P174 (Session D)

Targeting the autophagy pathway: towards a HPV-specific Head and Neck Cancer Therapy.

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Head and Neck cancer (HNC) is the 6th most common cancer worldwide. It accounts for 600.000 new cases and 350.000 deaths every year. Even if the main causative agents are tobacco and alcohol abuse, the role of the Human Papilloma virus (HPV) is clearly emerging. We can subdivide HNC in two distinct subgroups: HPV-positive (HPV+) and HPV-negative (HPV-), presenting very different features. They include: age, socioeconomic status, prognosis, genetic landscape, tissue differentiation. Despite this, all HNC cancer patients are treated with the same therapies, comprising chemo- and radiotherapy, and/or surgery.Specific therapies for HPV-positive HNC tumors will reduce the side effects and most likely improve the quality of life of the patient. Since autophagy is impinged by HPV in primary human keratinocytes, it could be the right pathway to be targeted to obtain a specific response by HPV-positive tumors. In this scenario, a rigorous study of autophagy pathway in HNC, as well as HPV-mediated autophagic impairment will permit the design of tailored therapies.The aims of this project are: (i) to characterize autophagy pathway in HNC; (ii) to understand the molecular mechanisms of HPV-mediated impairment of autophagy; (iii) test drugs that tackle autophagy pathway and specifically kill HPV-positive cells; (iv) design a combination of drugs able to treat HPV-positive HNC cells with the lowest toxicity for healthy cells.Our preliminary results show that autophagy machinery is downregulated in HPV+ with respect to HPV- HNC cells. We also show that HPV oncoproteins E6/E7 localize in the autophagosomes. In addition, we see the downregulation of E6/E7 upon autophagy induction, with an increased expression of the tumor suppressor p53. Moreover, we show the induction of apoptosis upon autophagy induction in HPV+ but not in HPV- HNC cells. Our results open up to new specific e more tailored therapies for HNC.

P175 (Session D)

In vitro evaluation of synergistic antioxidant and anti-inflammatory activity of curcumin and rosuvastatin

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Antioxidants and anti-inflammatory agents play crucial role in prevention of different disorders. Recent studies in modern biomedical science that concerns antioxidants and anti-inflammatory agents are based both on evaluation of natural and synthetic substances. In process of novel drug development natural substances have taken the leading role and trends in pharmaceutical industry are based on combined therapies of natural and synthetic agents. Among studied natural compounds, curcumin, which can be extracted from the rhizome powder of plant Curcuma longa, has drawn special attention of researches due to wide range of pharmacological properties and various molecular targets. Rosuvastatin, a synthetic statin that is described as an advance in pharmacological and clinical properties of hypolipemics, is used for treatment of hyperlipidemia but also posses other significant pharmacological properties. Both substances, curcumin and rosuvastatin, are highly pleiotropic molecules that demonstrated pharmacologically interesting in vitro and in vivo activities that included antioxidant and anti-inflammatory activity. So far, synergistic activity of curcumin and rosuvastatin has not been examined. The aim of present study was to investigate antioxidant and anti-inflammatory activity of curcumin, rosuvastatin and combination of curcumin and rosuvastatin in various concentrations by applying most frequently used in vitro models. For evaluation of antioxidant activity of tested substances we applied test for scavenging free radicals which is based on reaction between stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) and antioxidant. Antioxidant activity of tested substances was compared with ascorbic acid, a well-known and documented antioxidant. While for evaluation of anti-inflammatory activity we applied test by protein denaturation and activity of tested substances were compared to ibuprofen. Although both substances have demonstrated antioxidant and anti-inflammatory activity, results of our study indicated that synergistic activity is more efficient than individual compounds but further research is required on in vivo model.

P176 (Session D)

Unravel Gene drivers that define Luminal BCA Populations after Treatment Resistance and Metastasis

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Metastasis is the major cause of death from Breast Cancer (BCa). During its progression, divergent opinions have arisen regarding the hormonal status of the BCa subtypes.The mammary gland is composed of different cell linages, including the basal and the luminal cells (LCs). The last ones can be divided in estrogen receptor positive (ER+) and negative (ER-).Luminal tumors, which usually express ER+, may reappear after a long period of time on a process called latency or dormancy. Of importance, in Luminal BCa it is unknown which population and which is the driver of tumor initiation.It has been shown that Luminal A tumors can suffer molecular changes and switch to the Luminal B type at the metastatic site. Whether these changes are passenger or have consequences on latency and metastasis it is still unclear.We aim to elucidate if the heterogeneity in Luminal BCa tumors comes from a preexisting ER+/ER- population or due to the plasticity acquired from tumor evolution.

P177 (Session D)

Ketamine alters excitatory synaptic currents in the medial prefrontal cortex of acutely stressed rats

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The cellular and functional changes underlying the adaptive or maladaptive behavioral effects of an acute stressor are not well understood. In the medial prefrontal cortex (mPFC) of male rats, 40 min foot-shock protocol (FS), rapidly increases the number of excitatory synapses, the readily releasable Glu vesicle pool in synaptosomes, and the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) recorded in pyramidal neurons (Pyr). Within 24 hrs, FS induces shrinkage of apical dendrites.Miniature excitatory synaptic currents (mEPSCs) have been suggested to have a neurotrophic and homeostatic role, but the effects of FS on mEPSCs are unknown. To understand the sustained effects of FS on Glu transmission in the mPFC and its regulation by ketamine at antidepressant dosage, synaptic currents were recorded 24 hrs after FS in visually identified layer 2/3 Pyr of prelimbic mPFC in slices from adult male rats. Animals subjected to a 40-min session of inescapable FS (FS group), animals injected with ketamine (10mg/kg) 6 hrs after FS, and controls (CTR) were compared. The amplitude, area, rise, decay, and inter-event intervals of mEPSCs and sEPSCs were analyzed. mEPSCs in the FS group showed only a tendency to minor changes in frequency (small increase) and ampitude (small decrease) vs CTR. Ketamine after FS increased mEPSC frequency and peak amplitude and accelerated rise and decay with no change in area, with respect to CTR. sEPSCs frequency in the FS group had a tendency to a small decrease, with no change in waveform vs CTR. Ketamine after FS produced similar effects on sESPCs as for mEPSCs. Overall, this work indicates that, 24 hrs after FS, no or minor changes occur in miniature and spontaneous synaptic currents at layer 2/3 excitatory synapses of the mPFC. Ketamine modulation of the Glu synaptic currents of stressed animals suggests changes in synapse morphology and/or dendritic localization.

P178 (Session D)

Development of novel experimental genetic approaches in Plasmodium falciparum

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Malaria caused by Plasmodium parasites threatens almost half of the global population, with Plasmodium falciparum causing the highest mortality and morbidity. Characterization of P. falciparum genes important for pathogenesis has been greatly advanced by CRISPR based gene editing techniques. While the establishment of knockouts has been greatly aided by this development, there is still room for improvement since obtaining pure mutant parasite populations remains difficult. Furthermore whilst the knockout (KO) approach toolbox has expanded, there is still a lack of reliable and functional knockdown (KD) tools to use in Plasmodium. In this study we aimed to expand both genetic toolboxes by developing a fluorescence based screening method to select pure knockout populations, and to establish the use of the ground-breaking Cas13 protein as a novel and reliable knockdown system. To facilitate fluorescence based screening we employed a procedure in which a target gene is swapped with a fluorescent marker, enabling drug-free separation between fluorescent KO parasites and non-fluorescent wild-type (WT) parasites using cell sorting by flow cytometry. Using this knockout approach, a previously uncharacterized member of the PHIST protein family was successfully targeted, resulting in fluorescent parasites with a significant growth defect. We furthermore demonstrate successful sorting of as little as 10 GFP-positive KO parasites, capable of steady propagation without the occurrence of WT contaminations. The described genetic approaches facilitate characterization of the intricate biology of P. falciparum, enabling development of novel therapies to combat malaria.

P179 (Session D)

Metrological traceability chain of hepcidin

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Introduction: Hepatic hormone hepcidin regulates systemic iron levels and plays a role in diagnostics of iron metabolism disorders. However, hepcidin concentrations measured by various methods differ considerably, complicating interpretation.Objective: Therefore, we established a metrological traceability chain, which describes an unbroken calibration hierarchy from a measurement result to a defined reference in SI units. Methods: We developed a secondary reference material (sRM) by applying technical procedures described by the International Consortium for Harmonization of Clinical Laboratory Results and evaluated its effect in two round robins (sample send outs). Comprehensive purity analysis of a candidate primary RM (pRM) was performed by state of the art procedures. To define the analyte, we studied plasma protein binding of hepcidin using the relation between molecular weight and clearance. To this end, we measured hepcidin along with known freely circulating and partly bound analytes, as reference, in blood and peritoneal fluid of patients undergoing a peritoneal equilibration test. Results: The sRM was found to be stable, commutable and significantly reduced the inter-assay CV of participating hepcidin methods. We assigned its value using a pRM with certified purity and a calibrated candidate reference mass spectrometry method. Based on this calibration, we recalculated both adult and child reference values. Since the measured clearance of hepcidin was found to be compatible with its molecular weight, we concluded that hepcidin predominantly circulates freely which adds a key aspect to the metrological traceability chain by defining the analyte. Conclusion: We established a metrological traceability chain for hepcidin, which enables the use of this analyte in clinical practice and research if the reference materials and the derived reference values are implemented internationally.

P181 (Session D)

The long pentraxin PTX3 has a non-redundant role in the control of Streptococcus pneumoniae invasive infections

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Pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule which has served as a paradigm for linking the cellular and humoral arms of innate immunity. PTX3 is an important component of host resistance to pulmonary infections for selected pathogens. Our aim was to investigate the role of PTX3 in the control of pneumococcal infections caused by Streptococcus pneumoniae, the most common causative bacteria in community-acquired pneumonia and an important cause of mortality world-wide.By using a model of invasive pneumococcal infection in young-adult mice, we observed a strong expression of PTX3 by non-hematopoietic cells. Comparing the pneumococcal load and survival of infected mice, we observed a higher sensitivity of Ptx3-/- animals during the invasive phase of the infection which could be restored by a systemic administration of recombinant PTX3. Infected Ptx3-/- mice also showed an increased inflammatory profile. Furthermore, the local exogenous instillation of PTX3 during the ongoing infection was able to the pulmonary pneumococcal load. We also observed that PTX3 specifically bind on S. pneumoniae but not in physiological conditions found during in vivo infection. Different models in vivo and in vitro excluded PTX3 as an effective opsonin on S. pneumoniae. Our last results show that exogenous instillation of PTX3 reduces local inflammation and using another model of neutrophil depletion during the ongoing infection of Ptx3-/- animals damps the higher susceptibility of the defective mice, thus strongly suggest that PTX3 exert it's protective effect against pneumococcal by modulating the infection induced inflammation.Our results suggest a non-redundant role of PTX3 in the control of S. pneumoniae infections.

P182 (Session D)

Characterization of hiPSC-derived kidney organoids for electrolyte transport properties

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Increased global prevalence of kidney diseases such as chronic kidney disease, emphasizes the need for models better resembling the human physiology and pathophysiology than the existing animal models and cell lines. Human induced pluripotent stem cells (hiPSCs) derived kidney organoids may constitute 3D structures recapitulating in vivo kidney physiological mechanisms, thus providing a platform for disease modelling, developmental and regenerative studies. So far, nephrotoxicity and dye uptake assays suggested the functionality of proximal segments within these organoids. However, the physiological function of the distal tubules in hiPSC derived kidney organoids and their equivalence to the in-vivo counterparts have not been addressed. In this regard, we aim to find a suitable marker to sort segment specific populations in order to establish a monolayer culture for transport and functional studies. Kidney organoids were obtained from Leiden University and established by directed differentiation of hiPSC following published protocols. Both kidney organoids contained tubular segments expressing several specific transporters/channels, assessed by quantitative real time PCR and immunohistochemistry. Organoids were investigated for the possibility of using the lectin, peanut agglutinin (PNA) and Mucin-1 as epithelial markers to sort distal segment specific cells. Immunohistochemistry showed colocalization of PNA with distal tubule and proximal tubule markers. On the contrary absence of co-localization of a proximal marker with Mucin-1 suggests the possibility of using it to sort distal segments. The identification of Mucin-1 as a marker for distal tubules within hiPSC derived kidney organoids will allow the sorting this population of cell and their functional characterization.

P184 (Session D)

Quantification of extracellular matrix proteins of COPD vs. control by mass spectrometry

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic bronchitis and emphysema, leading to airflow obstruction. COPD lungs show an irreversible loss of alveolar tissue with destruction of extracellular matrix (ECM). Novel strategies aimed at the regeneration of alveolar tissue are urgently needed. The use of mesenchymal stem/stromal cells (MSCs) is promising as they produce anti-inflammatory factors, growth factors and ECM proteins, constituting a niche for alveolar repair. However, damaged alveolar ECM in COPD patients may hamper stem cell engraftment and regenerative function. In this study, mass spectrometry analysis was performed to elucidate changes within the ECM composition of COPD lungs. Furthermore, ECM produced by lung derived MSCs (LMSCs) from COPD patients was compared to ECM produced by control LMSCs. For assessing the ECM composition, tissue was collected from lung cancer patients undergoing pneumonectomy or lobectomy, which were included as COPD or control based on lung function data. Alveolar tissue was isolated from non-tumor regions using laser micro-dissection. For assessing ECM produced by LMSCs, LMSCs were isolated from peripheral lung tissue from COPD patients undergoing lung transplantation or lung volume reduction surgery and from non-COPD controls undergoing tumor resection surgery. LMSCs were grown in 6-well culture plates until confluency. The cell layer, containing both LMSCs and LMSC-produced ECM, was collected. For both experiments, ECM was extracted according to the extraction procedure by Barret et al. [1] before MS analysis. Assessment of the differences between ECM of COPD and control tissue as identified by mass spectrometry will enable the development of new strategies inducing alveolar repair in COPD.This work is financially supported by the Dutch 'Longfonds' (project 6.1.15.017) and 'Stichting Astma Bestrijding' (project 2017/038).Literature: 1. Barrett AS, et al. Journal of Proteome Research. 2017; 16 (11), 4177-4184.

P185 (Session D)

Nongenomic relaxatory effect of thyroxine on rat skeletal muscle arteries is associated with suppression of the extracellular matrix signaling in smooth muscle cells

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Introduction: Thyroid hormones (TH) regulate the circulatory system by genomic and nongenomic mechanisms. Fast nongenomic relaxatory effects of TH were observed in several arteries, but underlying mechanisms are still unclear and may differ among the vascular beds. In this study, we explored the mechanisms of TH nongenomic effects in rat skeletal muscle arteries. Methods: The experiments were performed on Wistar rats (m=300-450 g). Gastrocnemius feed (sural) arteries (d=270-400 micron) were isolated and studied in a wire myograph. In some experiments, the endothelium was removed with rat whisker. Arterial responses were compared using 2-way ANOVA, n was 6-12 for each group. Results: T4 but not T3 induced prominent concentration-dependent relaxation of the arteries preconstricted by methoxamine (a1-adrenoceptor agonist), the minimal effective concentration was 2 microM. Further, preincubation with T4 (3 or 10 microM) depressed the contractile responses to methoxamine (both maximum force and pD2 were reduced). This effect was abolished by tetrac (3 microM), a competitive inhibitor of integrin avβ3. T4 reduced contractile responses in the presence of L-NNA (100 microM) as well as after endothelium removal. Moreover, the relaxatory effect of T4 on methoxamine-induced contraction was not attenuated by Y27632 (Rho-kinase inhibitor, 3 microM) and iberiotoxin (BKCa inhibitor, 0.1 microM). However, the inhibition of integrin-linked kinase (ILK) by Cpd22 (10 microM) abolished the relaxatory effect of T4 in endothelium-denuded arteries. Conclusion: T4 induces nongenomic endothelium-independent relaxation of the sural artery by suppression the extracellular matrix signaling via integrin avβ3 and downstream integrin-linked kinase. The mechanism of nongenomic vasorelaxation does not include Rho-kinase inhibition or BKCa activation. T4-induced nongenomic vasorelaxation may contribute to the decrease of total peripheral resistance associated with hyperthyroidism and should be considered in case of hormone replacement therapy of hypothyroid states. Supported by the RFBR (Grant N19-015-00482).

P186 (Session D)

Clinical and genetic spectrum of hypomagnesaemia, seizure, and intellectual disability syndrome

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Introduction: Hypomagnesaemia, seizure, and intellectual disability (HSMR) syndrome is a rare disorder caused by mutations in the Cyclin M2 (CNNM2) gene. CNNM2 is expressed in the kidney and is involved in basolateral Mg2+-extrusion from the distal convoluted tubule to the blood compartment, thereby contributing to Mg2+-homeostasis. Only a few cases of HSMR syndrome patients have been described, hindering recognition of the disease. Objective Here, we aimed to characterise novel HSMR syndrome patients on a clinical and molecular level in order to develop clinical criteria for the diagnosis of HSMR syndrome. Methods: HEK293 cells overexpressing wild type (WT) and novel identified CNNM2 mutants were subjected to 25Mg2+-uptake assays to assess function. Subsequently, cell surface biotinylation assays and confocal microscopy were employed to investigate localisation and processing of CNNM2. Lastly, a literature study was performed of all HSMR syndrome cases for phenotypic analysis. Results: We identified eleven novel mutations in CNNM2 in patients with suspected HSMR syndrome and eight mutations displayed a reduced functionality (12-49% of WT). CNNM2 variants affected by truncations showed lowered expression at the plasma membrane. Two mutations in the N-terminal were located in the signal peptide and confocal microscopy revealed defective cleavage of this peptide and perinuclear retention of the protein in one variant (p.Leu48Pro). Moreover, we showed, by combining data from earlier reported cases that patients (14 cases) diagnosed with HSMR syndrome and defective CNNM2 all suffered from hypomagnesaemia (0.45 - 0.70 mmol/L) which could not be corrected by Mg2+-supplementation. Furthermore, the majority of patients experienced refractory seizures (89%) and intellectual disability (92%). Interestingly, obesity was observed (91%) as novel hallmark. Conclusion: Physicians are recommended to perform genetic testing and verification studies for CNNM2 if patients present with a mild hypomagnesaemia, intellectual disability, transient seizures and obesity.

P187 (Session D)

Effect of rare coding Variants in the CFI gene on factor I expression levels

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Age-related macular degeneration (AMD) is a major cause of visual impairment among elderly in the western world. AMD is a multifactorial disease with genetic variation in the complement system being a major contributor to disease risk. Various variants in the complement factor I (CFI) gene have been associated with AMD. However, interpreting the clinical relevance of rare coding variants remains challenging. Therefore, we measured FI levels in plasma samples of carriers of rare heterozygous missense variants and in vitro in the supernatants of cells expressing FI.In total 114 plasma samples of 93 donors were available for FI measurement with ELISA. The donors carried 20 different rare missense variants in CFI, for seven variants levels have not been reported in literature previously. For seven variants we observed reduced FI levels, both in plasma samples of carriers and in vitro. For three variants we noted slightly reduced expression levels in vitro (70-85% of wild-type [WT] expression), but FI levels in carriers were within reference range. Since all carriers are heterozygous, small changes in expression are likely masked by expression of the WT allele. Nine variants did not show reduced expression in vitro compared to WT, and also in plasma samples FI values were within the normal range. One splice variant, which was reported to cause exon skipping, leads to reduced levels in vivo, but this could not be evaluated in vitro since recombinant protein expression does not assess the effect of splice variants. Concluding, recombinant expression of FI can reveal changes that are masked by expression of WT FI in plasma of heterozygous carriers, but effects of exon skipping are not detected. Here we report the effects of seven variants that have not yet been reported previously, and confirm previous findings for the remaining 13 variants.

P189 (Session D)

Unravelling the mode of action of a novel class of antimalarials

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Malaria parasites strongly rely on the extracellular supply of pantothenate (vitamin B5). This is required to generate coenzyme A (CoA) that acts as a cofactor for many metabolic processes, including energy supply, lipid synthesis, and acetylation. Due to the emerging resistance against all front-line antimalarials, there is an urgent need for development of drugs that target novel pathways and affect multiple stages of the parasite. We have synthesized potent pantothenate derivatives, pantothenamides, that kill the pathology-causing blood-stage parasites and prevent transmission to the mosquito. These compounds target CoA-dependent processes by producing drug-CoA analogs that may act as antimetabolites and inhibit acetyl-CoA synthetase (ACS) and acyl-CoA synthetase 11 (ACS11) as was revealed by metabolomic profiling and gene editing techniques.From this multistage targeting class of compounds, we have selected MMV693183 as the clinical candidate. Surprisingly, transmission-blocking activity of this pantothenamide originates with the preferential killing of female parasites. We further investigated compound-specific effects and identified a group of compounds that specifically target either asexual or sexual blood-stage parasites. In both stages, we showed that acetyl-CoA is reduced and that mutations in ACS and ACS11 cause resistance to the compounds, suggesting that differential killing is not due to an off-target effect. The difference in mechanism of action of pantothenamides on different stages and sex of the malaria parasite is still unclear and is currently being investigated. We aim to unravel the coenzyme A pathway and processing of pantothenamides to their antimetabolites in different parasite stages in order to better understand the mode of action. Overall, we have discovered a potent multistage antimalarial with a novel mode of action that is under consideration for future clinical use.

P190 (Session D)

Platelet CD34 expression in a patient with a partial deletion of transcription factor CBFB

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CD34 is a hematopoietic stem and progenitor cell marker implicated in cell-cell interactions in the bone marrow niche. In healthy cells, CD34 expression is switched off upon maturation. Previously, aberrant CD34 expression on mature platelets has been associated with monogenic bleeding disorders that are caused by mutations in the transcription factors GFI1B (Growth Factor Independence 1B) and RUNX1 (RUNT related transcription factor 1). These familial disorders are characterized by a bleeding diathesis, caused by disturbed megakaryocyte development, which results in the formation of dysfunctional platelets. In addition, these disorders are accompanied by abnormal retention of CD34 on platelets, which is not lost during differentiation. Here, we report a patient with a bleeding disorder associated with a partial deletion of the transcription cofactor CBFB (Core Binding Factor B) and CD34 expression on platelets. Together with GFI1B and RUNX1, CBFB is now the third hematopoietic transcriptional regulator that associates with abnormal platelet CD34 expression and a bleeding tendency. This case underscores that mutations in various hematopoietic transcription factors may result in abnormal platelet development associated with increased CD34 expression. We recommend the inclusion of CBFB as potential bleeding disorder gene in the diagnostic workup of inherited bleeding disorders.

P191 (Session D)

The potential role of XCR1 as tumor escape mechanism

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Dendritic cells (DCs) orchestrate the immune response by presenting antigens to CD4+ and CD8+ T-cells. Human cDC1, the rarest subset of blood circulating DCs, was identified to be efficient at cross-presentation and elusively express the XCR1 receptor. The ligand of XCR1, the chemokine XCL1, is secreted by activated CD8+ T-cells and acts as a chemoattractant towards XCR1+ cells. Pre-clinical data revealed that the presence of XCL1 in a tumor correlates with a higher infiltration of XCR1+ DCs and thereby increases the infiltration of antigen-specific CD8+ T-cells in the tumor microenvironment (TME). Since XCR1 expression was also detected on tumor cells we hypothesize that tumors could overexpress XCR1 as an escape mechanism to arrest XCL1 in the tumor microenvironment. XCR1 expression of tumor cell lines and tumor samples was assessed via flow-cytometry staining, immunohistochemical staining (IHC), and on RNA level via qPCR. cDC1s were isolated from human PBMCs with magnetic microbeads and in vitro migration assays were performed with transwell plates. The XCR1 expression on the analysed tumor cell lines could not be determent since the expression levels varied between the different experiment and methods. However, one breast cancer and one head and neck tumor sample were detected XCR1+ via IHC and on RNA level via qPCR. cDC1s showed a decreased migration towards XCL1 and XCR1+ cells and in addition, increased migration to XCL1 with XCR1- cells. Both effects seem to depend on XCL1 concentration. All together suggest that tumor cells could express XCR1, which could impact the migration of cDC1s. Nevertheless, providing more insides on the suppressive effect of XCR1+ cells in the TME could help to enhance the infiltration of CD8+ T-cells in the TME, which is associated with a better clinical outcome of cancer patients.

P192 (Session D)

Increased risk of high-grade dysplasia and colorectal cancer in inflammatory bowel disease patients with recurrent low-grade dysplasia

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Background and aims: The impact of recurrent/persistent low-grade dysplasia (LGD) on the risk of high-grade dysplasia (HGD) and colorectal cancer (CRC) in inflammatory bowel disease (IBD) patients is unknown. In addition, it is unclear how a neoplasia-free period after index LGD impacts this risk. We aimed to (1) determine the impact of recurrent/persistent LGD on the advanced neoplasia (HGD/CRC) risk in IBD patients with a history of LGD and (2) evaluate the impact of a neoplasia-free time period after initial LGD diagnosis on advanced neoplasia risk. Methods: This is a nationwide cohort study using data from the Dutch National Pathology Registry (PALGA) to identify all IBD patients with LGD and =1 follow-up colonoscopy between 1991 and 2010 in the Netherlands. Follow-up data were collected until January 2016. We compared the cumulative advanced neoplasia incidence between patients with and without recurrent/persistent LGD at first follow-up colonoscopy using log-rank analysis. We subsequently studied the impact of a neoplasia-free period after initial LGD on the advanced neoplasia incidence. Results: We identified 4,284 IBD patients with colonic LGD with a median follow-up of 6.4 years. Patients with recurrent/persistent LGD at first follow-up colonoscopy had a higher cumulative incidence of advanced neoplasia (HR 1.66 (95%CI 1.22-2.25, p=0.001). A neoplasia-free period of 3 years after initial LGD was associated with a reduced risk of advanced neoplasia (incidence rate 8.5/1,000 patient-years).

P193 (Session D)

Pharmacokinetics of 111In-anti-mPD-L1 in immune challenged tumor-bearing mice

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Introduction: Immune checkpoint inhibitors show impressive anti-tumor efficacy in cancer patients. However, mixed treatment responses and serious side effects call for predictive biomarkers. Preclinical studies show that microSPECT/CT using radiolabeled anti-programmed death-ligand 1 (PD-L1) antibodies can be used to quantify PD-L1 expression in vivo. However, the activation status of PD-L1+ immune cells may influence anti-PD-L1 antibody pharmacokinetics. Therefore, we investigated the effects of lipopolysaccharide-mediated (LPS) immune activation on the PK and tumor-targeting of indium-111-anti-PD-L1. Methods: The effect of immune activation on anti-PD-L1 in vivo biodistributions was evaluated in three conditions; healthy BALB/c, Renca tumor-bearing BALB/c, and LPS-challenged (0.6 mg/kg body weight) Renca tumor-bearing BALB/c mice. Mice were intravenously injected with 30 or 100 µg indium-111-labeled anti-mouse-PD-L1. Pharmacokinetics was assessed by taking blood samples and biodistribution was quantified by microSPECT/CT and ex vivo biodistribution studies 72h after tracer injection. PD-L1 expression in organs of interest was evaluated immunohistochemically. Results: There were no statistically significant differences in the in vivo biodistribution of indium-111-anti-PD-L1 between tumor-bearing and non-tumor-bearing mice. However, in immune-challenged mice, splenic tracer uptake significantly increased compared with non-LPS-challenged tumor-bearing mice (65.0 $\pm$ 10.3 %ID/g vs. 35.2 $\pm$ 4.9 %ID/g; $p<0.001$), resulting in accelerated blood clearance and reduced tumor targeting (Blood 24h: 4.2 $\pm$ 0.3 %ID/g vs. 9.3 $\pm$ 3.1 %ID/g; $p<0.05$, Tumor:7.9 $\pm$ 5.3 %ID/g vs. 25.9 $\pm$ 11.6 %ID/g; $p<0.05$). Increasing the tracer dose to 100 µg resulted in reduced splenic uptake (27.3 $\pm$ 4.9 %ID/g vs. 12.1 $\pm$ 3.5 %ID/g; $p<0.05$) and slower blood clearance (Blood 24h: 12.2 $\pm$ 2.5 %ID/g vs. 14.3 $\pm$ 2.6 %ID/g; ns), and restored tumor targeting (18.1 $\pm$ 1.7 %ID/g vs. 15.3 $\pm$ 4.3 %ID/g; ns). Conclusions: This study shows that systemic inflammatory responses can significantly alter pharmacokinetics and tumor targeting of anti-PD-L1 antibodies. Increasing the anti-PD-L1 antibody dose saturates splenic uptake and restores efficient tumor targeting. This information is essential to better understand alterations in in vivo anti-PD-L1 antibody biodistribution and to avoid suboptimal antibody-dosing.

P195 (Session D)

Development of an imaging agent for early-stage atherosclerosis: PLGA-perfluorocarbon nanoparticles for imaging macrophage-like-smooth muscle cells in lesions

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Introduction: Atherosclerosis is an inflammatory disease which forms the basis for potential fatal diseases such as myocardial infarction and stroke. More recently, smooth muscle cells (SMCs) and their inflammatory role in the disease process is gaining interest. It is believed that these cells contribute to inflammation by taking up LDL and forming foam cells (macrophage-like-SMCs or MAC-SMCs), and might be an important regulator in plaque vulnerability. Atherosclerosis is usually silent until a clinical event occurs. Noninvasive imaging could provide early information, however, conventional imaging does not suffice, especially as no precise imaging agents exist. We believe we can use multimodal nanoparticles (NP), suitable for amongst others 19F MRI, as a contrast agent and target MAC-SMCs. The aim of this in vitro study is to investigate the NP uptake by macrophage(like) cells. Methods: NPs consist of poly(lactic-co-glycolic acid) (PLGA) with perfluoro-15-crown-5-ether (PFCE) entrapped, produced by sonication. A fluorescent dye will also be entrapped. The NPs will be analyzed with Dynamic Light Scattering (DLS) for size and Nuclear Magnetic Resonance (NMR) for fluorine content.Three cell types will be used: monocyte derived dendritic cells (mDCs), macrophages and MAC-SMCs. mDCs will be derived from donor blood and macrophages will be matured from a monocyte cell line (THP-1). The SMCs will be harvested from murine thoracic aortas after enzymatic digestion; SMC origin will be confirmed by a-actin reactivity. After 72 hours of incubation with cholesterol, SMCs will be differentiated into MAC-SMCs. The cells will be incubated with the NP.NP uptake will then be assessed by flow cytometry and confocal microscopy. Phagocytosis and efferocytosis will be studied using fluorescent latex beads and fluorescent labeled apoptotic cells respectively. Results and discussion: The experiments have to be optimized and executed yet. If necessary to improve uptake, we will vary (the coating of) the NPs.

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