14th Annual Conference on Life Sciences

29th & 30th September 2021

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GGL
International Giessen
Graduate Centre for the Life Sciences

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

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Graduate Centre for the Life Sciences

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14th GGL Conference on Life Sciences

29th - 30th September 2021

Justus Liebig University

Giessen - Germany

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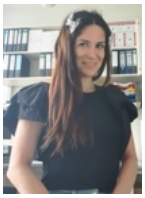
Conference Organising Committee Members 2021

PhD students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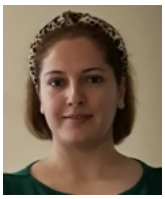
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14th GGL Annual Conference: Wednesday, September 29th, 2021

Time slot	Programme	Speaker	Chairperson
09:00 - 09:15	**Opening Remarks**	**Prof. Dr. Eveline Baumgart-Vogt**	
Talks of guest speakers			
09:15 - 09:45	**Keynote Section 7**	**Prof. Gisbert Schneider** ***De novo Drug Design with Machine intelligence*** **ETH Zürich/ETH Center Singapore**	**Nadine Sella**
09:45 - 10:00	**Break**		
10:00 - 10:30	**Keynote Section 5**	**Dr. Aniko Korosi** ***How does early-life stress lead to increased vulnerability to develop cognitive and metabolic dysfunction? A synergistic action of stress, inflammation and nutrition*** **University of Amsterdam, The Netherlands**	**Osama Elyamany**
10:30 - 10:45	**Break**		
10:45 - 11:15	**Keynote Section 3**	**Prof. Ghazwan Butrous** ***The Complex Interaction of Infection and Pulmonary Vascular Pathology*** **The Universities of Greenwich and Kent at Medway, UK**	**Mohammad Rashedul Alam**
11:15 - 11:30	**Break**		

Talks of doctoral candidates: **time**	**virtual room 1**	**virtual room 2**	**virtual room 3**	**virtual room 4**
	Section 3 Part 1	**Section 5 Part 1**	**Section 7 Part 1**	
GGL-Staff:				
Chairs:	**Zeki Ilker Kanbagli**	**Sara Shabani**	**Anjani Nayak**	
11:30 - 11:40	Paulin Brosinsky	Julia Diago Perez	Fabian Tann	
11:40 - 11:50	Leili Jafari	Aya Alserw	Wendell Albuquerque	
11:50 - 12:00	Kathrin Malkmus	Melina Kahl	Beatrice Tobisch	
12:00 - 12:10	Julie Antoine	Daniela Daume	Nadine Sella	
12:10 - 12:20	Reshma Jamal	Osama Elyamany	Anjani Nayak	
12:20 - 12:30	Mohammad Rashedul Alam			
12:30 - 13:30		**Lunch Break**		
	Section 3 Part 2	**Section 5 Part 2**	**Section 2 Part 1**	
GGL Staff:				
Chairs	**N.N.**	**Osama Elyamany**	**Yukino Kobayashi**	
13:30 - 13:40	Marie Dippel	Jessica Hernandez	Philipp Wolf	
13:40 - 13:50	Edibe Avci	Rebecca Claßen	Maria Wille	
13:50 - 14:00	Dima Hamarsheh	Benedicta Mensah	Dordia Anindita Rotinsulu	
14:00 - 14:10	Laureen Czech	Dominic Osei	Parviz Ghezellou	
14:10 - 14:20	Paniz Adibi	Sara Shabani	Jasmin Bazant	
14:20 - 14:30	Abdullah Al-Najjar		Pia Naujack	
14:30 - 14:45	**Break**			
		Section 4 Part 1	**Section 2 Part 2**	
GGL Staff:				
Chairs		**Janek Börner**	**N.N.**	
14:45 - 14:55		Nicole Bazant	Juan Velez	
14:55 - 15:05		Doudou Kong	Daniela Grob Guerra	
15:05 - 15:15		Jihed Gharred	Lisa Segeritz	
15:15 - 15:25		Nicole Schmid	Camilo Larrazabal	
15:25 - 15:35		Meike Schwan	Selina Ohl	
15:35 - 15:45			Corinna Heck	
15:45 - 16:00	**Break**			
	Section 1 Part 1	**Section 4 Part 2**		
GGL Staff:				
Chairs	**Bernhard Hellmann/Julia Beranek**	**N.N.**		
16:00 - 16:10	Julia Beranek	Janek Börner		
16:10 - 16:20	Silvana Hof-Michel	Maria Weller		
16:20 - 16:30	Bernhard Hellmann	Timo Schlemmer		
16:30 - 16:40	Stefan Baumanns	Fatimah Alabudeeb		
16:40 - 16.50	Mengmeng Zhou	Corinna Ulshöfer		
16:50 - 17.00	Alina Struff			
17:00 - 17:15	**Break**			

Talks of guest speakers **Time slot**	**Programme**	**Speaker**	**Chairperson**
17:15 - 17:45	**Keynote Section 1**	**Prof. Christian Sina** ***Digital therapeutics based on personalized nutrition- does it work?*** **Universitätsklinikum Schleswig-Holstein, Lübeck**	**Bernhard Hellmann**
17:45 - 18:00	**Break**		
18:00 - 18:30	**Keynote Section 4**	**Dr. Kaspar Burger** ***Nucleolar accumulation of the RNA-binding protein NONO promotes DNA repair*** **University of Würzburg**	**N.N.**

14th GGL Annual Conference Thursday, September 30th, 2021

Time slot	Programme	Speaker	Chairperson
Talks of guest speakers			
09:00 - 09:30	**Keynote Section 6**	**Prof. Robin Hobbs** ***Maintenance and regeneration of the male germline*** **Monash University / Hudson Institute of Medical Research, Clayton, Australia**	**Hiba Hasan**
09:30 - 09:45	**Break**		
09:45 - 10:15	**Keynote Section 2**	**PD Dr. Michael Mühlebach** ***Platform-based Vaccines – Short-cut to Protection*** **Paul Ehrlich Institute, Langen**	**Mohammed Samer Shaban**
10:15 - 10:30	**Break**		
10:30 - 11:00	**Keynote Section 9**	**Prof. Lars Tanvik** ***The fate and role of terrestrial organic matter in inland waters, from molecular to global scale*** **Uppsala University, Sweden**	**Alexander Konrad**
11:00 - 11:15	**Break**		

Talks of doctoral candidates: **time**	**virtual room 1**	**virtual room 2**	**virtual room 3**	**virtual room 4**
	Section 6 Part 1	**Section 10 Part 1**	**Section 2 Part 3**	**Section 9 Part 1**
GGL Staff:				
Chairs	**Christine Rager**	**Hendrik Lehmann**	**Oliver Puckewaldt**	**Michael Hauschild**
11:15 - 11:25	Hiba Hasan	Rebecca Hasseli	Lu Liu	Kai Jansen
11:25 - 11:35		Reem Jamous	Benadict Vincent Albert	Alexander Konrad
11:35 - 11:45	Hang Yan	Michaela Melzer	Mohammed Samer Shaban	Marigona Morina Gashi
11:45 - 11:55		Veronika Lehner	Xuesong Li	Leonhard Sommer
11:55 - 12:05	Rashidul Islam	Carla Doll	Svenja Gramberg	Niklas Schnepel
12:05 - 12:15	Wei Peng	Alina Hagen	Mudassar Mughal	Philipp Koellmann
12:15 - 13:15	**Lunch Break**		Monique Überall	
	Section 6 Part 2	**Section 10 Part 2**	**Section 2 Part 4**	**Section 9 Part 2**
GGL Staff:				
Chairs	**Shanjid Shiplu**	**Michael Cekay**	**Svenja Gramberg**	**Leonhard Sommer**
13:15 - 13:25	Shashika Kothalawala	Hendrik Lehmann	Mandy Beutler	Ferdinando Binacchi
13:25 - 13:35	Magdalena Kuchta	Anna-Lena Proksch	Max Möscheid	Michael Hauschild
13:35 - 13:45	Jane Maoga	Sebastian Stricker	Yukino Kobayashi	Eva Völker
13:45 - 13:55	Christine Rager	Jiawen Yong	Eric Springer	Marvin Rades
13:55 - 14:05	Agnes Mwaura	Fabian Edinger	Hicham Houhou	Vanessa Tirpitz
14:05 - 14:15		Wenjie Sheng	Oliver Puckelwaldt	Annalena Barth
14:15 - 14:30	**Break**			Catarina Martins
	Section 6 Part 3	**Section 10 Part 3**	**Section 8 Part 1**	
GGL Staff:				
Chairs	**Shashika Kothalawa**	**N.N.**	**Julian Schneemann**	
14:30 - 14:40	Vishnu Kumar	Paul Brunk	Darya Dudko	
14:40 - 14:50	Hassan Kabbesh	Ruth Dartsch	Felix Graf	
14:50 - 15:00	Dingding Ai	Manuel Richter	Parab Haque	
15:00 - 15:10	Shanjid Shiplu	Michael Cekay	Julia Büttner	
15:10 - 15:20	Sèyi Vanvanhossou	Philipp Arndt	Usman Ali	
15:20 - 15:30		Nazli Salik	Katrin Wiltschka	
15:30 - 15:45	**Break**			
		Section 10 Part 4	**Section 8 Part 2**	
GGL Staff:				
Chairs		**Jiawen Yong**	**Usman Ali**	
15:45 - 15:55		Salisa Kruijning	Nils Anschütz	
15:55 - 16:05		Chaoyu Zhang	Domenic Dreisbach	
16:05 - 16:15		Lucas Kimmig	David Lüke	
16:15 - 16:25		Anca-Laura Amati	Julian Schneemann	
16:25 - 16:35		Martin Reichert	Azar Rezaei	
16:35 - 16:45			Michael Waletzko	
16:45 -17.00	**Break**		Katja Wiedemann	

Talks of guest speakers **Time slot**	**Programme**	**Speaker**	**Chairperson**
17:00 - 17:30	**Keynote Section 8**	**Prof. Stephan Becker** ***Emerging viruses: Challenges for the Global Village*** **Institute for Virology, Philipps University Marburg**	**Felix Graf**
17:30 - 17:45	**Break**		
17:45 - 18:15	**Keynote Section 10**	**Prof. Stefan Hippenstiel** ***Charité 3R – 3R implementation at a university hospital - How can a medical faculty enhance application of the 3Rs?*** **3R Centre, Charité, Berlin**	**Anna-Lena Proksch**
18:15 - 18:45	**Closing Ceremony**		

Section 1 - Nutrition and Metabolism

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Day 1: Wednesday, September 29th, 2021

Section 1 - Nutrition and Metabolism Chairpersons: Berhard Hellmann & Julia Beranek & Bernhard Hellmann

17:15-17:45	**Prof. Christian Sina** (Universitätsklinikum Schleswig-Holstein, Lübeck, Germany) ***Digital therapeutics based on personalized nutrition — does it work?***
16:00-16:10	**Julia Lisa-Marie Beranek**: *Impact of glyphosate and its formulation Roundup® on proliferation, differentiation and metabolism of equine adipose tissue derived mesenchymal stem cells*
16:10-16:20	**Silvana Hof-Michel**: *Lithocholic acid as a potential modulator of age-associated inflammatory changes in the intestine of Drosophila melanogaster*
16:20-16:30	**Bernhard Hellmann**: *Erinacine C the cure against dementia?*
16:30-16:40	**Stefan Baumanns**: *Octanoic acid attenuates amyloid-β-induced toxicity in an Alzheimer's disease model of the nematode Caenorhabditis elegans through energy supply and reduction of amyoid-β aggregation*
16:40-16:50	**Mengmeng Zhou**: *Glutaredoxin 5 as a novel target for β-cell survival and regeneration*
16:50-17:00	**Alina Lucia Struff**: *Impact of Perfluorobutane Sulfonic Acid (PFBS) and Perfluorooctane Sulfonate (PFOS) on proliferation, differentiation and the metabolism of equine adipose tissue derived mesenchymal stem cells*

T1

OCTANOIC ACID ATTENUATES AMYLOID-B-INDUCED TOXICITY IN AN ALZHEIMER'S DISEASE MODEL OF THE NEMATODE *CAENORHABDITIS ELEGANS* THROUGH ENERGY SUPPLY AND REDUCTION OF AMYOID-B AGGREGATION

Baumanns, S[1], Wenzel, U[1]

[1]*Institute of Nutritional Sciences, Molecular Nutrition Research, Interdisciplinary Research Center, Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany*

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia. The pathogenesis is a complex process, in which the proteotoxicity of amyloid-β (Aβ) was identified as a significant factor. Furthermore, mitochondrial dysfunctions frequently occur at an early stage of the pathogenesis, preceeding the irreversible loss of neurons. Octanoic acid (OA), also known as caprylic acid, is a medium-chain fatty acid, which could contribute to maintain normal mitochondrial function through several pathways. Our aim was to investigate the effect of OA on Aβ-induced toxicity and its underlying mechanisms using the nematode *Caenorhabditis elegans*.

Computer-based analysis of motility was used as a measure of Aβ-induced toxicity in the Aβ-overexpressing strain GMC101. For the investigation of molecular mechanisms, gene knockdowns were induced through RNA-interference by feeding *E. coli* HT115 expressing dsRNA derived from specific *C. elegans* gene fragments. Mitochondrial function was determined by measurement of adenosine triphosphate (ATP) with luciferase reaction and mitochondrial membrane potential (MMP) as well as reactive oxygen species (ROS) with fluorescence microscopy using the fluorescent probes TMRE and MitoTracker Red CM-H2Xros, respectively. Additionally, Aβ-aggregation was measured with the fluorescent probe NIAD-4.

OA was able to improve the motility impaired by Aβ and therefore to attenuate Aβ-toxicity in GMC101. The effect of OA was lost under RNA-interference versus the SDHC ortholog mev-1, which encodes for a subunit of the electron transport chain (ETC) complex II. Furthermore, incubation with OA increased ATP, MMP and ROS-level, while reducing Aβ-aggregation.

In conclusion, this study provides evidence, that OA could be a potential therapeutic agent for AD. Whereas the mechanistic link between OA and reduced Aβ-aggregation needs to be further elucidated, OA attenuates Aβ-induced toxicity by serving as an energy fuel through β-oxidation, metabolization in the citric acid cycle and subsequent ETC.

T2

IMPACT OF GLYPHOSATE AND ITS FORMULATION ROUNDUP® ON PROLIFERATION, DIFFERENTIATION AND METABOLISM OF EQUINE ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

Beranek, J[1], Failing, K[2], Arnhold, S[3], Mazurek, S[1]

[1] *Institute of Veterinary Physiology and Biochemistry, Justus Liebig University of Giessen*
[2] *Unit for Biomathematics and Data Processing, Veterinary Faculty, Justus Liebig University of Giessen*
[3] *Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University of Giessen*

Glyphosate-based formulations are globally used as broad-spectrum herbicides in agricultural practice. A popular and widely used commercial glyphosate-formulation is Roundup®.
In our project, we investigate the impact of glyphosate as pure substance compared to its Roundup® formulation on proliferation, migration, differentiation as well as on the metabolism of equine adipose tissue derived mesenchymal stem cells (ADSC). The multilineage differentiation potential of the ADSC allows investigations in an undifferentiated state as well as during and after the differentiation of the stem cells.
Dose finding studies with undifferentiated proliferating ADSC as well as adipogenic-differentiated stem cells revealed higher IC50-values for the pure substance glyphosate in both cell culture approaches in comparison to the Roundup® -formulation.
Metabolic turnover rate determinations after Roundup® supplementation in the cell culture supernatants showed a significant increase in glucose consumption and lactate production as well as alanine, serine and glutamate release. In contrast, the same concentration of pure glyphosate exhibited no effect on glycolysis or amino acid metabolism when compared to mock treated controls.
If supplemented to the medium during the entire 14-day period of adipogenic differentiation, Roundup® induced an increase in triglyceride synthesis and storage. At the same concentration, pure glyphosate had no effect on adipogenic differentiation.
During osteogenic differentiation pure glyphosate as well as the Roundup®-formulation reduced the calcification level of the osteocytes compared to mock treated controls.

Together these results imply a not inconsiderable influence of solvent-based ingredients in the Roundup®-formulation on the cell division rate, the metabolism and the differentiation of ADSC.

T3

ERINACINE C THE CURE AGAINST DEMENTIA?

Hellmann, B[1], Zorn, H[2], Eckert, G[1]

[1] *Institut für Ernährungswissenschaft, AK Prof. Dr. Eckert, Wilhelmstrasse 20, 35392 Giessen*
[2] *Institut für Lebensmittelchemie und Lebensmittelbiotechnologie, Justus Liebig University Giessen Heinrich-Buff-Ring 17,D-35392 Giessen*

Mitochondria are the power plants of the cell. They have the ability to adapt to cellular bioenergetic changes. A loss of this adaptive response has the potential to compromise cellular function and increases the risk for neuro-degenerative diseases such as Alzheimer's disease. Hericium erinaceus is an edible medicinal fungi used in Asia as a remedy against cognitive impairments. We aimed to test the self-isolated and characterized secondary metabolite erinacnine C on mitochondrial parameters.

Hererefore, we hypothesize an effect of erinacine C via the TrkB receptor. We testes its effect on mitochondrial parameters in a cellular neuronal Alzheimer's disease model. Furthermore, we were interested in the cell viability, neuroplasticity and mitochondrial genes.

ATP levels were elevated for erinacine C in SH-SY5Y-MOCK and SH-SY5Y-APP cells. The mitochondrial genes TFAM and NRF1 showed a model specific response. Additionally we could measure a strong neuritogenesis effect.

We could show Erinacine C promising effects on human neuronal cells and in an Alzheimer's disease model.

T4

LITHOCHOLIC ACID AS A POTENTIAL MODULATOR OF AGE-ASSOCIATED INFLAMMATORY CHANGES IN THE INTESTINE OF *DROSOPHILA MELANOGASTER*

Hof-Michel, S[1], Wagner, AE[1]

[1] *Institute of Nutritional Sciences, Justus Liebig University Giessen, Wilhelmstrasse 20, 35392 Giessen*

In humans, bile acids play an important role in the digestion and absorption of diet-derived lipids. About 95 % of primary bile acids, being produced from cholesterol, undergo enterohepatic circulation whereas the remaining 5 % are converted by colonic bacteria to the secondary bile acids lithocholic acid (LCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA). These secondary bile acids have recently been recognized as signalling molecules, e.g., mediating changes in glucose homeostasis as well as in energy and lipid metabolism.
LCA has been shown to exert anti-proliferative and pro-apoptotic abilities in cancer cells, and a lifespan-prolonging effect in *Caenorhabditis elegans* as well as in the fruit fly *Drosophila melanogaster*. However, the underlying mechanisms for these lifespan-prolonging effects are still unclear. Since annihilation of intestinal bacteria in the fruit fly reversed the lifespan-prolonging effect of LCA, a link to the intestinal microbiome has been suggested. Previous experiments showed that a dysbiosis of the intestinal microbiome is linked to an impaired intestinal barrier function and inflammation, often observed during aging. As also epigenetic effects have been discussed to be centrally involved in the process of aging, we hypothesize that LCA regulates the microbial balance in the intestine and modulates inflammatory processes via epigenetic mechanisms, thus improving the barrier function and subsequently leading to lifespan prolongation. To unravel the potential underlying mechanism, epigenetic marker genes (e.g. histone acetylases and -deacetylases) have been analyzed by qPCR. Furthermore, the so-called Smurf-Assay which provides information on the functional state of the fly's gut barrier has been performed.

T5

REGULATION OF HUMAN AND MICROBIAL TRANSGLUTAMINASES IN THE CONTEXT OF COELIAC DISEASE

Stricker, S[1], Rudloff, S[1,2], de Laffolie, J[1], Zimmer, K-P[1]

[1] *Department of General Pediatrics & Neonatology, Justus Liebig University, Giessen, Germany*
[2] *Institute of Nutritional Sciences, Justus Liebig University, Giessen, Germany*

Enzymatic modification of gliadin peptides by human tissue transglutaminase (TG2) is a central step in Coeliac Disease (CD) pathogenesis as it markedly increases immunogenicity of gliadin peptides which is a precondition for the following immune response. Besides TG2, microbial transglutaminase (mTG) is able to catalyse the same enzymatic reaction and might take place in CD pathogenesis either as food additive or as an active enzyme released by our intestinal microbiota. Our project aimed to investigate potential regulatory mechanisms of both enzymes using an *in vitro* and cell culture-based assay.
Transamidation activity was evaluated either using immobilized mTG and TG2 or the intestinal epithelial cell line Caco2. After treatment with inhibitory or stimulatory substances, transamidation of the substrate 5BP (5-(biotinamido)pentylamine) was quantified using photometric or fluorometric assays.
Using the *in vitro* assay, we could demonstrate redox regulation of TG2 by the proteins ERp57 and TRX1 (124 ± 13 %; $p < 0.05$) as well as a competitive inhibition by the molecule ERW1041 (100 µM ERW1041 61 ± 26 %, $p < 0.01$). The small molecule PX12 showed a strong, direct inhibitory effect on TG2 (53 ± 19 %; $p < 0.05$). In contrast to this, mTG activity remained unaffected by all those substances. Using unpermeabilized Caco2 cells, we could display TG2 activity on the cell surface that could be influenced by ERW1041 (74 ± 7 %; $p < 0.01$), DTT and glutathione.
Here we could demonstrate oxidoreductive as well as competitive inhibitory mechanisms on TG2 activity. The competitive inhibition by ERW1041 appeared to be TG2-specific, as mTG activity remained unaffected. As mTG does not underly a strict regulation, it might increase the load of immunogenic peptides in the gut lumen either as active enzyme released by the intestinal microbiota or by creating neo-epitopes in mTG-treated food products.

T6

IMPACT OF PERFLUOROBUTANE SULFONIC ACID (PFBS) AND PERFLUOROOCTANE SULFONATE (PFOS) ON PROLIFERATION, DIFFERENTIATION AND THE METABOLISM OF EQUINE ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

Struff, A[1], Failing, K[2], Arnhold, S[3], Mazurek, S[1]

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[2] *Unit for Biomathematics, Justus Liebig University, Frankfurter Strasse 95, 35392 Giessen*
[3] *Institute for Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Frankfurter Strasse 94, 35392 Giessen*

Perfluorobutane sulfonic acid (PFBS) is used as a short chain replacement for the toxic long chain surfactant Perfluorooctane sulfonate (PFOS), both of which belong to the group of Perfluorinated alkyl substances (PFAS). PFAS are ubiquitous organic compounds of anthropogenic origin that confer dirt-repellent, waterproof and non-stick properties when added to consumer goods.
A dose finding study with undifferentiated equine adipose tissue derived mesenchymal stem cells (ADSC) revealed an about six times higher IC50 value for PFBS (1400µM) in comparison to PFOS (240µM). This result shows that PFOS has a much stronger inhibitory effect on the division activity of the ADSC compared to the short chain PFBS.
Measurements of the metabolic conversion rates of the culture medium supernatants revealed a significant increase of alanine production when cells were treated with 240µM PFOS. In presence of 240µM PFBS an increase of both glucose and glutamate consumption was observed whereas treatment with 1400µM PFBS led to a shift from an aspartate production to a significant aspartate consumption compared to the control group.
The IC50 concentrations of PFOS and PFBS were supplemented into the medium during the entire period of adipogenic (14 days) and osteogenic (21 days) differentiation. During adipogenic differentiation, both PFOS and PFBS induced an about 40 to 50 percent reduction of the cell number/well. Moreover, adipogenesis of PFOS and PFBS exposed ADSC was enhanced and cellular lipid accumulation significantly increased in comparison to mock-treated ADSC.
Quantification of Alizarin-Red staining of the osteogenic differentiation showed a more inhibitory effect of PFOS on the calcification of ADSC than PFBS.

Furthermore, the analysis of the effect on cell migration in the presence and absence of PFOS and PFBS is in progress.

T7

ANTHELMINTIC ACTIVITY OF BIARYLALKYL CARBOXYLIC ACIDS AGAINST *SCHISTOSOMA MANSONI* - CHARACTERISATION OF AN ALDOSE REDUCTASE AS A POTENTIAL TARGET PROTEIN

Überall, ME[1], Czermak, P[2,3], Grevelding, CG[1]

[1] *Justus Liebig University Giessen, BFS, Institute of Parasitology, Giessen, Germany*
[2] *Institute of Bioprocess Engineering and Pharmaceutical Technology (IBPT), Technische Hochschule Mittelhessen (THM) - University of Applied Sciences, Giessen, Germany*
[3] *Justus Liebig University Giessen, Faculty of Biology and Chemistry, Giessen, Germany*

Schistosomiasis, caused among others by the parasitic trematode species *Schistosoma mansoni*, leads to chronic inflammation and finally liver fibrosis. If untreated, the disease can cause life-threatening complications. The inflammation in the liver and other organs, such as the gut, is caused by the eggs of the parasite. The current treatment of schistosomiasis is based on a single drug, Praziquantel (PZQ). Due to the frequent use of PZQ, there is upcoming fear of emerging resistance. Therefore, it is necessary to find alternative drugs.
A structural approach for active compounds against schistosomes are biarylalkyl carboxylic acid derivatives (BACADs). In pilot experiments with BACADs, *in vitro* cultured schistosomes have shown phenotypic changes such as reduced pairing stability, reduced egg production rates, and/or tegumental damage. The structures of BACADs are based on a series with eight inhibitors of the human aldose reductase (AR). Besides other genes coding for proteins with detoxifying functions, an orthologue of AR (Smp_150700) was found in the genome data for *S. mansoni*. This enzyme is the focus of this work as potential drug target. In a previous study with adult S. japonicum, an AR inhibitor decreased the vitality of the worms. This study reinforced the interest in AR as a potential drug target in *S. mansoni*.
First results aim at the recombinant expression of SmAR in BL21(DE3) *E. coli* and LOBSTR-BL21(DE3)-RIL *E. coli* and the optimisation of yield and purity of the recombinant protein. For future characterisation of SmAR, an enzyme assay will be established. Furthermore, the molecular characterization of SmAR is planned, which includes gene expression and localization experiments in adult and larval *S. mansoni*.

T8

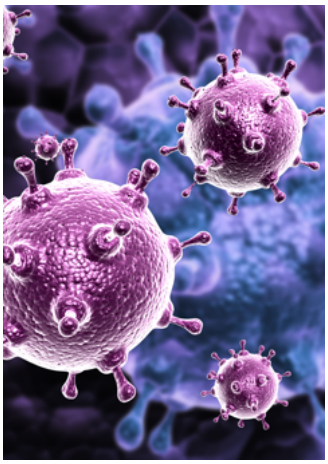
GLUTAREDOXIN 5 AS A NOVEL TARGET FOR B-CELL SURVIVAL AND REGENERATION

Zhou, M[1], Petry, SF[1], Linn, T[1]

[1] *Clinical Research Unit, Center of Internal Medicine, Justus Liebig University, 35392 Giessen, Germany.*

Insulin-producing pancreatic β-cells are crucial for the maintenance of glucose homeostasis in mammals. Glutaredoxin 5 (Glrx5) is a mitochondrial enzyme of the group of thioredoxin proteins that exerts essential tasks for the respiratory chain and cellular iron homeostasis. There is evidence suggesting that iron overload is the main pathological factor behind the Glrx5-deficiency. Since iron promotes the production of ROS, Glrx5-deficiency eventually leads to iron-induced apoptosis, which is entitled "ferroptosis". Previous research has shown that a deficiency of Glrx5 is associated with impaired insulin secretion and β-cell decay in diabetic mice model and murine β-cells (MIN6). Therefore, the current study was designed to confirm the previously gathered data regarding the potential protective benefit of Glrx5 on β-cells and gain new insights into the underlying mechanisms by employing a Glrx5-overexpressing mouse model as well as human β-cell-cultures (EndoC-βH3).
Islets of Glrx5-overexpressing mice presenting with uncontrolled diabetes will be assessed in terms of Glrx5 and insulin content of the islets, islet mass, and insulin secretion, ROS production, and markers of ferroptosis. EndoC-βH3 cell function, Glrx5 expression, ROS production, and markers of ferroptosis will be analyzed after exposure to glucotoxicity, lipotoxicity, and a mix of inflammatory cytokines.
So far, breeding of homozygous (Glrx+/+) mice and wild-type controls (Glrx5WT/WT) has been successfully conducted in our animal facility. It has been ensured that expression of Glrx5, Ins1, and Ins2 mRNA expression as well as Glrx5 and insulin protein levels in islets of homozygous animals are elevated. Also, the EndoC-βH3 cell line is being cultured. While no concrete results have yet been collected, it is expected that our observations on MIN6 cells can be confirmed in human β cells. Then, this will allow further study of the Glutaredoxin-regulated pathways involved in β-cell survival and regeneration.

Section 2 - Infection and Immunity



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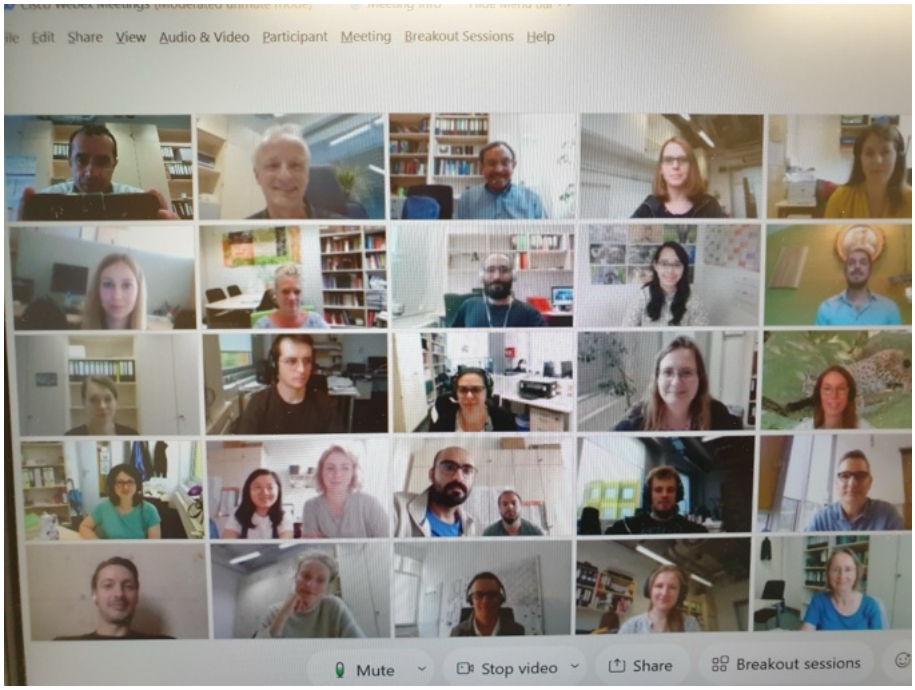

Day 2: Thursday, September 30th, 2021

Section 2 - Infection and Immunity

Chairpersons: Yukino Kobayashi &
N.N. &
Oliver Puckelwaldt &
Svenja Gramberg &
Mohammed Samer Shaban

09:45-10:15	**PD Dr. Michael Mühlebach** (Paul Ehrlich Institute, Langen, Germany) ***Platform-based Vaccines – Short-cut to Protection***
11:15-11:25	**Lu Liu**: *Functional impact of a specific M1 protein phosphorylation site on influenza A virus propagation*
11:25-11:35	**Benadict Vincent Albert**: *Functional analysis of human genes regulated by HCoV-229E using genome-editing by CRISPR-Cas9*
11:35-11:45	**Mohammed Samer Shaban**: *Chemical ER Stress Inhibits Replication of three human Coronaviruses in Cultured Cells*
11:45-11:55	**Xuesong Li**: *Identification of GPCR-neuropeptide interaction and their functional analysis in Schistosoma mansoni*
11:55-12:05	**Svenja Gramberg**: *2D transcriptome analyses of tissues in the liver fluke Fasciola hepatica*
12:05-12:15	**Mudassar Mughal**: *First Insights into the Autophagy Machinery of Adult Schistosoma mansoni*
12:15-12:25	**Monique Evelyn Überall**: *Anthelmintic activity of biarylalkyl carboxylic acids against Schistosoma mansoni - Characterisation of an aldose reductase as a potential target protein*
13:15-13:25	**Mandy Beutler**: *Expression of Schistosoma mansoni proteins in E. coli and HEK cells*
13:25-13:35	**Max Möscheid**: *First insights in the role of the pairing-dependently and ovary-preferentially expressed thyroid hormone receptor (THRβ) of Schistosoma mansoni in the development of vital eggs*
13:30-13:40	**Philipp Wolf**: *Cholinergic Regulation of ATP-mediated Release of the Pro-Inflammatory Cytokine Interleukin-1β – Comparison between healthy Volunteers and Crohn's disease Patients*
13:35-13:45	**Yukino Kobayashi**: *Contribution of unique regions of Alp1 and Alp2b in Plasmodium transmission*
13:40-13:50	**Maria Wille**: *Site-directed mutagenesis of epidemic antibiotic-resistance plasmids to understand their success strategies*
13:45-13:55	**Eric Springer**: *Real-time measurement of ATP dynamics in Plasmodium falciparum using genetically encoded fluorescent probes*
13:50-14:00	**Dordia Anindita Rotinsulu**: *Analysis of whole genome sequencing data of Streptococcus equi ssp. equi isolates from equines*
13:55-14:05	**Hicham Houhou**: *Identification of aldehyde dehydrogenase as novel anthelmintic target in the liver fluke Fasciola hepatica*
14:00-14:10	**Parviz Ghezellou**: *Comparative Venomics of Medically Important and Poorly Known Endemic Iranian, Macrovipera razii, and Cypriot, Macroviper lebetina lebetina, Giant Vipers*
14:05-14:15	**Oliver Puckelwaldt**: *High-resolution transcriptomics of the liver fluke Fasciola hepatica*
14:10-14:20	**Jasmin Bazant**: *The effect of hydrogen peroxide and the antioxidant ebselen on the activity of the pore forming toxin pneumolysin*
14:20-14:30	**Pia Franziska Marie Naujack**: *Site-directed mutagenesis elucidates molecular IgE-IPSE/alpha-1 interaction responsible for basophil activation*
14:45-14:55	**Juan Velez**: *Cryptosporidium parvum infection triggers rapid changes in metabolic signatures of bovine small intestinal explants*
14:55-15:05	**Daniela Grob Guerra**: *Sarcoptes scabiei induces oxidative responses and calcium efflux but weak NET formation in bovine polymorphonuclear neutrophils*
15:05-15:15	**Lisa Segeritz**: *Autochthonous metastrongyloid lungworm infections in wild Eurasian lynx (Lynx lynx) in Germany and new insights into gastrointestinal protozoan and helminth fauna*
15:15-15:25	**Camilo Larrazabal**: *Ca++ signaling in Besnoitia besnoiti tachyzoite invasion and development in primary bovine host endothelial cells*
15:25-15:35	**Selina Ohl**: *Immune-metabolic changes in the synovium that define remission in arthritis*
15:35-15:45	**Corinna Heck**: *Characterization of fibroblast-mediated alteration of neovascularization in SCID mouse model of rheumatoid arthritis*

K2

PLATFORM-BASED VACCINES – SHORT-CUT TO PROTECTION

Dr. Michael D. Mühlebach[1]

[1]Paul-Ehrlich-Institut, Bundesamt für Impfstoffe und biomedizinische Arzneimittel, Div. Veterinary Medicine

The current COVID-19 pandemic illustrates the need for fast availability of safe and effective vaccines, when a significant pathogen fast spreads world-wide from its wild-life pool. Well described for flu pandemics with few interlaying decades in the past, respiratory pathogens will be a constant future challenge even long after the situation around SARS-CoV-2 has normalized.
Without a preformed preparedness of the scientific and regulatory community in the past, it would have been impossible to shrink vaccine development times from 10 to 15 years to less than 1 year. This presentation will outline the vaccine development concept that allowed the fast development of the highly effective and safe available COVID-19 vaccines: The use of platform technologies that can be adapted to different emerging pathogens.
Using measles vaccine virus (MeV) as an example, this talk will outline why and how such well known and tried and tested vaccines can be used to also protect against other diseases of interest. The measles vaccines are among the most effective vaccines utilized so far, which would permit eradication of this most contagious disease. Recombinant MeV can be modified to additionally present foreign antigens to the immune system after vaccination, and thereby trigger immune responses that can protect against the secondary emerging pathogen of choice. Using this example, the presentation will visualize what is needed for prove of concept of such a vaccine, and why the development in general can progress on virtually another time-scale without impairing the thoroughness of the process.

T9

FUNCTIONAL ANALYSIS OF HUMAN GENES REGULATED BY HCOV-229E USING GENOME-EDITING BY CRISPR-CAS9

Albert, BV[1], Werner, S[1], Meier-Sölch, J[1], Mayr-Buro, C[1], Weiser, H[1], Shaban, MS[1], Hain, T[3], Schmitz, L[2], Kracht, M[1]

[1] Rudolf Buchheim Institute of Pharmacology, Justus Liebig University, Giessen, Germany
[2] Institute of Biochemistry, Justus Liebig University, Giessen, Germany
[3] Institute of Medical Microbiology, Justus Liebig University, Giessen, Germany

Human coronavirus 229E is a member of the genus Alphacoronavirus associated with mild upper respiratory tract infections. The virus replicates in the host cell cytoplasm and triggers immunomodulatory and ER-associated stress responses by poorly characterized mechanisms. To obtain more insight into the functional relevance of human genes in viral replication, we used an unbiased set up to identify the genes needed for HCoV-229E replication based on the genome-scale CRISPR-CAS9 knockout (GECKO) lentiviral sgRNA library consisting of more than 100,000 sgRNAs targeting the human genome with six sgRNAs per gene. We have successfully generated stably transduced Huh7 cells with the GECKO library. The screening strategy was then based on analyzing the enriched integrated sgRNAs by deep sequencing in the cells surviving HCoV-229E infection. So far, our deep sequencing data identified numerous genes which are required for replication and, or cell survival. Further validation of these hits will provide insights into their functional relevance in context to different stages of CoV replication (viral entry, viral-host response, and budding).

T10

THE EFFECT OF HYDROGEN PEROXIDE AND THE ANTIOXIDANT EBSELEN ON THE ACTIVITY OF THE PORE FORMING TOXIN PNEUMOLYSIN

Bazant, J[1], Abu-Mraheil, M[1], Chakraborty, T[1]

[1]Institute for Medical Microbiology, Biomedizinisches Forschungszentrum Seltersberg, Justus Liebig University, Schuberstrasse 81, 35392 Giessen

The human pathogen Streptoccocus pneumoniae (Spn) is the main cause of community-acquired pneumonia. The WHO estimates that pneumococcal infections are responsible for 1.6 million deaths each year, including approximately 716,000 deaths among children < 5 years of age. Spn releases two major extracellular virulence factors Pneumolysin (PLY), a member of the family of Cholesterol-dependent cytolysins (CDCs) and Hydrogen peroxide (H_2O_2). Both virulence factors induce epithelial and endothelial barrier damage in the lung during infection, which in turn promotes spread of Spn. The reduction of PLY significantly enhances its hemolytic activity. This suggests that the modification of the unique cysteine residue in PLY may considerably alter the activity of the toxin. Although the production of high amounts of H_2O_2 by Spn is known to be important for Spn infection, little is known about how or whether H_2O_2 affects the activity PLY.
We found that addition of H_2O_2 decreases the activity

of purified PLY. This can be a result of sulfenylation of the unique cysteine residue in PLY and/or by increased oxidation of PLY due to elevated H_2O_2 concentration in the medium. Addition of ebselen (EB), an organoselenium compound that degrades H_2O_2 by consuming GSH (gluthathione) and can form selenosulfides with thiol groups of cysteine-containing proteins, enhanced the PLY activity by degradation of H_2O_2. Our findings show that EB inhibits the growth of Spn, while Spn deletion mutants with impaired H_2O_2 production were found to be less sensitive to EB.
In preliminary experiments, we found that the substitution of unique cysteine residue of the pore-forming toxin Listeriolysin O (LLO) abolished the inhibition effect of H_2O_2. Therefore, we assume that cysteine modification caused by H_2O_2 (sulfenylation) is the reason for the decreased PLY activity in the presence of H_2O_2.

T11

EXPRESSION OF *SCHISTOSOMA MANSONI* PROTEINS IN *E. COLI* AND HEK CELLS

Beutler, M[1], Moreira, B[1], Falcone, FH[1], Grevelding, CG[1]

[1] *Institute for Parasitology, Justus Liebig University Giessen, Schubertstr. 81, 35392 Giessen*

For successful protein expression, the choice of a suitable expression system is important. Here, we show different protein expression attempts (expression in *E. coli* and HEK cells) for *Schistosoma mansoni* aldehyde dehydrogenase (SmALDH_022), tyrosine kinase 6 (SmTK6) and abelson-like kinases 1 and 2 (SmAbl1 and 2). We cloned the SmAbl sequences in full length and additionally as tyrosine kinase domain (TKD) into expression vectors for the HEK cell system and *E. coli*. Expression of SmAbl's succeeded only as TKD's in *E. coli*. The fusion of SmALDH_022 and SmTK6 to the maltose binding protein showed no increase of soluble protein in the lysate fraction.

T12

COMPARATIVE VENOMICS OF MEDICALLY IMPORTANT AND POORLY KNOWN ENDEMIC IRANIAN, MACROVIPERA RAZII, AND CYPRIOT, MACROVIPER LEBETINA LEBETINA, GIANT VIPERS

Ghezellou, P[1], Dillenberger, M[2], Kazemi, SM[3], Spengler, B[1]

[1] *Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany*
[2] *Biochemistry and Molecular Biology, Interdisciplinary Research Center, Justus Liebig University Giessen, Germany*
[3] *Zagros Herpetological Institute, Qom, Iran*

Venomous snakes include the largest and medically most important venomous animals, causing the majority of envenoming and fatalities in humans and their domestic animals. There are at least 1.8–2.7 million human snakebites worldwide annually, resulting in more than 130,000 deaths. More recently, the World Health Organization (WHO) recognized snakebite envenoming as a "priority category A neglected tropical disease (NTD)". Yet, despite the considerable effort of WHO, the snakebite as the world's most lethal NTD remains a hidden health crisis. Increasing knowledge about the main components of snake venoms is key to improving our understanding of clinical pharmacology and the efficiency of antivenom, providing valuable information on essential toxins that necessitate neutralizing by antivenom. Here, we report the comparative proteomic characterization of the venoms of adult specimens of medically important and poorly known two giant vipers from Iran, Macrovipera razii, and Cyprus, Macroviper lebetina lebetina, which belong to the same genus, Macrovipera. The crude venoms were de-complexed in two steps, reverse-phase HPLC fractionation and the analysis of each chromatographic fraction via SDS-PAGE. Subsequently, the protein spots were digested with trypsin, and the resulting peptides were analyzed via liquid chromatography high-resolution tandem mass spectrometry (LC-HR-MS/MS). The obtained data showed that both viper's venom proteomes comprised a complex arsenal of peptides and proteins. Among the venom proteins, snake venom Zn^{2+}-dependent metalloproteinases (SVMPs) represent the most abundantly expressed gene family, following serine proteinases (svSPs), phospholipases A2 (PLA2), and C-type lectin-like (CTLs) toxin families. They are considerably involved in the clinical observation of Macrovipera species envenoming, including disrupting the extracellular matrix of the vascular subendothelium (SVMPs), preventing blood clots (svSP), platelet aggregation (e.g., CTLs), and hemolytic or

myotoxic effects (PLA2).

T13

2D TRANSCRIPTOME ANALYSES OF TISSUES IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Gramberg, S[1], Puckelwaldt, O[1], Häberlein, S[1]

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The liver fluke *Fasciola hepatica* is a parasitic flatworm, which causes chronic liver disease in humans and animals, and which leads to huge economic losses in the livestock industry worldwide. As therapeutic options are extremely limited and resistance to commonly used drugs is increasing, the identification of new drug targets is needed. To do this, it is first necessary to better understand the flukes' biology, including its metabolic and developmental adaptions inside a host. Of particular interest are the surface layers of the parasite: The tegument and the branched intestine are of substantial metabolic activity, both exposed to the hosts' environment and essential for fluke development and viability. Therefore, these are interesting targets for drug development.

While global transcriptomics studies on *F. hepatica* are well established, knowledge on tissue-specific gene expression is very limited and has been based on studies of single genes. Performing Spatial Transcriptomics on *F. hepatica* will allow deep insights into tissue-specific gene expression patterns throughout the flukes' body. This cutting-edge method will be applied for the first time in helminth research. During the workflow, glass slides covered with thousands of oligonucleotide-coated spots are used to bind mRNA from a tissue section. mRNA is then reverse-transcribed and sequenced. Finally, RNA-sequencing data is combined with histological data from the tissue section, which is made possible by a spatial barcoding technology, that allows back-mapping of each mRNA to a specific spot on the slide. As a result, the whole transcriptome can be visualized in 2D, according to the original morphological context.

Spatial gene expression of selected genes will be independently confirmed by *in situ* hybridization. Whether candidate genes are essential for gut development and fluke viability will be studied with post-genomic tools such as RNA interference. Some of these genes could be later explored as potential drug targets.

T14

SARCOPTES SCABIEI INDUCES OXIDATIVE RESPONSES AND CALCIUM EFFLUX BUT WEAK NET FORMATION IN BOVINE POLYMORPHONUCLEAR NEUTROPHILS

Grob, D[1], Conejeros, I[1], Larrazabal, C[1], Lopez-Osorio, S[1,2], Velásquez[1], ZD[1,2], Taubert, A[1], Hermosilla, C[1]

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[2] *Grupo de Investigación CIBAV, Universidad de Antioquia UdeA, Medellín 050034, Colombia*

Sarcoptes scabiei is the causal agent of sarcoptic mange. The life cycle includes four stages which develop in the skin of hosts including humans and animals. Clinical symptoms develop as consequence of allergic reactions leading to severe dermatitis, pruritus and exudation. Based on its dermal localization, *S. scabiei* is constantly exposed to different components of the innate immune system of the host, such as epithelial cells and phagocytes. In this context, the direct contact between polymorphonuclear neutrophils (PMN) and *S. scabiei* mites will inevitable occur. PMN use different effector mechanism against foreign pathogens: phagocytosis, degranulation of antimicrobial molecules and neutrophil extracellular trap (NET) formation. Aim of current work was to characterize early responses of bovine PMN against both, S. scabei mites and antigens (*Sc*Ag). Bovine PMN were isolated from peripheral bovine blood and exposed to *S. scabiei* stages and *Sc*Ag. Immunofluorescence analyses detecting DNA (DAPI), histones and neutrophil elastase (NE) were performed to demonstrate the presence of NET. *Sc*Ag-induced PMN activation was estimated on the level of oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) via Seahorse technology (Agilent). Calcium signals were fluorimetrically detected using the Ca++-sensitive dye fluo-4 (Invitrogen). Live cell 3D tomographic microscopy (3D Explorer, Nanolive) was performed to analyze *Sc*Ag-induced morphological changes in PMN.
Immunofluorescence analyses revealed that *S. scabiei* mites and *Sc*Ag were not able to strongly induce NET formation; nevertheless, stimulation with *Sc*Ag resulted in effective PMN activation as documented by a rapid induction of neutrophil oxidative responses on the level of oxygen consumption and proton efflux. Furthermore, *Sc*Ag stimulation induced a short increase in Ca++-driven signals over time in PMN. In contrast, mite- or *Sc*Ag-driven NETosis could only be detected on a very low level.

T15

CHARACTERIZATION OF FIBROBLAST-MEDIATED ALTERATION OF NEOVASCULARIZATION IN SCID MOUSE MODEL OF RHEUMATOID ARTHRITIS

Heck, C[1], Kürsammer, D[1], Frommer, K[1], Arnold, M[1], Rehart, S[2], Müller-Ladner, U[1], Neumann, E[1]

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[2] Dept. of Orthopedics and Trauma Surgery, Agaplesion Markus Hospital, Frankfurt, Germany

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune-mediated disease that is primarily characterized by inflammation of the synovial tissue and subsequent destruction of cartilage and underlying bone. RA synovial fibroblasts (RASF) actively contribute to joint destruction through production of cytokines, chemokines and matrix-degrading enzymes and by actively migrating to and invading into joint cartilage. Matrix degradation leads to a release of matrix proteins such as the collagen-fragments endostatin, arresten and canstatin, known as endogenous angiogenic inhibitors. Neovascularization in RA is increased and results from insufficient supply of oxygen and nutrients as well as the inflammatory environment in the synovial tissue. Neovascularization includes processes such as angiogenesis and arteriogenesis, which are based on endothelial cell (EC) activation, proliferation and sprouting. In the SCID mouse model of RA, RASF induce the formation of helix-like vessels. To analyse specific RASF/EC interactions and angiogenesis *in vitro*, a 2D tube formation assay with human umbilical vein endothelial cells (HUVEC) on matrigel coating was used. Effects of the collagen-fragments on tube formation in mono- and co-culture with RASF were tested. For immunohistochemical evaluation of the helix-like vessels, the vascular markers angiopoietin-2 and ephrin-B2 were established. Our results demonstrate that RASF directly influenced tube formation by arranging into the tubes. Stimulation of HUVEC with endostatin as well as with arresten resulted in disturbed tube formation, while addition of RASF attenuated the endostatin effect. Canstatin stimulation did not show any effect on tube formation in monoculture, but resulted in disturbed tube formation in co-culture. To further analyse the effects of collagen-fragments on RASF/EC interactions in RA neovascularization, we will perform functional assays regarding migration and adhesion. We will further test them in the SCID mouse model of RA concerning the formation of RASF-induced helix-like vessels.

T16

IDENTIFICATION OF ALDEHYDE DEHYDROGENASE AS NOVEL ANTHELMINTIC TARGET IN THE LIVER FLUKE *FASCIOLA HEPATICA*

Houhou, H[1], Rennar, G[2], Gallinger, T[2], Mäder, P[2], Hardt, M[3], Grünweller, A[2], Strube, C[4], Maule, A[5], Schlitzer, M[2], Grevelding, GC[1], Häberlein, S[1]

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[5]Parasitology & Pathogen Biology, The Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK

The liver fluke *Fasciola hepatica* is a threat for both animal and human health. Fasciolosis causes economic losses in cattle and sheep farming worldwide, and it has been listed as a neglected tropical disease of humans. Several reports showed the spread of resistance against the main effective drug, triclabendazole. Therefore, finding alternative treatment options is highly demanded.
Aldehyde dehydrogenases (ALDHs) are involved in cellular detoxification of reactive aldehydes. Our real-time qPCR analyses have shown that ALDH orthologues are expressed by various life stages of *F. hepatica*. Among others, we detected ALDH transcripts in gonadal and gastrodermal tissues using *in situ* hybridization. Therefore, we next assessed the possible fasciolicidal effects of the known ALDH inhibitor disulfiram, an approved drug for use in humans. Newly excysted juveniles (NEJs), immature, and adult flukes were exposed for three days to disulfiram or chemically optimized derivatives with lower cytotoxicity *in vitro*. Disulfiram in a concentration of 20 μM already led to severe effects on NEJ motility. Overall efficiency was even enhanced when a novel disulfiram derivative was used, which showed severe effects on NEJs already at 2 μM. This derivative exhibited improved efficacy also against immature and adult flukes. Furthermore, real-time qPCR analyses of treated adults revealed a significant increase in the expression of oxidative stress genes such as SOD and SODex as well as both ALDH orthologues, which might reflect a potential mode of action. Next, the specificity of the inhibitors on our target enzymes will be tested in an enzymatic assay. For this aim, both orthologues were successfully cloned and expressed.
Our findings suggest that ALDH may represent a potential target and disulfiram a promising basis for the

design of novel anti-fasciolid compounds.

T17

CONTRIBUTION OF UNIQUE REGIONS OF ALP1 AND ALP2B IN *PLASMODIUM* TRANSMISSION

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Plasmodium is an apicomplexan parasite that causes malaria. The infection spreads through the bite of infected *Anopheles* mosquitoes, where the highly motile form of the parasite is deposited into the human host. For the completion of its life cycle, both mosquito and human stages are critical. Therefore, blocking the transmission between these hosts is an effective measure to supress malaria infections. In my project, two Actin-like proteins are being investigated; Alp1 and Alp2b. Both are apicomplexan-specific proteins and we have previously shown these to be critical for the parasite's motility and sexual development in transmission stages. In comparison to canonical actin, which is also known as a central component of the parasite motility, Alp1 and Alp2b have very divergent amino acid sequence identity (< 34 %). Furthermore, they also contain several insertion and deletion sites that are highly conserved across the *Plasmodium* species. The aim of this study is therefore to 1) elucidate the protein structures by crystallography and conduct biochemical functional assays, 2) observe *in vivo* localisation of the proteins using fluorescent tags and 3) generate mutants of those unique insertion and deletion regions to study their impacts on the parasite phenotypes, in order to ultimately understand their roles during the transmission and potential properties as a transmission-blocking drug target. So far, despite the successful expression of Alp1 and Alp2b in *E. coli*, their insolubilities posed major challenges for the IMAC purification. Although the utilisation of lysis detergents such as FC12 significantly improved the overall solubility, it affected the quality of the proteins and could potentially inhibit their activities. In the meantime, a stable *P. berghei* culturing system was successfully established. The robustness of the system was verified through repetitive exflagellation and motility assays, in which the results were comparable to our previous study and literature values.

T18

CA++ SIGNALING IN *BESNOITIA BESNOITI* TACHYZOITE INVASION AND DEVELOPMENT IN PRIMARY BOVINE HOST ENDOTHELIAL CELLS

Larrazabal, C[1], Grob, D[1], Velasquez, ZD[1], Hermosilla, C[1], Taubert, A[1], Conejeros, I[1]

[1] *Institute of Parasitology, Biomedical Research Center Seltersberg, Justus Liebig University Giessen*

Besnoitia besnoiti is the causal agent of bovine besnoitiosis characterized by massive cyst formation in parasitized skin and mucosa. During early phase of infection, fast proliferating tachyzoites invade and replicate within primary host endothelial cells in a Ca++-dependent mechanism. In non-excitable cells, extracellular signals activate the inositol-triphosphate/calcium (InsP3/Ca++) pathway, dependent of the phospholipase C (PLC) activation and increase of IP3 levels inducing Ca++ release from intracellular stores. Despite understanding of Ca++ signalling, less is known in coccidian infections. Aim of this study was to characterize possible role of InsP3/Ca++ pathway during *B. besnoiti* invasion and Ca++ dynamics during intracellular development in bovine primary endothelial cells (BUVEC).

The InsP3/Ca++ signalling during invasion was evaluated by infection rate (i.r.) estimation of tachyzoites pre-treated with Ca++ chelators (BAPTA and EGTA) or PLC inhibitors (U73187 and D609). Moreover, Ca++ signals were fluorimetrically evaluated in free tachyzoites loaded with Fluo-4AM and stimulated with PLC activators (m-3m3FBS or ethanol) in presence of PLC inhibitors. Additionally, Ca++ dynamics during tachyzoite replication was determined in *B. besnoiti*-infected BUVEC loaded with Fluo-4AM at different time points post infection (p.i.).

Current data show that treatments with BAPTA but not EGTA affected the invasion process reducing the i.r. by 85.4 ± 9.3, thus suggesting that intracellular Ca++ sources are relevant for invasion. In line, treatments with U73187 and D609 reduced i.r. by 79.3 ± 9.4 % and 49.7 ± 8.9 %, demonstrating that PLC signaling is critical for host cell invasion. Additionally, we found that m-3M3FBS induced a PLC-independent Ca++ flux, thereby limiting its usage as PLC activator. Finally, we observed an increase in total Ca++ signal in *B. besnoiti*-infected BUVEC over time, since the signal was mainly originated from tachyzoites within infected host cells we suggest a parasite replication-driven process.

T19

IDENTIFICATION OF GPCR-NEUROPEPTIDE INTERACTION AND THEIR FUNCTIONAL ANALYSIS IN *SCHISTOSOMA MANSONI*

Li, X[1], Weth, O[1], Haimann, M[1], Grevelding, CG[1]

[1]*Institute for Parasitology, Biomedical Reserach Center Seltersberg, Justus Liebig University Giessen*

Schistosomes are parasitic flatworms that cause one of the most prevalent but neglected infectious diseases, schistosomiasis. Standard treatment of schistosomiasis relies on a single drug, praziquantel. Neuropeptides are important messenger molecules that act via G protein-coupled receptors (GPCRs) as neurotransmitters, neuromodulators, or hormones in the nervous system. Due to GPCR's pharmacological importance, and proven drug ability, GPCRs are promising targets for new anthelmintics.
Comparative transcriptomics of paired and unpaired worms and their gonads revealed 59 differentially regulated GPCR genes putatively involved in *Schistosoma mansoni* neuronal processes. According to the current analysis, 23 of 27 listed *S. mansoni* neuropeptide precursor (Sm_npp) genes expressed in adult *S. mansoni* show higher transcript levels in the head part of males (paired or unpaired) and unpaired females, which possibly indicates a function in the nervous system. However, our knowledge of *S. mansoni* GPCRs and their ligands are still fragmentary. Goal of this study is to confirm Sm_npp-GPCR interactions by biochemical methods and to characterize the appropriate partners at the molecular level including functional analysis.
To this end, we analyzed the function of a presumptive rhodopsin orphan receptor (Smp_084270), and its candidate ligands (Smp_071050) and (Smp_004710) by double-stranded RNA (dsRNA)-mediated RNA interference (RNAi) and subsequent phenotype studies. The results showed a significant knockdown of gene transcripts by quantitative real-time PCR. Phenotypically, we observed a substantial decline in egg production compared with the untreated control group. Consistent with the decreased egg production, CLSM analyses revealed morphologic changes in the gonads of both sexes. However, no detectable differences were observed in the pairing stability of dsRNA-treated schistosome couples.
These results suggest that the rhodopsin orphan receptor (Smp_084270) and its candidate ligands (Smp_071050) and (Smp_004710) exert a direct effect at the level of male-female molecular communication to control or at least influence the reproductive biology of female *S. mansoni*.

T20

FUNCTIONAL IMPACT OF A SPECIFIC M1 PROTEIN PHOSPHORYLATION SITE ON INFLUENZA A VIRUS PROPAGATION

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Phosphorylation is a post-translational modification known to regulate the functions of proteins including proteins encoded by influenza A viruses (IAV). We previously compared the Ser/Thr and Tyr phosphoproteomes from the mouse-adapted SC35M virus or its non-adapted SC35 counterpart at early and late time points after infection of murine lung epithelial cells. The results identified 12 novel phosphorylation sites and confirmed 8 previously identified sites on several viral proteins. We then analyzed all published phosphorylation sites described to date, and comprehensively collected their structural context. From this analysis we selected several highly conserved phosphorylation sites with a functional implication. Using reverse genetics, subcellular fractionation and confocal laser-scanning microscopy we here provide experimental evidence that a specific phosphorylation site in the M1 of SC35M affects intra-cellular M1 transportation. Compared to wild type M1 the loss of this phosphorylation site affects the cytoplasmic and nuclear accumulation of M1 and NP, thereby leading to the failure of viral rescue. Furthermore, a proteomics-based screen using antibody-based affinity purification coupled to mass spectrometry was performed to reveal the effects of this M1 phosphorylation site on protein/protein interactions. Overall, our data reveals a functional impact of a specific phosphorylation site on IAV replication by regulating intracellular localization and trafficking of IAV proteins.

T21

FIRST INSIGHTS IN THE ROLE OF THE PAIRING-DEPENDENTLY AND OVARY-PREFERENTIALLY EXPRESSED THYROID HORMONE RECEPTOR (THRB) OF *SCHISTOSOMA MANSONI* IN THE DEVELOPMENT OF VITAL EGGS

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Schistosoma mansoni, a parasite of humans and animals, is the cause of the widespread infectious disease schistosomiasis. Schistosomes have a complex life cycle, which is typical for trematode parasites. Furthermore, the female schistosome reaches sexual maturity only if constantly paired with a male, which is subject of ongoing studies. Comparative transcriptomic studies of adult schistosomes and their (isolated) gonads have uncovered among others that genes coding for nuclear receptors (NRs) are regulated in a pairing-dependent manner. In addition, some of these NRs seemed to be expressed in a tissue-specific manner with a preference for the female ovary. Members of the nuclear receptor superfamily are ligand-activated transcription factors that play diverse roles in cell differentiation and development, cell proliferation, and cell metabolism, emphasizing their biological importance. The binding of the ligand to its corresponding nuclear receptor results in transactivation of specific genes within a target tissue, which we aimed to characterize in more detail. In vertebrate systems, it has been described that NR-subfamily members are associated with post-embryonic development and adult homeostasis. However, recent studies have shown that retinoic acid (RA) and thyroid hormones (TH) are also involved in embryogenesis. In recent experiments, we focused on a potential but yet uncharacterized thyroid hormone receptor β (THRβ), for which we obtained evidence for its involvement in early embryogenesis.

To characterize THRβ we performed WISH, and RNAi knock-down experiments, which showed a potential role of THRβ for egg formation and embryogenesis. To further characterize THRβ, inhibitor assays employing DIPTA (a thyroid hormone analog) are planned. Since THRs and RARs bind their target sequence as heterodimers with RXR as the central binding partner, the respective binding partners will be determined via a bacterial expression system.

T22

FIRST INSIGHTS INTO THE AUTOPHAGY MACHINERY OF ADULT *SCHISTOSOMA MANSONI*

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Schistosomiasis is a neglected tropical disease caused by blood flukes (schistosomes) of the genus Schistosoma. This chronic disease is a significant public health problem with > 200 million people infected. The main pathogenicity is caused by eggs laid by the female worm, which requires the female to be in a constant pairing with a male partner. We hypothesize a role for a fundamental cellular process called autophagy in the regulation of key processes in schistosome biology. Autophagy, a process activated during starvation or cellular stress, is known as essential pathway involved in regulating cell survival, reproduction, organ and body reshaping in various organisms, but autophagy has been basically neglected in schistosome research. Here, for the first time, we shed light on the autophagy machinery, its involvement in reproduction of *Schistosoma mansoni*. We identified autophagy genes by in-silico analyses and quantified their transcript level by qRT-PCR in female and male worms prior and after *in vitro* culture. Furthermore, worms were treated with autophagy inhibitors (bafilomycin A1, wortmannin and spautin-1) or an autophagy inducer (rapamycin) to evaluate effects on worm vitality and reproduction as well as autophagy protein expression. Among the identified autophagy genes were Beclin, Ambra1, Vps34, Dram, DAP1, and LC3. The damage-regulated autophagy modulator DRAM was significantly higher transcribed in males compared to females, while for the death-associated protein DAP1 it was opposite. The conversion of the autophagy protein LC3, a key marker for autophagic activity, was impaired by bafilomycin but induced by rapamycin. All inhibitors affected worm fitness and egg production. Furthermore, the morphology of gonads was negatively affected. In summary, we identified autophagy genes in *S. mansoni*, of which some show an interesting sex-dependent expression pattern. Pharmacological manipulation of autophagy induced detrimental effects, which encourages future studies on identifying antischistosomal targets within the autophagy machinery.

T23

SITE-DIRECTED MUTAGENESIS ELUCIDATES MOLECULAR IGE-IPSE/ALPHA-1 INTERACTION RESPONSIBLE FOR BASOPHIL ACTIVATION

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IPSE/alpha-1 (IL-4 inducing principle of *S. mansoni* eggs) is a 134 amino acid glycoprotein secreted by the eggs of the blood trematode *Schistosoma mansoni*, the causative agent of schistosomiasis/bilharziasis, an important neglected tropical disease affecting more than 250 Mio people worldwide.
Natural IPSE/alpha-1 occurs as a dimer, in which an unpaired C-terminal cysteine (C132) is responsible for intermolecular homodimerization, while six other cysteines provide three intramolecular disulfide bonds. IPSE/alpha-1 has been shown to bind to IgE, resulting in the release of IL-4 and IL-13 from basophils and mast cells. The classical mechanism of IgE-dependent activation consists of cross-linking of receptor-bound IgE by multivalent cognate allergen binding to the antigen-recognition variable region of the corresponding immunoglobulin. While IPSE/alpha-1 must occur as homodimer for successful IgE binding, this protein appears to activate basophils by binding to IgE in a 1:1 stoichiometry without any typical cross-linking.
The aim of this study is to investigate the molecular details underlying this unique interaction between IPSE/alpha-1 and IgE. Using site-directed mutagenesis, we created single and double mutants (C132A, T92Y, T92Y/R127A and T92Y/R127L), based on the knowledge that neither IPSE monomers nor the T92Y/R127L mutant, are able to activate basophils. Wildtype and mutant forms of His-tagged IPSE were recombinantly expressed in HEK293–6E suspension cells, followed by affinity chromatography (IMAC) for purification. The ability of all IPSE/alpha-1 forms to activate an IgE-reporter-system was evaluated using humanized NFAT-DsRed rat basophilic leukemia (RBL) reporter cells.
Our results show that none of the mutants was capable to activate basophils. We conclude that T92 must be a key residue involved in IgE binding and thus basophil activation. However, this assumption will be validated by experiments using R127 single mutants (R127L, R127Y) and by providing evidence that the generated mutants are properly folded. Ultimately, X-ray crystallography will reveal a detailed model of interaction.

T24

IMMUNE-METABOLIC CHANGES IN THE SYNOVIUM THAT DEFINE REMISSION IN ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease which affects the synovial tissue and causes destruction of bone and cartilage. Since the disease cannot be cured so far, remission is the therapeutic goal of RA. In order to achieve remission, early diagnosis and start of treatment are crucial. There are different types of medication available for treatment of RA which improved treatment success of RA in the last decades. Even though the number of patients achieving remission is increasing, the prognostic outcomes of the patients differ significantly. So far, the molecular mechanisms of why some patients are able to stop their medication while others are permanently dependent on continuing their medication to stay in remission are unclear. Therefore a better understanding of remission is necessary. The aim of this project is the characterization of remission in RA by histological and immunohistochemical investigation of the synovial tissue in combination with identification of metabolic changes. RA patients receiving joint replacement surgery were clinically classified into subgroups (remission vs. active stage). 37 synovial tissue samples (20 active, 17 remission) were scored on hematoxylin/eosin stained sections using the Krenn-Score. The results showed a significant decrease of hyperplasia in RA patients in remission compared to active disease. Sub-analyses regarding different types of medication confirmed the decrease in all medication groups, especially in patients receiving biological DMARDs. Based on these results, the lining layer will be excised using laser-microdissection and analysed by RNA sequencing. Identification of specific synovial fibroblast and macrophage subpopulations will be performed by immunofluorescence staining using antibodies against podoplanin, and CD90 for lining/sublining fibroblasts and CD14, CD16, CD68 and CD163 for macrophage subtypes. Mass spectrometry will be used to study metabolic changes in RA synovial tissue and fibroblasts. Future results will be compared with patients suffering from psoriatic

arthritis and spondyloarthritis.

T25

HIGH-RESOLUTION TRANSCRIPTOMICS OF THE LIVER FLUKE *FASCIOLA HEPATICA*

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The globally distributed liver fluke *Fasciola hepatica* causes the infectious disease fascioliasis. This is not only a neglected tropical disease affecting some million people, but also leads to huge economic losses by infesting sheep and cattle. Treatment relies mainly on triclabendazole, for which resistance is emerging. Understanding the basic cell biology of this parasite is key to the development of new alternative treatment approaches.

Classical RNAseq workflows work with whole organisms or organs, which are disrupted and RNA isolated from the resulting homogenate. Due to this technical limitation, the data represents a transcriptomic average of all cells present in the mixture, potentially masking rare transcripts or heterogeneity in transcriptomes of low abundant cell types. Using the technology "Single cell RNA-seq", we plan to generate a transcriptome atlas for the liver fluke that preserves the single cell information. This will unveil cell- and organ-specific gene expression such as for stem cells and the trematode-specific tegument, both preferential targets for anthelminthic drugs.

We established a protocol to perform a scRNAseq experiment. To this end, we first optimized the dissociation of adult *F. hepatica*, accomplished a successful FACS sort for these cells and were then able to perform a scRNAseq experiment. After sequencing, further analysis of these cells, using the analysis package Seurat, revealed several cell clusters with a distinct transcriptome. Based on known marker genes, some cell clusters were identified belonging to tissues like the tegument or vitellarium. The next steps for this project are the detailed validation of the predicted cell clusters via *in situ* hybridisation, as well as the generation of additional scRNAseq datasets from adult flukes, to create a more detailed view of the cell heterogeneity. We will also include lineage tracing analysis to shine light on the differentiation paths of cell types from stem cell-like neoblasts.

T26

ANALYSIS OF WHOLE GENOME SEQUENCING DATA OF *STREPTOCOCCUS EQUI* SSP. *EQUI* ISOLATES FROM EQUINES

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Streptococcus equi ssp. *equi* (*S. ee*) is a bacterial pathogen that causes strangles, a highly contagious disease of equids that occurs worldwide. This study aims to enlighten the genetic diversity of *S. ee*. *S. ee* isolates from equines in Germany, Denmark, The Netherlands, Austria, Luxembourg, and Indonesia (n=191, 2002–2020) were investigated. Whole genome sequencing (WGS) data were submitted to *seM* allele typing, multilocus sequence typing (MLST), and core genome MLST (cgMLST) based on 1.286 genes using PubMLST and Pathogenwatch, respectively.

MLST yielded only two and highly related STs, namely ST151 (74 %) and ST179 (26 %). However, 32 different *seM* alleles representing three *seM* groups were discovered, including 19 novel *seM* alleles and excluding 10 isolates that contained deletions in *seM*. Using cgMLST, 159 genotypes were identified that were assorted to three of the six globally recognized clusters, namely BAPS-2, BAPS-5, and BAPS-6 containing 181, 9, and one isolates, respectively. The isolates differed from one another by 0–110 pairwise core-genome SNPs (cgSNPs). All isolates with ST151 belonged to BAPS-2, whereas isolates with ST179 were distributed in BAPS-2, BAPS-5, and BAPS-6. All isolates that belonged to groups *seM*-6, -8, -9 clustered to BAPS-5, BAPS-6, and BAPS-2, respectively. German isolates (n=177) were distributed in BAPS-2 (95 %), BAPS-5 (4.5 %) and BAPS-6 (0.5 %). Some isolates from the same districts were identical (cgSNPs=0) or clustered together in the same sub-groups. All Indonesian isolates (n=7, ST179, *seM* allele 166) differed from each other by only 2

to 14 cgSNPs and built an exclusive sub-group in BAPS-2.

Our results confirm that the genetic diversity of *S. ee* is low with respect to MLST and that cgMLST schemes are needed to resolve the population structure appropriately. Nonetheless, *seM* typing may be helpful for tracking strangles if strains with rare *seM* alleles are involved.

T27

AUTOCHTHONOUS METASTRONGYLOID LUNGWORM INFECTIONS IN WILD EURASIAN LYNX (*LYNX LYNX*) IN GERMANY AND NEW INSIGHTS INTO GASTROINTESTINAL PROTOZOAN AND HELMINTH FAUNA

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The Eurasian lynx (*Lynx lynx*) is the largest feline apex predator in Europe and its habitat in Central Europe is limited to protected areas with lynx reintroduction and conservation projects. In Germany, the Eurasian lynx is listed as a highly threatened species. This wild carnivore can be infected by a wide range of parasites, affecting its health condition and lynx population performance. Therefore, this study was conducted to analyse the actual parasite fauna of free-ranging Eurasian lynxes in Germany.

In total, 25 individual faecal samples were collected between 2010 - 2019 in the Harz National Park. Samples were coprologically analysed by *Giardia/Cryptosporidium* coproantigen-ELISAs, sodium acetate acetic acid formalin (SAF)-technique and carbolfuchsin-stained faecal smears. A subset of ancylostomatid-positive samples was additionally examined by PCR and sequencing to identify parasites to species level.

Overall, 15 taxa of parasites were detected in lynx scat samples, with eight nematode species (*Uncinaria stenocephala*, *Crenosoma* sp., *Angiostrongylus* sp., *Aelurostrongylus abstrusus*, *Toxascaris leonina*, *Toxocara cati*, *Cyclospirura* spp. and *Capillaria* spp.), one cestode species (*Dibothriocephalus* sp.), one trematode species (Heterophylidae) and five protozoan species (*Toxoplasma/Hammondia*, *Cystoisospora rivolta*, *Sarcocystis* spp., *Giardia* spp. and *Cryptosporidium* spp.).

This study represents the first report on *Angiostrongylus* sp., *Crenosoma* sp. and *A. abstrusus* infections in Eurasian lynxes, detected by a non-invasive method. It gives not only new insights into the protozoan and helminth endoparasite fauna but also into anthropozoonotic parasites (*T. cati*, *U. stenocephala*, *Dibothriocephalus* spp., *Cryptosporidium* spp. and *Giardia* spp.) circulating in these wild felids.

T28

CHEMICAL ER STRESS INHIBITS REPLICATION OF THREE HUMAN CORONAVIRUSES IN CULTURED CELLS

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Coronaviruses are RNA viruses with large genomes that infect a broad range of species, including humans. The four "common" human CoV (HCoV-229E, -NL63, -HKU1, -OC43) cause a spectrum of mild symptoms mostly limited to the upper respiratory tract. The three highly pathogenic CoV, severe acute respiratory syndrome (SARS) CoV 1 and 2 and middle east respiratory syndrome CoV (MERS-CoV) cause significant disease burden and mortality. We found that CoVs strongly activate the ER stress protein kinase PERK that is well known to confer translational suppression. This prompted a large study to reveal the role of ER stress for CoV replication and the host response. Here, we provide evidence that pharmacological reprogramming of ER stress pathways can be exploited to suppress CoV replication.
To investigate the role(s) of chemically-induced ER stress for CoV replication, we used several types of model systems, including Huh7, MRC-5 and Vero6 cell lines. The cells were infected with HCoV-229E, MERS-CoV or SARS-CoV-2 in the presence or absence of the ER stress trigger thapsigargin. Expression of viral or host proteins or mRNAs were examined using cell lysates for immunoblotting, mass spectrometry or RT-qPCR. In parallel, the super-

natants were used for ELISA assays and plaque assays. Immunofluorescence assays were utilized to visualize subcellular sites of viral replication. Proteomic analyses were performed to globally uncover virus and thapsigargin-regulated components, pathways and protein networks.
We found that thapsigargin efficiently inhibits replication of all three CoV in different cell types in the lower nM range with favorable selectivity indices (SI, CC50/EC50 >50). Mechanistically, the compound partially restores virus-induced translational shut-down and counteracts the CoV-mediated downregulation of IRE1α and the ER chaperone BiP. The proteome-wide data sets further revealed multiple upregulated factors that are likely to contribute to the thapsigargin-induced antiviral state, including HERPUD1, an essential factor of ER quality control and ER-associated protein degradation complexes and p62/SQSMT1, a regulator of selective autophagy. The data show that thapsigargin hits a central mechanism required for CoV replication, suggesting that thapsigargin (or derivatives thereof) may be developed into broad spectrum of anti-CoV drugs.

T29

REAL-TIME MEASUREMENT OF ATP DYNAMICS IN *PLASMODIUM* FALCIPARUM USING GENETICALLY ENCODED FLUORESCENT PROBES

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Malaria is a serious infectious disease and remains one of the greatest public health burdens for humankind. In 2019, an estimated 229 million cases occurred worldwide. Of these, around 409,000 people died, with the majority of victims being children under five. Of all malaria-causing *Plasmodium* (P.) species, *P. falciparum* has the highest impact on global health causing most infections as well as the deadliest form of the disease. With resistance formation on the rise, basic research for development of new drugs remains critical for global health.
Because of its pivotal role, the energy metabolism of *P. falciparum* is an interesting target for drug design and its central metabolite adenosine triphosphate (ATP) a most interesting factor to analyse. So far, only cell disruptive or intensiometric ATP assays were developed for *P. falciparum* with different drawbacks and partly inconsistent results. Therefore, we stably integrated fluorescent probes, based on förster resonance energy transfer (FRET) and known as Adenosine 5'-Triphosphate indicator based on Epsilon subunit for Analytical Measurements (ATeam), into the genome of *P. falciparum*. ATeams are capable of measuring ATP levels in a ratiometric manner, thereby, facilitating *in vivo* measurements of ATP dynamics in real-time using fluorescence microscopy, plate reader detection, and flow cytometry, while overcoming many obstacles of established ATP analysing methods.
We produced recombinant ATeam protein for *in vitro* characterisation and stably integrated ATeams into the *P. falciparum* genome. Thereby, we already gained proof of principle for cytosolic *in vivo* measurements using a plate reader spectrofluorometer. Besides measuring ATP levels in the cytosol, we also targeted the sensors to different subcellular compartments of the parasite, as well as to the erythrocyte host cytosol. In this way, we want to uncover so far unknown mechanisms of action of selected antiparasitic compounds and get new insights into parasite-host interactions.

T30

CRYPTOSPORIDIUM PARVUM INFECTION TRIGGERS RAPID CHANGES IN METABOLIC SIGNATURES OF BOVINE SMALL INTESTINAL EXPLANTS

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The apicomplexan parasite *Cryptosporidium* is still on the front line of foodborne/waterborne parasitoses worldwide, causing thousands of human deaths each year. The analysis of *C. parvum*-driven metabolic impacts on its host cell in a scenario more close to the *in vivo* situation will provide important insights into the parasite strategy of cell building block scavenge and eventually reveal new promising anti-cryptosporidial therapeutic targets. In this sense, bovines represent the most important reservoir of the common zoonotic subclade *C. parvum*. Therefore, we here analyzed metabolic signatures of *C. parvum*-infected bovine small intestinal explants (BSIE) propagated under physioxic conditions. Thus, bovine small intestinal explants from crossbreed cattle were infected with *C. parvum* sporozoites. *C. parvum* development in this *ex vivo* model was monitored by

qPCR and SEM-analysis and early conversion rates of key metabolites were estimated in supernatants of *C. parvum*-infected explants. Metabolic analyses were performed in parallel under physioxia (5% O2) and hyperoxia (21% O2) to elucidate parasite-driven metabolic effects at physiological oxygen conditions. Metabolic signatures of *C. parvum*-infected BSIE revealed a change in metabolic responses being characterized by an initial down-regulation of conversion rates of key metabolites, such as glutamate, glutamine, glucose, lactate, serine and aspartate at 3 hours p. i. (hpi). By contrast, a rapid increase of above mentioned conversion rates was detected after trophozoite establishment (i. e. 6 hpi), suggesting a succesfull parasite modulation on the metabolic performance of its epithelial niche. Of note, metabolic signatures-based principal component analyses (PCA) confirmed atmospheric-dependent-clustering of *C. parvum*-driven metabolic responses, suggesting physioxia as pivotal factor on conversion of fuels by *C. parvum* infected-cells.
Here, we described metabolic insights into a bovine *ex vivo* model of *C. parvum* infection, which revealled physioxia as driving factor of metabolic responses from *C. parvum*-infected bovine intestinal epithelium.

T31

SITE-DIRECTED MUTAGENESIS OF EPIDEMIC ANTIBIOTIC-RESISTANCE PLASMIDS TO UNDERSTAND THEIR SUCCESS STRATEGIES

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Plasmids are circular, autonomously, double-stranded DNA molecules present in bacteria and archaea. They do not encode essential genes for bacteria to survive, but they can give them advantages over their competitors. Many properties of plasmids are still poorly understood in particular genes involved in the successful spread of epidemic plasmids. This work deals with epidemic plasmids of the incompatibility groups IncN and InX4 that are wide-spread in Enterobacteriaceae worldwide and carry resistance genes encoding resistance towards last-resort antibiotics (e.g. colistin, carbapenem). The aim is to investigate and characterize these plasmids to understand the mechanisms of their success.
For IncX4-plasmids, deletion mutants have been produced in an earlier work and are characterized in this project in detail. Firstly, the p9 mutant of the IncX4-plasmid was selected because the *Escherichia coli* host showed a reduced growth rate and an increased conjugation rate in comparison to the wildtype (WT). The p9 mutant was successfully complemented. In further steps, the growth and conjugation rates of the complemented mutant will be investigated, and the interaction partners of p9 will be determined using a 6xHis-tag.
For the IncN-plasmid, deletion mutants using the red recombinase system of phage lambda and a sequence-specific homologous recombination have to be generated. For the first mutagenesis attempt, gene segments were selected that are found only in a specific subset of IncN-plasmids. The PCR template used for this is usually pKD4, which actually carries a kanamycin resistance cassette. Because pKD4 cannot be used for the mutagenesis of the IncN-plasmid due to the many resistances encoded on the IncN-plasmid, a hygromycin resistance cassette was successfully introduced in pKD4 by intermediate cloning steps in pUC19. The generated PCR fragments were introduced into the IncN-plasmid. Next, the mutants will be identified and the growth and conjugation rates compared to the WT will be determined.

T32

CHOLINERGIC REGULATION OF ATP-MEDIATED RELEASE OF THE PRO-INFLAMMATORY CYTOKINE INTERLEUKIN-1B – COMPARISON BETWEEN HEALTHY VOLUNTEERS AND CROHN'S DISEASE PATIENTS

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The pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 are mainly produced by monocytes and macrophages. Increased levels of these cytokines contribute to the pathogenesis of Crohn's disease (CD). Previously, we have identified a novel anti-inflammatory mechanism that efficiently inhibits the ATP-mediated release of IL-1β from human mono-

cytic cells via activation of nicotinic acetylcholine receptors (nAChRs). Here, we tested if this cholinergic mechanism is active in CD patients.

Basic research was performed on monocytic THP-1 cells, THP-1-derived M1-like macrophages and primary human monocytes, which were negatively enriched from blood samples of healthy volunteers and CD patients. In addition, similar experiments on mouse bone marrow-derived macrophages (BMDMs) were performed. After priming the cells with lipopolysaccharide (LPS) for 3–5 hours, the ATP-induced release of pro-inflammatory cytokines (IL-1β/IL-18) was studied in the presence and the absence of classical (e.g. acetylcholine) and unconventional (e.g. phosphocholine) nAChR agonists. To test for the involvement of nAChRs, the antagonistic conopeptides RgIA4 and [V11L;V16D]ArIB were used.

The ATP-mediated release of IL-1β by THP-1 monocytes and THP-1-derived M1-like macrophages was inhibited using classical and unconventional nAChR agonists. This inhibitory effect was reversed by the specific conopeptides RgIA4 and ArIB indicating the involvement of nAChRs containing subunits $\alpha 7$, $\alpha 9$ and/or $\alpha 10$. Moreover, the ATP-mediated release of IL-1β and IL-18 by freshly isolated primary monocytes from healthy human volunteers and CD patients was efficiently inhibited by the nAChR agonists.

In conclusion, we found, that the ATP-mediated release of IL-1β and IL-18 by monocytic cells, M1-like macrophages, mouse BMDMs and primary human monocytes is inhibited using classical and unconventional nAChR agonists. Furthermore, we provide evidence that the cholinergic mechanism is functional in primary monocytes from CD patients. The cholinergic control of pro-inflammatory cytokines such as IL-1β and IL-18 could be a promising opportunity for the development of therapies against inflammatory diseases like CD.

Section 3 - Heart, Lung and Blood Vessels

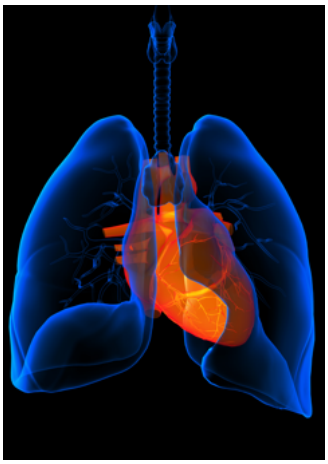

Image: colourbox.com

Day 1: Wednesday, September 29th, 2021

Section 3 - Heart, Lung and Blood Vessels Chairpersons: Zeki Ilker Kanbagli & N.N. & Mohammad Rashedul Alam

10:45-11:15	**Prof. Ghazwan Butrous** (The Universities of Greenwich and Kent at Medway, UK) ***The Complex Interaction of Infection and Pulmonary Vascular Pathology***
11:30-11:40	**Paulin Brosinsky**: *Does cell-type specific silencing of MAO-B interfere with the development of right ventricle (RV) hypertrophy or RV failure in pulmonary hypertension?*
11:40-11:50	**Leili Jafari**: *The transcriptional landscape of human right ventricle in chronic thromboembolic pulmonary hypertension*
11:50-12:00	**Kathrin Malkmus**: *Classical transient receptor potential proteins 1, 3 and 6 play a role in chronic hypoxia-induced pulmonary hypertension*
1220-12:30	**Mohammad Rashedul Alam**: *Role of peroxisomes in type II alveolar epithelial cells (AECII) and surfactant metabolism in the lung*
12:00-12:10	**Julie Antoine**: *Role of the apoptosis-related ER-stress factors C/EBP homologous protein (CHOP) and Apoptosis signal-regulating kinase 1 (ASK1) in the development of pulmonary fibrosis*
12:10-12:20	**Reshma Jamal**: *Can the lungs develop outside the body? Unfolding the in-vitro isolated lung model in the postnatal alveolarization period*
13:30-13:40	**Marie Suzanne Dippel**: *Protective function of acute phase proteins in lung and ischemia/reperfusion injury*
13:40-13:50	**Edibe Avci**: *Role of HDAC9 deficiency on alveolar epithelium during chronic inflammation in aged lung*
13:50-14:00	**Dima Hamarsheh**: *Solitary chemosensory cells in the respiratory tract of man*
14:00-14:10	**Laureen Czech**: *The role of uncoupling protein 2 (UCP2) in the myocardial tissue in response to hypoxia*
14:10-14:20	**Paniz Adibi**: *Identification of Growth Factors and Regulatory Cytokines during Postnatal Cell Cycle Exit in Cardiomyocytes*
14:20-14:30	**Abdullah Al-Najjar**: *MicroRNA analysis in monocyte subsets of patients with vascular disease*

K3

THE COMPLEX INTERACTION OF INFECTION AND PULMONARY VASCULAR PATHOLOGY

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Infectious diseases are one of the leading causes of pulmonary hypertension in the world, particularly in the developing countries. A wide range of parasites, such as worms, bacteria, viruses, and fungi, are involved. They have not been thoroughly studied and there has been no worldwide epidemiological assessment, only estimates and speculation. However, research is underway on schistosomiasis and HIV. Schistosomiasis may cause a critical inflammatory response that helps reshape the pulmonary vascular system. HIV proteins interfere with several molecular pathways that facilitate significant pulmonary vascular remodelling. The study of infectious diseases in pulmonary hypertension helps improve understanding of the complex role of inflammation and the different molecular pathways of the various mechanisms in other aetiologies of pulmonary hypertension.

T33

IDENTIFICATION OF GROWTH FACTORS AND REGULATORY CYTOKINES DURING POSTNATAL CELL CYCLE EXIT IN CARDIOMYOCYTES

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Cardiac growth during embryonic development is primarily mediated by cardiomyocyte proliferation, which in mice drops continuously after birth, such that adult cardiomyocytes are almost unable to divide. If postnatal cell cycle withdrawal is induced by the extrauterine environment, cardiomyocyte numbers per heart might be reduced after preterm compared to term birth, which can impact heart function later in life. To determine the onset of cardiomyocyte cell cycle exit, we performed immunofluorescence staining for proliferation markers on mouse heart sections at the fetal stage E18.5 and immediately after birth (NB18.5). E18.5 and NB18.5 mice share the same gestational age but only NB18.5 mice were shortly exposed to the extrauterine environment. Our results revealed a decline in overall cell cycle activity and a reduction in mitotic cardiomyocytes in murine ventricles at NB18.5 compared to E18.5. Cardiac proliferation is influenced by growth promoting signaling pathways. Comparing E18.5 and NB18.5 via western blot, we noticed that phosphorylation of Akt, the mTORC1 target S6 and MAP kinase Erk1 dropped significantly directly after birth. These signaling pathways are at least partially regulated by growth factors. Consequently, we hypothesized that cardiomyocyte cell cycle exit immediately after birth is influenced by altered growth factor and cytokine availability in the postnatal compared to the intrauterine environment. Our *in silico* screen demonstrated that among 161 studied growth factors and cytokines, 68% exhibited variation in their RNA expression pattern after birth compared to fetal stages in mouse and human hearts. Moreover, antibody array screenings revealed variations in 12 out of 141 investigated cytokines and growth factors in E18.5 versus NB18.5 hearts. In upcoming studies, candidate growth factors and cytokines will be selected for *in vitro* studies to test their ability to induce proliferation in isolated neonatal mouse cardiomyocytes. Therefore, our study could have implications for cardiomyocyte endowment in humans born preterm.

T34

ROLE OF PEROXISOMES IN TYPE II ALVEOLAR EPITHELIAL CELLS (AECII) AND SURFACTANT METABOLISM IN THE LUNG

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Among the 40 different cell types in the lung, peroxisomes are most abundant in type II alveolar epithelial cells (AECII) and bronchiolar club cells. To date, only limited information is available on the regulation of the peroxisomal compartment, its functions, and metabolic pathways in AECII. The aim of the project is to investigate the metabolic role of peroxisomes, the regulation of the peroxisomal compartment and its dependent gene expression as well as the surfactant metabolism in murine AECII cells and the T7 AECII model cell line that was isolated from the H-2kb-tsA58 transgenic mouse ("immortomouse"). As shown, T7 cells are capable of producing surfactant phospholipids and secrete all surfactants protein (SP-A, SP-B, *SP-C*, and SP-D), however, it is not clear until now whether and how peroxisomes contribute to AECII biologies, such as protection against ROS and surfactant synthesis. Our results showed that a much higher abundance of peroxisomes in differentiated T7 AECII (33° C + Int-γ) in comparison to

cells in the proliferation state (39° C - Int-γ). As revealed by immunofluorescence, qRT-PCR and Western blotting experiments showed a marked elevation of the mRNAs and proteins of the peroxisomal biogenesis proteins (PEX13p, PEX14p), peroxisomal β-oxidation enzymes (MFP2 and ACAA1), and ether lipid synthesizing enzymes (AGPS and GNPAT) in differentiated T7 AECII in comparison to proliferated T7 AECII. The future outlook will be to generate via the Cre-LoxP-technology an AECII-cell-specific knockout mouse with peroxisome deficiency by disrupting the *Pex13* gene, coding for the peroxisomal biogenesis protein 13 (PEX13) involved in peroxisomal matrix protein import. The resulting complete peroxisomal dysfunction in AECII cells of the mouse lung will reveal the eventual effects of disrupted peroxisomal metabolic pathways in AECII cells on lung integrity and function, especially on ROS and surfactant metabolism in this organ.

T35

ROLE OF THE APOPTOSIS-RELATED ER-STRESS FACTORS C/EBP HOMOLOGOUS PROTEIN (CHOP) AND APOPTOSIS SIGNAL-REGULATING KINASE 1 (ASK1) IN THE DEVELOPMENT OF PULMONARY FIBROSIS

Antoine, J[1,2,6], Hadzic, S[3,6], Henneke, I[3,6], Klymenko, O[1,2,6], Seeger, W[1,2,3,4], Guenther, A[1,2,3,5,6,7], Korfei, M[1,2,6]

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Idiopathic pulmonary fibrosis (IPF) is an uncurable interstitial lung disease of unknown origin. Its main characteristic is maladaptive proapoptotic Endoplasmic Reticulum (ER)-stress in type-II alveolar epithelial cells (AECII). The resulting chronic epithelial injury and aberrant reparative response are fatal for IPF-patients. In order to study the role of ER-stress in the development of lung fibrosis as well as of its preventive blockade, we decided to investigate two genetically modified mouse lines.
First, we used a double-transgenic line we created, in which an AECII-specific overexpression of the proapoptotic ER-stress factor Chop can be induced by doxycycline feeding (*SP-C* rtTA/tetO7-Chop) as an upregulation of Chop was observed in AECII of IPF-patients. A nuclear Chop-overexpression was successfully induced in three months old Chop transgenic mice but without the development of a pronounced lung fibrosis. As IPF is an age-related disease, we investigated the age as a possible "second hit-factor" in the development of fibrosis, and to this purpose we induced the Chop-overexpression in "old" Chop transgenic mice (between 12 and 27 months old). We also treated doxycycline fed Chop-mice intratracheally with bleomycin and compared them to two control groups at different post-application times.
Previous analyses revealed an upregulation of ASK1-signalling in both the apoptotic AECII and proliferating fibroblasts of IPF-lungs. Apoptosis in AECII would be induced through the ASK1-p38 axis and the ASK1-JNK1/2 axis, while in lung fibroblasts, profibrotic responses would be mediated by the same axes. In order to evaluate the protective effect of blocked ASK1-signalling on development of lung fibrosis *in vivo*, we used *Ask1(-/-)*-knockout mice in combination with an intratracheal bleomycin application. We are currently phenotyping these mice. We further want to investigate a pharmacological inhibition of Ask1 by the small-molecule selonsertib in the bleomycin-mouse model of lung fibrosis as a potential therapeutic blockade which could be beneficial to IPF-patients.

T36

ROLE OF *HDAC9* DEFICIENCY ON ALVEOLAR EPITHELIUM DURING CHRONIC INFLAMMATION IN AGED LUNG

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Aging is characterized with altered metabolic profile in persistent low-grade pro-inflammatory state, influenced by epigenetic modifications. Histone deacetylase 9 (*HDAC9*) , a member of class IIa, is known

to catalyze the removal of acetyl groups from lysine residues in both histone and non-histone proteins. *HDAC9* plays role in gene expression in tumor formation, inflammation, cardiac hypertrophy, atherosclerosis, and metabolic diseases. The aim of study is to reveal the role of *HDAC9* deficiency leading to chronic inflammation in aged lung.
The study is performed in 3 different age groups (Group 1= 8–12 weeks old (young), Group 2= 30–34 weeks old (mild aged), Group 3= ≥ 48 week old (aged)). Aged *HDAC9 KO* exhibited reduced survival and decreased body weight. Hematoxylin and eosin stainings revealed that CD45+/CD68+ monocytes and CD45+/CD3+ T-cells were present in inflamed sites of lungs in aged *HDAC9 KO* mice. Interestingly, *HDAC9* deficiency led to reduced *PCNA* and *SP-C* positive cells. While vimentin expression in *HDAC9* KO-AT2 cells of young mice increased, *E-cadherin* decreased. Moreover, *HDAC9* KO-AT2 cells showed reduced proliferation and increased apoptosis. Importantly, *HDAC9* K0-AT2 cells promoted pro-inflammatory cytokine release in aged lung. Alveolar macrophages proliferated when cocultured of *HDAC9* KO-AT2 media. Furthermore, RNA seq-analysis showed that glycerophospholipid biosythesis is upregulated in *HDAC9* K0-AT2 cells. Metabolomic profiling of *HDAC9* KO-AT2 cells confirmed rewired choline metabolites as well as dysregulated free fatty acid metabolites.
These results indicate that *HDAC9* deficiency is involved in chronic inflammation and age-related functional and structural changes of the lung via rewiring metabolic state of AT2 cells. The findings of the study will provide important insights to reveal the role of *HDAC9* in the regulation of inflammaging-related lung remodeling.

T37

DOES CELL-TYPE SPECIFIC SILENCING OF MAO-B INTERFERE WITH THE DEVELOPMENT OF RIGHT VENTRICLE (RV) HYPERTROPHY OR RV FAILURE IN PULMONARY HYPERTENSION?

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Pulmonary arterial hypertension (PAH) is a disease caused by narrowed, blocked or destroyed blood vessels in the lung which can lead to hypoxia. The consequence would be slower blood flow through the lungs, but the blood pressure within the lung arteries rises. Thereby, the heart has to work harder to pump the blood through the lungs. To compensate this, the muscle cells which belong to the pulmonary arteries, increase and grow resulting in a hypertrophy. Finally the cardiac output decrease and a heart failure can occur. PAH caused by left-sided heart disease is largely investigated. In comparison, PAH caused by right-sided heart disease is mostly unexplored. It is indicated, that an increase of the reactive oxygen species (ROS)-production from mitochondria can play a crucial role in pulmonary hypertension caused by chronic hypoxia and right ventricular remodelling.
For this reason, further investigations of ROS molecules against the background of PAH seem to be a promising approach for the understanding of right-sided heart failure.
ROS molecules are generated by different compartments within the cell whereby mitochondria are known to generate the strongest ROS signal. Proteins of the outer mitochondrial membrane, called monoamine oxidases (MAO), can degrade neurotransmitters which can be released in various situations in the body whereby the ROS-production increases. It is already demonstrated that an inhibition of monoamine oxidase B (MAO-B) leads to a decrease of the ROS-production. Therefore, the effect of a decreased mitochondrial ROS generation should be analysed in this project by using heart-specific, inducible MAO-B-knockout mice. To achieve a situation of pulmonary arterial hypertension, "pulmonary artery banding" (PAB)- operations will be used.
With this model the relation between MAO-induced ROS-production, mitochondrial dysfunction and heart failure will be investigated. To do that, isolated cardiomyocytes will be analysed with molecular biological methods, as well as ROS- and respiratory-measurements will be performed. Additionally, *in vivo* echocardiography will be used to show morphological differences.

T38

THE ROLE OF UNCOUPLING PROTEIN 2 (UCP2) IN THE MYOCARDIAL TISSUE IN RESPONSE TO HYPOXIA

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Myocardial ischemia-reperfusion injury (IRI) develops after a period of ischemia and subsequent return of blood supply. Pathogenesis of myocardial IRI includes tissue damage due to generation of reactive oxygen species (ROS) by mitochondria. It is indicated, that during IRI a ROS molecule boost occurs which can lead to oxidative tissue damage. The uncoupling protein 2 (UCP2) is an inner mitochondrial

membrane protein and may contribute to the reduction of the mitochondrial membrane potential. Consistently, lowering the mitochondrial membrane potential influences the ROS formation and can have an impact on oxidative stress. The aim of these experiments is to evaluate the importance of UCP2 in myocardial IRI.
To investigate the influence of UCP2 in myocardial tissue during ischemia-reperfusion the Langendorff heart *ex vivo* was used. The Langendorff heart is an *ex vivo* technique that allows the examination of heart rate and contractile strength during ischemia-reperfusion. Additionally, experiments with isolated cardiomyocytes in response to hypoxia were performed by using the UCP2-deficient rat as a model (UCP2+/-).
First results indicate differences between wildtype, heterozygous and homozygous UCP2 knockout groups (LVDP recovery in post-ischemic hearts). In particular, UCP2-deficient rats displayed a better recovery at the initial reperfusion period. Moreover, cardiomyocytes were isolated from wildtype and heterozygous UCP2-deficient rats to analyze their contractile and relaxation activity as well as morphological changes in response to hypoxia and normoxia. We observed differences in cell morphology and contractile/relaxation behavior in the heterozygous UCP2 knockout group compared to the wildtype cells. The heterozygous group shows higher cell viability in response to hypoxia compared to the control group.
Our first results suggest that the UCP2 modifies the coupling between metabolism and function.

T39

PROTECTIVE FUNCTION OF ACUTE PHASE PROTEINS IN LUNG AND ISCHEMIA/REPERFUSION INJURY

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Pulmonary ischemia/reperfusion injury (IRI) develops after a transient cut off from blood and air supply. The early phase of the pathogenesis of lung IRI includes the recruitment of splenic monocytes, which travel via the portal vein through the liver before entering the lung. Here they interact with endothelial cells and release the pro-inflammatory cytokine interleukin-1β (IL-1β), which causes blood-air barrier leakage. Neutrophils subsequently extravasate and cause acute pulmonary damage, which can be life-threatening. Accordingly, the release of monocytic IL-1β plays a pivotal role in the pathogenesis of pulmonary IRI. Extracellular ATP originating from cells damaged by ischemia can activate the P2X7 receptor, induce inflammasome assembly and activation of caspase-1. The latter cleaves pro-IL-1β and mature IL-1β is quickly released from monocytes. Our research group recently described a pathway negatively regulating the P2X7 receptor function via nicotinic acetylcholine receptors. Apart from classical nicotinic agonists, this pathway can be initiated by several endogenous proteins including circulating acute phase proteins like α1-antitrypsin or C-reactive protein, which are produced by the liver in response to inflammation. We hypothesise, that the potential of splenic monocytes to secrete IL-1β in response to ATP is down-modulated in the anti-inflammatory hepatic milieu by high local concentrations of acute phase proteins. Consequently, infiltration of lung tissue by neutrophils and IRI are attenuated. We will isolate splenic monocytes, treat them *ex vivo* with control solution, α1-antitrypsin or C-reactive protein and transfer them into splenectomised mice during reperfusion of ischemic lungs. We will investigate the histopathology of the lung and measure cytokine concentrations in blood, bronchoalveolar lavage fluid and lung tissue. Life-cell imaging will be used to investigate the assembly of citrine-labelled inflammasomes and automated immunofluorescence mapping to analyse leukocyte populations infiltrating the damaged lung. This study might pave the way for the development of efficient therapies preventing IRI.

T40

SOLITARY CHEMOSENSORY CELLS IN THE RESPIRATORY TRACT OF MAN

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Specialized sensory epithelial cells sense noxious substances and initiate protective reflexes. They are found along the mammalian respiratory tract. Studies in mice suggested the presence of at least two populations: 1) neuroendocrine cells (marker: PGP9.5), 2) solitary cholinergic chemosensory cells (SCCC) (synonyms: brush or tuft cells; markers: GNAT3, PLCβ2, TRPM5). In humans, neuroendocrine cells are present but the existence of SCCC remains unknown.
Single- and multiple-labelling immunofluorescence with antibodies against established marker proteins was performed on specimens of human papillae vallatae (positive control) and respiratory tract (nose,

trachea, lung) obtained from anatomy body donors and pathology; TRPM5-eGFP reporter and C57BL6/J mice served as references. Publicly available single cell RNA sequencing (scRNAseq) data were analyzed *in silico*.
our study revealed that PLCβ2 antisera labelled cells in human taste buds, but not in the respiratory mucosa; TRPM5 and GNAT3-positive cells were not found. Accordingly, *in silico*-analysis revealed only minimal expression of these markers in human respiratory epithelial cells, in contrast to mice. Instead, scRNAseq data pointed to the endoplasmic reticulum protein LRMP (lymphoid-restricted membrane protein) as a human brush cells marker. LRMP-antibodies labelled rare (0.07–0.7 cells/mm basement membrane), slender epithelial cells along the entire airways reaching deep into the lung. Double labelling showed that they form an independent population, separate from ciliated, secretory, neuroendocrine cells and ionocytes. In mice, LRMP was also localized specifically to SCCC cells, which were restricted to extrapulmonary airways.
We concluded that these data identify chemosensory cells in human airways. Their distribution along the airway tree and expression of signaling pathway proteins differ from mice.

T41

EFFECT OF INFLAMMATION-MEDIATED ENDOTHELIAL METABOLIC SHIFT ON ENDOTHELIAL BARRIER FUNCTION

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The integrity of the endothelial cell barrier of the microvasculature is compromised by inflammation. The increased vascular permeability leads to tissue injury and organ dysfunction. In recent years, considerable advances have been made in the understanding of signalling mechanisms regulating the endothelial barrier integrity. The role of endothelial metabolism as a modulator of endothelial barrier integrity is not yet well-studied. The aim of the present study was to investigate the effect of inflammation on endothelial metabolism and its role in the maintenance of endothelial barrier integrity. The study was carried out on cultured human umbilical vein endothelial cells and rat coronary microvascular ECs. Inflammatory condition was simulated by treating cells with low concentrations (1 ng/mL) of TNFα for 24h. Total cellular ATP concentration was measured using luminescence-based commercial kit (ATPLite, PerkinElmer). Mitochondrial mass was analysed by the ratio of mitochondrial DNA (mtDNA) to nuclear DNA (nDNA). The cellular glucose uptake was measured by fluorescent microscopy using a fluorescent analogue of glucose (2-NBDG).
Treatment of human endothelial cells with TNFα resulted in significant suppression of mitochondrial and upregulation of glycolytic ATP synthesis rate, suggesting a metabolic switch. This was accompanied by a reduction in mitochondrial content (mtDNA/nDNA), reduction in total cellular ATP levels, an enhanced expression of glycolytic enzymes 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and phosphofructokinase 1 (PFK1), and enhanced glucose uptake by endothelial cells (n=5; $p<0.05$ for all parameters tested). The study demonstrates that TNFα induces metabolic switch towards glycolysis in endothelial cells. Moreover, the data suggest that upregulation of glycolysis may serve as an endogenous metabolic adaptation to the TNFα-induced suppression of mitochondrial ATP synthesis, which protects endothelial barrier integrity.

T42

THE TRANSCRIPTIONAL LANDSCAPE OF HUMAN RIGHT VENTRICLE IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

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Chronic thromboembolic pulmonary hypertension (CTEPH) is defined as a subtype of pulmonary hypertension (PH). CTEPH characterized by fibrotic obstructions in the pulmonary arteries that lead to in-

creased pulmonary vascular resistance. The resulting pulmonary hypertension causes right ventricular (RV) remodeling and finally leads to right heart failure and death. Unlike other forms of PH, CTEPH is curable by pulmonary endarterectomy (PEA) surgery, which removes residual thrombus material from the vessel wall and causes improvements in pulmonary hemodynamics and RV function.
The aim of this study is to use RNA-sequencing (RNA-seq) data from human RV tissue to identify the signaling pathways, potential biomarkers, and master regulators, that are specifically involved in the effect of PEA on the RV of CTEPH patients.

RNA-seq followed by bioinformatics analysis performed on the RV biopsies obtained from CTEPH patients before PEA surgery and, the results compared with those biopsies obtained during 12 months follow up evaluation.

Bioinformatic analysis of RNA-seq data showed 2799 genes (Log2 fold change $\geq$0.585; FDR$\leq$0.05) differentially regulated between pre and post PEA sample groups. This substantial number of differentially expressed genes (DEGs) indicates a major change in the transcriptional landscape of the RV in these patients. To further investigate the potential biomarker candidates from the large pool of 2799 DEGs, extensive bioinformatics analysis of different data sets shortlisted 250 DEGs that were functionally associated with cardiovascular development or disease. The findings of this study reveal prominent transcriptional changes that occur in response to PEA. Pathway analysis and gene ontology enrichment confirmed activation of the hypoxia response via hypoxia-inducible factor 1 (HIF-1), hippo and calcium signaling pathway, platelet-derived growth factor (PDGF) pathways, and proteoglycans after PEA compared with before PEA.

Extensive and unbiased transcriptomic profiling of RV biopsies from CTEPH patients, revealed a major change in the transcriptional landscape of these patients occurs following reduction the pressure overload of the RV by PEA.

T43

CAN THE LUNGS DEVELOP OUTSIDE THE BODY? UNFOLDING THE IN-VITRO ISOLATED LUNG MODEL IN THE POSTNATAL ALVEOLARIZATION PERIOD

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Lung development has been studied extensively in recent years, generating new cognizance into the origins of different cell types that exist in the lung as well as molecular pathways that regulate structural changes. Using transgenic mice often is limited due to a global lethal phenotype. This led to the development of tools such as organoids formation or precision cut lung sections. However, such tools described had a major missing component, such as breathing movement inducing stretch forces. Including these forces to mimic the in-vivo situation requested to develop an in-vitro isolated, perfused ventilated lung model.
This project involves the establishment and validation of in-vitro isolated ventilated and perfused lung model using both positive and negative pressure ventilation. Neonatal mouse lungs are ventilated and perfused in-vitro for periods of 4 until 12 hours starting from postnatal day 4 until postnatal day 14. Structural and cellular changes are compared with structural features of in-vivo grown respective neonatal mouse lungs. Optimizing ventilation and perfusion parameters in order to reach conditions of neonatal mouse physiology represents the first milestone of the project. Thus, prevention of ventilation induced lung damage and analyses of possible structural and cellular changes of isolated perfused and ventilated lungs versus structural features of in-vivo grown neonatal mouse lungs shall be achieved.
Establishment of an in-vitro method including all physiological aspects will pave way to characterize and modulate alveolarization using lineage tracing, cell depletion, pharmacological interventions and gene editing. Thus, cellular and molecular targets for the development of new therapeutic concepts for pulmonary structural diseases can be studied with our neonatal in-vitro lung ventilation and perfusion model.

T44

CLASSICAL TRANSIENT RECEPTOR POTENTIAL PROTEINS 1, 3 AND 6 PLAY A ROLE IN CHRONIC HYPOXIA-INDUCED PULMONARY HYPERTENSION

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Chronic hypoxia-induced pulmonary hypertension (CHPH) is a life threatening disease, which is accompanied by a rise in mean pulmonary arterial pressure (mPAP) above 25 mmHg. In addition, it is characterized by an increased right ventricular systolic pressure (RVSP) due to pulmonary vascular remodeling. Latter results in increased muscularisation of small pulmonary arteries and pulmonary vascular resistance leading to right heart hypertrophy and ultimately RV failure. The main cell type involved in vascular remodeling is the precapillary pulmonary arterial smooth muscle cell (PASMC). High level of intracellular calcium concentration is associated with abnormal proliferation and migration of PASMC, which is supposed to be the reason for the development of PH. Although CHPH has a high mortality, the underlying pathomechanism is still barely understood.

Classical transient receptor potential channels (TRPC) are non-specific cation channels especially gating calcium and sodium ions and are able to build homomeric and heteromeric channels, which is why we hypothesize that their composition effects their activatability. We suggest that TRPC channels are crucial in the pathogenesis of CHPH and that a loss of TRPC proteins 1, 3 and 6 improve the symptoms of CHPH. In fact, the results demonstrated that RVSP and right ventricular wall thickness of TRPC1/3/6 deficient mice (TRPC1/3/6-/-) are ameliorated when compared to wildtype controls whereas the muscularisation of small pulmonary vessels is unaltered. Additionally, intracellular calcium concentration of PASMCs was shown to be affected by the knockout of TRPC1, 3, and 6 proteins after activation of store- and receptor-operated calcium channels. Along this finding, our results indicate the importance of TRPC as a treatment target for CHPH.

T45

LONGITUDINAL RIGHT ATRIAL FUNCTION IN INCIDENT PULMONARY ARTERIAL HYPERTENSION

Richter, MJ[1], Zedlera, D[2], Berliner, D[2], Douschan, P[3], Gall H[1], Ghofrani, HA[1,4,5], Kremer, N[1], Olsson, KM[6], da Rochaa, BB[7], Rosenkranz, S[7], Seeger, W[1], Yogeswarana, A[1], Rako, Z[1], Khodr Tello, K[1]

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Background: Right atrial (RA) function has emerged as an important determinant of outcome in pulmonary arterial hypertension (PAH). However, studies exploring longitudinal RA function after initiation of specific pulmonary vascular treatment and its association with outcome in incident PAH patients are lacking.
Methods: Retrospectively, in 56 therapy naive PAH patients RA function was assessed as echocardiographic speckle-tracking peak longitudinal strain (PLS) and peak active contraction strain (PACS). Measurements were repeated during follow-up under specific mono- or combination therapy. In addition, time to clinical worsening was assessed while events were predefined. Patients were stratified in tertile according to the difference of baseline to follow-up RA PLS into worsened (tertile I: Δ-17.8 to -4.2 %), stable (tertile II: Δ -4.2 to 4.0 %) and improved RA function (tertile III: Δ 4.0% to 44.6%). In analog, PACS was stratified accordingly. Spearman rho correlation and linear regression analysis were used to determine the association of ΔRA PLS with Δ RV function. The impact of stratified RA function on time to clinical worsening were assessed using Kaplan–Meier and Cox regression analyses.
Results: Median time to echocardiographic follow-up was of 11 [9–12] month. 37 out of 56 (66%) patients

received specific dual or triple combination. ΔRA PLS during follow-up was significantly associated with the change of key hemodynamic and echocardiographic parameters. Using multivariate linear regression analysis the change of pulmonal vascular resistance and right ventricular end-systolic area emerged independently associated with ΔRA PLS. During a median time to clinical worsening of 12 [4 -18] months, 22 (39.3%) patients experienced a clinical worsening event. In multivariate cox regression analysis, worsening RA PLS during follow-up was significantly association with clinical deterioration (hazard ratio: 5.53; 95% confidence interval: 1.54–19.94; p 0.009). This was supported by Kaplan-Meier analysis as patients with worsening RA PLS had a significantly impaired prognosis than those with stable or improved RA function (log rank <0.001). Overall, stratification of PACS was not prognostic.
Conclusion: After initiation of specific pulmonary vascular therapy in PAH evolution of RA function is heterogeneous. Stratification according to worsening, stable or improved RA PLS emerged as a clinical and prognostic relevant finding. Improvements of RA PLS are driven by reduction of afterload and RV reremodeling.

Section 4 - Protein and Nucleic Acid Interactions

Image: colourbox.com

Day 1: Wednesday, September 29th, 2021

Section 4 - Protein and Nucleic Acid Interactions Chairpersons: Janek Börner & N.N. & N.N.

18:00-18:30	**Dr. Kaspar Burger** (University of Würzburg, Germany) ***Nucleolar accumulation of the RNA-binding protein NONO promotes DNA repair***
14:45-14:55	**Nicole Bazant**: *Arabidopsis phytochrome A (phyA) nuclear translocation is regulated by the phosphorylation status of the shuttle protein FHY1*
14:55-15:05	**Doudou Kong**: *Keeping meristems at balance – stem cell maintenance and termination in flowers with two meristem types*
15:05-15:15	**Jihed Gharred**: *Impact of water deficit and biochar on growth and photosynthetic parameters of Medicago ciliaris*
15:15-15:25	**Nicole Evelin Schmid**: *A lytic bacteriophage changes biofilm morphology of Shewanella oneidensis*
15:25-15:35	**Meike Schwan**: *How do bacteria restrict the assembly of a single polar flagellum?*
16:00-16:10	**Janek Börner**: *The conserved endoribonuclease RNase III affects formation of photosynthetic complexes in Rhodobacter sphaeroides*
16:10-16:20	**Maria Weller**: *Pig Retinal Explants as an intermediary model between in vitro and in vivo approaches for gene therapy*
16:20-16:30	**Timo Schlemmer**: *Plant extracellular vesicles and their role in RNAi-based plant protection*
16:30-16:40	**Fatimah Alabudeeb**: *Characterization of DNA repair mechanisms and genome editing efficiency in differentiated neurons*
16:40-16:50	**Corinna Ulshöfer**: *The role of IMP3 in mRNA localisation*

K4

NUCLEOLAR ACCUMULATION OF THE RNA-BINDING PROTEIN NONO PROMOTES DNA REPAIR

Kaspar Burger[1]

[1]Biocenter of the Julius Maximilian University Wuerzburg, Department of Biochemistry & Molecular Biology, Wuerzburg, Germany

DNA double-strand breaks (DSBs) are highly toxic lesions that threaten genome stability and lead to cancer, if left unrepaired. The DNA damage response (DDR) counteracts the accumulation of DSBs and requires long non-coding (lnc)RNA and RNA-binding proteins (RBPs) for efficient DSB repair (DSBR). We could previously show that RNA polymerase II (RNAPII) and the RNAi factor Dicer promote DSBR. RNAPII that is specifically phosphorylated at CTD tyrosine-1 residues accumulates at promoter-associated DSBs in a c-Abl kinase-dependent manner and produces damage-induced lncRNA. Such transcripts form double-stranded (ds)RNA intermediates and undergo processing by nuclear phosphorylated Dicer to facilitate the recruitment of repair factors like 53BP1 to DSBs.
However, the regulatory principles that integrate the RNA metabolism with DSBR remain poorly understood. We currently investigate the role of nuclear bodies for genome stability and are particularly interested in paraspeckles. Paraspeckles function as RNA metabolic hubs to regulate gene expression by retention of a subset of mRNA. The structural lncRNA NEAT1 and the multifunctional RBP NONO are core components of paraspeckles and frequently upregulated in tumours. NONO and NEAT1 are required for the efficient response to DSBs. The depletion of NONO or NEAT1 in human U2OS cells delays DDR signaling and increases persistent DNA damage. We observe that a subset of NONO accumulates on chromatin and in the nucleolus upon incubation with Etoposide, while NEAT1 levels are specifically elevated in the nucleoplasm of damaged cells. To investigate the molecular principles that link NONO and NEAT1 to genome stability we combined mass spectrometry, CLIP-seq, and site-directed mutagenesis to determine damage-induced changes in the NONO interactome. Our preliminary data suggest that the DDR redistributes a subset of NONO to the nucleolus in an RNA-dependent manner possibly to mitigate the accumulation of aberrant transcripts on damaged chromatin and promote DSBR pathway choice.

T46

CHARACTERIZATION OF DNA REPAIR MECHANISMS AND GENOME EDITING EFFICIENCY IN DIFFERENTIATED NEURONS

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Inherited retinal dystrophies is a group of heterogeneous disorders, which vary in severity and progression. There are more than 270 genes that have been associated with these diseases, most of them being expressed in photoreceptors. The genome editing field, in which the CRISPR-Cas9 technology is used to introduce DNA double strand breaks (DSB) that are subsequently repaired by the cells own repair systems, has gathered much attention in recent years. The major pathways for DSB repair are non-homologous end-joining (NHEJ) and homology directed repair (HDR). The main obstacle for its application in the retina is the lack of adequate information of DNA repair efficacy in photoreceptors. The goal of this project is to identify and to study DNA repair and to improve genome editing efficacy in mature neurons. We standardized cell culture conditions for iNGN cells, which are inducible pluripotent stem cells that differentiate to mature neurons upon activation of the TET ON system by adding doxycycline to the culture media. In addition, we will compare the efficacy of DNA repair in iNGN vs iNGN-Tet3KO to determine the effect of the Tet3 protein, a master regulator of neuron cell type specific gene expression. In order to generate a standard cell line of iNGN expressing the traffic light reporter 3 system, which allows to study NHEJ and HDR efficiency, we transfected the iNGN cells with piggyBac transposon system, which contains a fluorescence based reporter system (TLR-3), and selected using puromycin. For the purpose of greater precision and less off-target activity, we will be using a CRISPR-Cas version that is inducible with 4-hydroxy tamoxifen prior to differentiation. As a control, we transfected HEK293T cells with different BRET reporters and guides applying the inducible system. In conclusion, we aim to study the efficacy of the different genome editing approaches in mature neurons and to analyze the impact of Tet3 on the DNA repair system.

T47

ARABIDOPSIS PHYTOCHROME A (PHYA) NUCLEAR TRANSLOCATION IS REGULATED BY THE PHOSPHORYLATION STATUS OF THE SHUTTLE PROTEIN FHY1

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Plants use different photoreceptors to determine light quality and quantity to adjust growth and development of the light environment. Phytochrome A (phyA) is a red (R) and far-red (FR)- light sensing photoreceptor. Absorption of R light converts phyA into the physiologically active far-red absorbing Pfr form, which translocates into the nucleus and subsequently regulates gene expression. FHY1 (FR elongated hypocotyl 1) and its homolog FHL (FHY1-like) are required for nuclear translocation of phyA. In previous work, conserved serines that might be potential phosphorylation sites, were identified in FHY1 and FHL proteins. In addition, it has been shown that FHY1 mutants in which conserved serines have been replaced by aspartate, which acts as a phosphomimic amino acid, are unable to transport phyA (Pfr) into the nucleus (Helizon *et al.*, 2018). This findings suggesting that the phyA transport is regulated by the phosphorylation state of FHY1. In this work, FHY1 is purified from seedlings undergoing deetiolation, to study the *in vivo* secondary modifications of FHY1 by MS. The interaction of FHY1 with PP2AA2, which is a subunit of the protein phosphatase 2A, could be shown by bimolecular fluorescence complementation assays and yeast two- and three-hybrid assays (Helizon 2015, PhD thesis). This findings and the observation of a delayed phyA transport in pp2aa2-mutant protoplasts support the idea that the phyA transport is regulated through the change of the phosphorylation status of FHY1. The results presented will demonstrate the role and regulation of FHY1 on phyA nuclear translocation.

T48

THE CONSERVED ENDORIBONUCLEASE RNASE III AFFECTS FORMATION OF PHOTOSYNTHETIC COMPLEXES IN *RHODOBACTER SPHAEROIDES*

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Bacteria are frequently adjusting their transcriptome to adapt to changes in their environment. This does not only take place on level of transcription, but also involves ribonucleases (RNases) that control RNA processing and turn-over. RNase III (*rnc*) is a highly conserved endoribonuclease, which is present in all known eukaryotes (Dicer and Drosha are RNase III enzymes) and prokaryotes, and was shown to be an important regulator of gene expression in many organisms. The enzyme can contain one or two nucleolytic active RNase III domains (RIIID) that harbour a 9 amino acids RNase III signature motif. Bacterial RNase III enzyme typically contain a single RIIID, often followed by a C-terminal dsRNA binding domain.
Rhodobacter sphaeroides is a facultative phototrophic alpha proteobacterium that can perform aerobic respiration in presence of oxygen, as well as anoxygenic photosynthesis, anaerobic respiration or fermentation in absence of oxygen. To better understand the role of RNase III in adjusting the *R. sphaeroides* transcriptome to changes in the environment, we constructed a mutant strain lacking RNase III activity by exchanging two highly conserved amino acids in the signature motif. An obvious phenotype of this mutant was its lighter red color indicating that formation of photosynthetic complexes differs to the wild type. Indeed, we could confirm lower amounts of bacteriochlorophyll and carotenoids and lower levels of photosynthetic complexes in the mutant. Quantification of several mRNAs encoding structural proteins of photosynthetic complexes or enzymes required for pigment synthesis, revealed that their levels are influenced by RNase III. These results demonstrate, how much a single RNase can influence important physiological processes.

T49

A SMALL DEPOLARIZING TOXIN CONFERS CONDITIONAL PERSISTENCE AGAINST DIFFERENT ANTIBIOTICS

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Persister cells are phenotypic variants within isogenic bacterial populations. Due to their high tolerance towards stress, persister cells contribute to the survival of populations under harmful conditions by a bet-hedging strategy. The persistent state is characterized by a particular low metabolic turnover, and is probably controlled by toxin-antitoxin (TA) systems.
The type I toxin-antitoxin system TisB/IstR-1 of *Escherichia coli* was linked to bacterial persistence due to its mode of action. In the case of DNA damage, the small hydrophobic toxin TisB is expressed, conferring cellular depolarization and ATP depletion to its host. Translation of TisB is tightly regulated by its RNA antitoxin IstR-1 and a complex RNA structure formed by the first 41 nucleotides of the 5'-UTR of the tisB mRNA. Previous investigations showed a highly persistent phenotype in an *E. coli* strain, in which both regulatory RNA elements were deleted (strain ΔistR Δ1–41), but the molecular basis remained elusive.
Flow cytometry analysis of highly persistent strain ΔistR Δ1–41 revealed the presence of a growth-inactive subpopulation, which is absent in *E. coli* wild-type cultures. Cell sorting experiments determined that the non-growing population contributes to the high persister frequency against Fluoroquinolone antibiotics. This defense mechanism is however conditional. Against DNA cross-linking agents such as mitomycin C, the highly persistent strain is even more susceptible as the wild type.

T50

IMPACT OF WATER DEFICIT AND BIOCHAR ON GROWTH AND PHOTOSYNTHETIC PARAMETERS OF MEDICAGO CILIARIS

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Water shortage is the major constraint affecting fodder production and yield stability in most arid and semi-arid regions (Shao *et al.*, 2009). However, desertification is growing fast and the predicted global climate change will enhance a further dilatation.

When faced with this climatic change and increasing water demand for agriculture, the selection of economically feasible and ecological sustainable plants resistant to water deficit stress and able to grow on a wasteland has high priority.

We intended to study the impact of biochar on drought resistance of crop plants, native in the arid Mediterranean area.

Medicago ciliaris was selected as a test species because it can reduce soil erosion, suppress weeds, and is known as a mother plant in row crop production. Moreover, Medicago is a Leguminosae with a high capacity of symbiotic nitrogen fixation, often used to improve soil fertility and particularly soil nitrogen content.

Our results showed that drought leads to a significant reduction of growth, CO_2/H2O Gas exchange, and Water use efficiency (WUE) of Medicago ciliaris. Moreover, drought leads to a significant increase in oxidative stress

On the other hand, biochar reduces significantly the negative impact of drought on growth, CO_2/H2O Gas-exchange, Water use efficiency (WUE), and oxidative stress

T51

KEEPING MERISTEMS AT BALANCE – STEM CELL MAINTENANCE AND TERMINATION IN FLOWERS WITH TWO MERISTEM TYPES

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Floral meristems are dynamic systems that generate floral organ primordia at their flanks and, in most species, terminate while giving rise to the gynoecium primordia. However, we find species with floral meristems that generate additional ring meristems repeatedly throughout angiosperm history. Ring meristems produce only stamen primordia, resulting in polystemous flowers (having stamen numbers more than double that of petals or sepals), and act independently of the floral meristem activity. Most of

our knowledge on floral meristem regulation is derived from molecular genetic studies of *Arabidopsis thaliana*, a species with a fixed number of floral organs and, as such of only limited value for understanding ring meristem function, regulation, and ecological value. In this project we will to analyze *Pteridophyllum racemonsum*, the most basal Papaveraceae and *Eschscholzia californica*, an emerging genetic model system well established in our group. P. racemosum has the autapomorphic state of the Papaveraceae with two sepals, four petals and four stamens opposite the petals. *E. californica* has, as most Papaveraceae several whorls with varying numbers of stamens ranging from 20–30. Thus, we can compare the ancestral state (fixed) found in P. racemosum with the derived state (polystemous) found in *E. californica* to identify and understand the genetic regulatory mechanisms that can balance two meristem types in the flower.

T52

PLANT EXTRACELLULAR VESICLES AND THEIR ROLE IN RNAI-BASED PLANT PROTECTION

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Extracellular vesicles (EV) are droplets encapsulated by a phospholipid bilayer and released by the fusion of multivesicular bodies with the plasma membrane. EVs are speculated mediators of RNA signalling molecules between plants and their interacting microbes such as short interfering (si)RNAs which are necessary for RNA interference (RNAi) a gene regulation mechanism. During RNAi, siRNA derived by double stranded (ds)RNA precursor molecules guide Argonaute (AGO) proteins by base-pair homology to their targeted messenger RNA which is in the following degraded by the AGO exonuclease activity. The dsCYP3RNA which causes resistance against Fusarium graminearum (Fg) can be derived by endogenous RNA-expressing transgenes or by exogenously applied foliar RNA spray application. We found CYP3RNA derived siRNAs in EVs isolated from CYP3RNA expressing *Arabidopsis thaliana* (Ath) plants and from CYP3RNA sprayed barley leaves. Additionally, ESCRT mutants of CYP3RNA expressing Ath lost their resistance phenotype towards Fg and target gene downregulation was not longer visible, supporting the hypothesis of EVs responsible for siRNA transport between both species. We further tested, if purified EVs from CYP3RNA sprayed barley leaves trigger growth inhibition of Fg and downregulation of the target genes. We observed that plant EVs neither derived by CYP3RNA expressing Ath nor CYP3RNA sprayed barley leaves cause growth reduction when applied to Fg in liquid culture or lead to target gene downregulation, but stress symptoms were observed as colony discolouration after applying EVs from CYP3RNA sprayed barley leaves on solid Fg agar plates.

T53

A LYTIC BACTERIOPHAGE CHANGES BIOFILM MORPHOLOGY OF *SHEWANELLA ONEIDENSIS*

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Surface-attached bacterial cells, which are embedded in a self-produced extracellular matrix are referred to as biofilms. The extracellular matrix consists of various substances such as polysaccharides and DNA. Cells in biofilms are more protected against several environmental stressors. Hence, biofilm formation is a considerable issue in medical and industrial settings. The potential usage of viruses (phages) that infect and lyse bacteria to prevent biofilm formation has been discussed for several years. Surprisingly, there exist differential data. Besides a phage-dependent elimination of biofilms, it could also been shown that phages promote biofilm formation due to a moderate lysis and release of extracellular DNA.
To study the influence of phages on biofilm formation, we used *Shewanella oneidensis* MR-1 as a model organism and isolated natural phages that can infect cells of this species. Among others, the isolated phages Thanatos and Phonos were characterized. Thanatos and Phonos are both lytic phages with completely different lysis behaviours. Cultures infected with Thanatos are quickly lysed while lysis due to Phonos can be barely observed.
Biofilm experiments with Thanatos or Phonos showed that biofilm formation is increased when phages are added at an initial phase. In contrast, only a treatment with Thanatos leads to reduced biofilm biomass in later stages of biofilm formation. Interestingly, it could also be shown that Thanatos-mediated cell lysis can change the biofilm morphology of a strain with a deficit in biofilm formation positively. Taken together, these results show that the social biology of phages

is extremely complex and not only based on simply killing the host.

T54

HOW DO BACTERIA RESTRICT THE ASSEMBLY OF A SINGLE POLAR FLAGELLUM?

Schwan, M[1], Blagotinsek, V[2], Mrusek, D[2], Khaledi, A[3], Glatter, T[4], Häussler, S[3], Bange, G[2], Thormann, K[1]

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Flagella are organelles of locomotion and multi-protein complexes whose positioning, number and assembly requires complex spatiotemporal control. Generally, flagellar assembly is thought to be controlled in several transcriptional tiers, which is mediated through several master regulators. Here, we have studied the regulation of flagellar genes in polarly flagellated gammaproteobacteria by the regulators FlrA, RpoN (σ54) and FliA (σ28) in *Shewanella putrefaciens* as a general modelorganism.
The major regulators, FlrA and RpoN, activate the transcription of the basal body building blocks, the flagellar export gate and FliA the regulator of late proteins. Notably, only a few of the early flagella proteins appear to be regulated at the protein level while most are present at normal levels also in the absence of the master regulators. The data indicates that strict control at both the transcriptional and protein level only occurs for key components for initiation of flagellar assembly (such as some early C-ring components), motor activation (such as MotY) and for highly abundant proteins, such as the outside structures (such as hook proteins and flagellins).
In addition, we investigated the regulation of the MinD-like ATPase FlhG, which is in many bacteria species a central factor to numcerical control of flagella. The deletion of flhG in polarly flagellated bacteria leads to polar hyperflagellation. Here, we have studied the molecular mechanism of this numerical control of flagella. A negative feedback of the FlrA-dependent gene expression is triggered by the interaction of FlhG with the HTH-domain of FlrA. Not only the cellular copy number of FlhG but also its subcellular localization is critical for its function in the numerical regulation of flagella. This study demonstrates another level of regulatory complexity underlying the spatio-numerical regulation of flagellar biogenesis, and implies that flagellar assembly transcriptionally regulates the production of initial building blocks.

T55

THE ROLE OF IMP3 IN MRNA LOCALISATION

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The Insulin-like growth factor 2 mRNA binding protein (IGF2BP, IMP) family is known to regulate RNAs in terms of stability, translation and localisation. In mammals, three paralogues of these multi-domain RNA-binding proteins (RBPs) exist, and all are recognised tumour markers, especially IMP1 and IMP3. Research focussed mainly on IMP1, since the role of IMP3 was often underestimated. Thus, the exact function of IMP3 still remains elusive. In this study, we explore the role of IMP3 in RNA localisation. Preliminary data of our lab implicate that IMP3 might function in the secretory pathway, in which mRNAs are guided to the endoplasmic reticulum (ER) for translation and the resulting protein translocated into the ER lumen. To obtain more insight on a global transcriptome level, we combined subcellular fractionation with next-generation RNA-sequencing (RNA-seq) and individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) in human ES-2 cells. We focussed on mRNAs with changes on the gene expression level in the membrane organelle fraction upon siRNA-mediated IMP3 knockdown. Combining the RNA-seq and iCLIP datasets, we found a subset of direct mRNA targets downregulated upon IMP3 knockdown. Biochemical validation by IMP3 RIP experiments and RT-qPCR of subcellular fractions confirmed our results and revealed mRNAs of the secretory pathway to be regulated by IMP3. In sum, we provide evidence for a novel distinct function of IMP3 in mRNA localisation.

T56

PIG RETINAL EXPLANTS AS AN INTERMEDIARY MODEL BETWEEN *IN VITRO* AND *IN VIVO* APPROACHES FOR GENE THERAPY

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Genome Editing is one of the most exciting developments in medical research over the last years. But there is a long way until an idea can actually be transferred into a treatment option for human patients. The standard route would lead from *in vitro* straight to *in vivo* in animals, but there are a lot of preconceptions on testing such treatments in animal models right away. An organotypic intermediate model like retinal explant cultures therefore might be the go-to approach to test efficacy and safety of the potential treatment candidates beforehand to reduce animal experimentation.

In the present study, porcine retinal explants were obtained from eyes of healthy adult pigs and cultured on a semipermeable membrane for up to 28 days. Different medium compositions were tested with regard to their effect on tissue integrity. Immunohistochemistry with different primary antibodies on frozen retinal explant sections was used to detect the quality of tissue preservation. Furthermore, as our planned gene therapy approach is based on adeno-associated viruses (AAV) as a shuttle, different AAV serotype-capsid-combinations encoding for the green fluorescent protein (GFP) were compared in their ability to transduce photoreceptors in retinal explant cultures.

Immunohistochemistry revealed changing morphology of the retinal explants over time. Ultimately one medium composition was chosen, where the changes were only barely noticeable so the explants could be kept in very good condition until at least day 20. Some vectors successfully transduced photoreceptor cells of the explants mostly in the periphery, while others allowed transduction of cells throughout the explant.

Since the project aims at testing gene therapeutic applications based on genome editing, subsequent experiments will focus on the application of CRISPR/Cas expression constructs to target stop mutations in the ABCA4 gene, which causes a cone mediated retinal degeneration.

Section 5 - Neurosciences

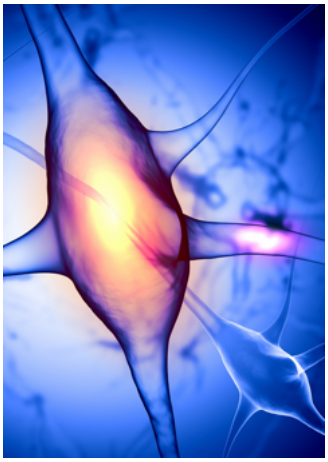

Image: colourbox.com

Day 1: Wednesday, September 29th, 2021

Section 5 - Neurosciences Chairpersons: Sara Shabani & Osama Elyamany

10:00 -10:30	**Dr. Aniko Korosi** (University of Amsterdam, The Netherlands) ***How does early-life stress lead to increased vulnerability to develop cognitive and metabolic dysfunction? A synergistic action of stress, inflammation and nutrition***
11:30-11:40	**Julia Diago Perez**: *Hypoxia-induced epigenetic reprogramming in tumour development*
11:40-11:50	**Aya Alserw**: *The role of 2-oxoglutarate homeostasis in tumour invasion and metastasis*
11:50-12:00	**Dominic Osei**: *Role of peroxisomes in BoDV-induced neuroinflammation under modified TNF signaling paradigms*
11:50-12:00	**Melina Kahl**: *A subpopulation of olfactory receptor neurons in the baso-lateral main olfactory epithelium of Xenopus laevis expresses S100z*
12:00-12:10	**Daniela Daume**: *Morphological and functional visualization of the secondary olfactory pathway in larvae of the African Clawed frog Xenopus laevis*
12:10-12:20	**Osama Elyamany**: *Top-Down and Bottom-up Modulation of a Dichotic Listening Task with simultaneous Electroencephalography (EEG)*
13:30-13:40	**Jessica Hernandez**: *Neutropenia enhances the brain and peripheral inflammatory response to LPS-induced hypothermic severe systemic inflammation*
13:50-14:00	**Rebecca Classen**: *Stimulation of muscarinic receptors by functionalised gold nanoparticles*
14:00-14:10	**Sara Shabani**: *The Role of Oxytocin in Sexual Sensation Seeking: A Candidate Gene Study Looking at rs3796863 and rs53576*
15:05-15:15	**Benedicta Mensah**: *Ligamentum arteriosum is an innervated contractile smooth muscle*

K5

HOW DOES EARLY-LIFE STRESS LEAD TO INCREASED VULNERABILITY TO DEVELOP COGNITIVE AND METABOLIC DYSFUNCTION? A SYNERGISTIC ACTION OF STRESS, INFLAMMATION AND NUTRITION

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Early-life stress (ES) is associated with increased vulnerability to cognitive impairments as well as metabolic disorders like obesity later in life. We investigate the role of a synergistic effect of stress, metabolic factors, nutrition and the neuroimmune system in this early-life induced programming.
We use an established model of chronic ES and expose mice to limited nesting and bedding material during first postnatal week and study the central and peripheral systems under basal and challenged conditions (i.e. LPS, amyloid accumulation, western style diet (WSD) and exercise) to gain further insight in the functionality of brain plasticity, neurogenesis microglia and adipose tissue. In addition, given the high nutritional demand during development, we propose that early nutrition is critical for programming of brain and body. We focus on essential micronutrients and fatty acids and propose that an early dietary intervention with a diet enriched with these nutrients might protect against ES-induced functional deficits.
We show that ES leads to cognitive impairments associated with reduced hippocampal neurogenesis at basal conditions as well as in response to exercise, primed microglia with exaggerated response to LPS or amyloid accumulation. Metabolically, ES mice exhibit a leaner phenotype but they accumulate more fat in response to WSD. Finally, with an early dietary intervention with micronutrient or fatty acid we were able to (at least partly) prevent ES-induced cognitive decline, likely mediated by modulation of microglia, without however affecting the ES-induced metabolic profile. These studies give new insights for the development of targeted dietary interventions for vulnerable populations.

T57

THE ROLE OF 2-OXOGLUTARATE HOMEOSTASIS IN TUMOUR INVASION AND METASTASIS

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[1] These authors contributed equally to this work*

Metabolic reprogramming, a recognized hallmark of cancer, has been associated with tumour invasion and metastasis. Epithelial/proneural-to-mesenchymal transition (EMT/PrMT) is a key process behind cancer cell dissemination, which is regulated by several transcription factors, including Snail. 2-oxoglutarate (2-OG) is a metabolite whose intracellular level depends on the activity of isocitrate dehydrogenase 1 (IDH1). The metabolite acts as a co-substrate for 2-OG-dependent dioxygenases (2-OGDDs) which regulate different cellular activities, including metastatic capacity. While mutant-IDH-induced, 2-OGDD-dependent tumorigenic mechanisms have been described in detail, it is less well understood how wild-type IDH and 2-OG levels control EMT and invasion. We aim to define the homeostatic function of IDH1 and 2-OG in the regulation of EMT and tumour progression primarily in glioblastoma, as well as in breast and lung cancer models.
Boyden chamber, western blot, immunofluorescence, RT-qPCR, RNAseq and metabolite mass spectrometry assays are employed to determine the role of IDH1 and 2-OG levels on EMT regulators and cell invasion. IDH1 knockdown and several functional assays are used to further explore the underlying mechanisms.
We observed that TGFβ; an established EMT inducer results in IDH1 downregulation, with a concomitant increase in Snail expression and invasion. Strikingly, 2-OG supplementation reverts TGFβ-induced Snail at the mRNA and protein level as well as cellular invasion *in vitro*. In line with a functional role of IDH1 in EMT, IDH1 deficiency promotes invasion and metastasis and enhances a cancer stem cell phenotype. Importantly, ongoing experiments are conducted to iden-

tify crucial mechanistic pathways involved in Snail regulation by 2-OG.
Collectively, our findings emphasize the interplay between metabolism and metastasis and reveal novel mechanistic insights into the role of wildtype IDH and intracellular 2-OG levels in the metabolic control of tumor invasion and EMT/PrMT.

T58

STIMULATION OF MUSCARINIC RECEPTORS BY FUNCTIONALISED GOLD NANOPARTICLES

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Nanomedicine has gained high attention in the last years due to the wide range of possible applications of nanostructures. These nanostructures can be functionalised by binding a high number of ligands on their surface and thereby they can be used for specific drug targeting. Due to the plenty of ligands stabilised on a single nanostructure, they can show multivalent interactions at the receptor side. In this project, the two ligands carbachol and atropine were bound to the surface of spherical gold nanoparticles with different diameters as the effects of nanoparticles are highly dependent from their chemical properties. Their influence on intestinal smooth muscle cells and epithelial secretion is tested in organ bath and Ussing chamber experiments. While carbachol, a muscarinic receptor agonist, leads to an enhanced contraction of the intestine and an increased chloride secretion, atropine, a muscarinic antagonist, inhibits these effects. Therefore, carbachol and atropine functionalised nanoparticles could serve as novel therapy options for intestinal diseases like postoperative ileus or spasms of the gut. Ussing chamber experiments revealed that the inhibition of chloride secretion by atropine functionalised nanoparticles is clearly size-dependent. The mucosal absorption of the nanoparticles is an important factor for possible oral application in the patient. Hence, functionalised nanoparticles were applied on the mucosal side of the intestine and were able to diminish intestinal contractions and chloride secretion. Transmission electron microscope images of the intestine will help to investigate how the nanoparticles can cross the intestinal epithelium.

T59

MORPHOLOGICAL AND FUNCTIONAL VISUALIZATION OF THE SECONDARY OLFACTORY PATHWAY IN LARVAE OF THE AFRICAN CLAWED FROG *XENOPUS LAEVIS*

Daume, D[1], Offner, T[1], Weiss, L[1], Hassenklöver, T[1], Manzini, I[1]

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In the vertebrate olfactory system, olfactory receptor neurons (ORNs) located in the olfactory epithelium (OE) send axons to the olfactory bulb (OB) where they synaptically connect to projection neurons (PNs). In *Xenopus laevis* larvae, two types of ORNs are present in the OE with differences in morphology, odor-sensitivity and signal transduction mechanisms. These two ORN populations form individual odor-processing streams, which target strictly separate OB regions. In the OB, projection neurons relay olfactory information to the olfactory cortex (OC). It is unknown if these odor-processing streams are also mapped to distinct areas on the level of the OC. In this study, we set out to investigate the secondary olfactory pathway of larval *Xenopus laevis*. We first morphologically characterized the bulbar PNs via immunohistochemical stainings. We further injected and electroporated groups and single PNs in whole-mount preparations of the brain with various fluorescent dyes. Multiphoton imaging allowed the visualization of the connections of the bulbar PNs to the OC. Several cortical target regions could be identified, including the medial and lateral amygdala, the dorsal pallium, and the preoptic area. Thereby, we found that axons of PNs located in the medial and lateral OB divide into two independent olfactory tracts targeting different areas of the OC. We recorded odorant-induced responses of cortical neurons by multiphoton calcium imaging in GCaMP6 transgenic animals, directly proving that OC regions are functionally connected with the OB. Together, this is the first functional description of the secondary olfactory pathway in an amphibian species. It provides a solid basis for further investigations aiming to understand olfactory information processing in the OC and indicates that the segregation in two odor-processing streams could be continuous in the secondary olfactory pathway.

T60

HYPOXIA-INDUCED EPIGENETIC REPROGRAMMING IN TUMOUR DEVELOPMENT

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One of the crucial questions in tumour biology is to understand how tumour cells can exploit the pro-tumour functions of hypoxia and overcome the growth inhibitory and stress signals to fully activate the hallmarks of cancer. Considering that cancer cells undergo hypoxia and re-oxygenation periods during metastasis, we have developed an *in vitro* model by using lung, breast and glioma cancer cells exposed to repeated hypoxia-reoxygenation cycles called intermittent (IM) hypoxia.

We aim to elucidate the molecular mechanisms that lead to a more invasive tumour cell phenotype under such conditions, focusing on potentially stable epigenetic changes. We hypothesise that hypoxia-inducible transcription factors (HIFs) may promote the induction and establishment of an adapted invasive phenotype.

Our results revealed that IM-hypoxia-treated cells exhibit increased invasiveness and elevated levels of Snail transcription factor that drives epithelial to mesenchymal transition (EMT) and cancer cell dissemination. Moreover, we observed increased inducibility of the hypoxia-inducible transcription factor 2 alfa subunit (HIF2α) and increased levels of the DNA demethylase enzyme, TET1. This led to hypothesise that a hypoxic-induced and epigenetically stable invasive phenotype could be established.

To uncover interplay between HIF2α, TET1 and Snail in detail and to evaluate their role in controlling the IM hypoxia gene regulatory programme, we are currently using RNAseq and genome-wide DNA modification analyses under control in HIF2α silenced conditions in breast cancer cells. Further experiments are planned to investigate crosstalk with histone methylation, particularly with histone lysine demethylases (KDMs), as several KDMs were described as cellular O2 sensors which potentially orchestrate the regulation of tumour progression with HIFs and TET1 under various hypoxic conditions. Taken together, these studies will reveal the regulatory mechanisms behind the establishment and maintenance of the IM hypoxia-induced phenotype and adaptive epigenome changes, which may have an impact on defining therapeutic targets to inhibit metastatic spreading of cancer cells.

T61

TOP-DOWN AND BOTTOM-UP MODULATION OF A DICHOTIC LISTENING TASK WITH SIMULTANEOUS ELECTROENCEPHALOGRAPHY (EEG)

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Dichotic Listening (DL) involves the presentation of two different sounds simultaneously to both ears (one to the right ear and the other to the left ear). Participants with left-hemispheric dominance report more sounds from the right ear (showing a right-ear advantage). During the left-ear report, the auditory information must be transferred from the right to the left (dominant) hemisphere. Accordingly, using Electroencephalography (EEG), functional connectivity between both auditory cortices increased during left-ear reports.

Having applied top-down modulation of DL (attention instructions) last year, we extended our work by adding bottom-up modulation. This was achieved by modulating the sound intensity of right or left ear sounds.

Therefore, 30 right-handed participants performed three blocks (360 sound pairs) of DL during simultaneous EEG recording. The intensity of right and left sounds was modulated to produce three different conditions: no sound attenuation, right or left sound attenuation.

During the non-attenuation (neutral) condition, participants exhibited the typical right-ear advantage. As hypothesised, the left-attenuation condition significantly increased right-ear reports while the right-attenuation condition decreased them.

In conclusion, bottom-up modulation of DL affects the behavioural outcome by increasing the reports from the ear with higher sound intensity. In addition to these behavioural results, we will present EEG findings of both top-down and bottom-up modulations.

T62

NEUTROPENIA ENHANCES THE BRAIN AND PERIPHERAL INFLAMMATORY RESPONSE TO LPS-INDUCED HYPOTHERMIC SEVERE SYSTEMIC INFLAMMATION

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The innate immune system plays a pivotal role in shaping acute inflammatory responses through immune-to-brain signaling. This includes central nervous system-induced sickness responses, such as fever, lethargy, and adipsia, which are known to occur during systemic inflammation. Previous studies have shown that leukopenia can alter sickness responses leading to a prolonged fever response. Moreover, neutropenic fever is a severe clinical status of unknown origin. We aimed to investigate the effects of neutropenia on the sickness response and immune-to-brain signaling during acute systemic inflammation in mice. To induce neutropenia and systemic inflammation, mice received an intraperitoneal injection of anti-polymorphonuclear serum (PMN) followed by a high dose intraperitoneal injection of lipopolysaccharide (LPS, 2.5 mg/kg) 24 or 48 hours later. Brains, peripheral tissue, and serum were collected at 4h or 24h after LPS-stimulation for detection of peripheral/brain inflammatory markers. To investigate the physiological significance of neutropenia, we continuously recorded locomotor activity, core body temperature, food, and water intake using a telemetric system over the course of the experiment. PMN reduced circulating neutrophils by approximately 20% compared to control mice that received NRS in dose response experiments. Initial experiments revealed that neutropenia inhibited recruitment of neutrophil granulocytes (NG) to the brain, brain nuclear factor interleukin-6-activation, and was associated with inhibited LPS-induced expression of the anti-inflammatory cytokine interleukin 10 and NG specific chemokine CXCL1 (48h). PMN-pretreatment exacerbated LPS-induced hypothermia compared to NRS controls (24h), while adipsia, anorexia, and loss in body weight were not affected by neutropenia (4h and 24h). Further analyses revealed that LPS-induced increase in corticosterone serum levels were higher (24h) and levels of circulating cytokines were enhanced (4h and 24h) in PMN mice when compared to NRS counterparts. Together, our ongoing experiments suggest an anti-inflammatory role of NG with neutropenia exacerbating sickness and immune responses during systemic inflammation.

T63

A SUBPOPULATION OF OLFACTORY RECEPTOR NEURONS IN THE BASO-LATERAL MAIN OLFACTORY EPITHELIUM OF *XENOPUS LAEVIS* EXPRESSES S100Z

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S100z is a member of the multigene family of S100 calcium-binding proteins, and exclusive to vertebrates. Calcium-binding proteins are of fundamental importance for the establishment and maintenance of neuronal activity and have previously been found in chemosensory cells of the amphibian *Xenopus laevis*. In this study, we analysed the expression pattern of S100z in the olfactory system of *Xenopus laevis* using immunohistochemistry. In larval animals, we found that S100z expression was specific to olfactory receptor neurons in the main olfactory epithelium (MOE) and was absent in cells within the vomeronasal organ (VNO). Interestingly, S100z-expressing neurons were distributed in the whole epithelium, but a large number of these cells were located basolaterally. Additionally, we observed that the axons of S100z-expressing neurons project into the lateral glomerular cluster of the olfactory bulb. In adult animals, S100z was exclusively expressed in receptor neurons in the middle cavity within the MOE. Based on the distribution pattern of the S100z-positive neurons, we assume that S100z is labelling microvillous olfactory receptor neurons, and is probably expressed by a specific subfamily of receptors. The most likely candidates are an ancient clade of vomeronasal type 2 receptors. Future experiments using in-situ hybridization will be needed to confirm this assumption. All in all, our findings provide an excellent basis for future studies to characterize olfactory receptor subfamilies and the functions of calcium-binding proteins in aquatic vertebrates.

T64

LIGAMENTUM ARTERIOSUM IS AN INNERVATED CONTRACTILE SMOOTH MUSCLE

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The ligamentum arteriosum (LA) is generally considered a mere remnant of the embryonic bypass (ductus arteriosus) from the pulmonary circulation to the aortic arch, obliterating soon after childbirth. This study set out to elucidate the morphology, innervation and neurochemistry of LA innervation.

LA of human, pig, wild-type and transgenic mice were studied using routine and special histological staining methods, single- and double-immunofluorescence labeling using antibodies directed against structural markers, neuropeptides, and transmitter synthesizing enzymes such as α-smooth muscle actin (αSMA), neuropeptide Y (NPY), and tyrosine hydroxylase (TH) respectively. Ultrastructural studies of pig and mice LA using transmission electron microscopy (TEM) was employed. Contractility of pig LA was assessed in organ bath experiments, applying electrical field stimulation and increasing cumulative dose of noradrenalin.

Contrary to a canonical ligament, the LA was mainly made up by αSMA-positive cells in all three species. TEM confirmed the presence of vascular smooth muscle cells within LA tunica media. Myofilaments, dense bodies and bands, all relevant in the formation of the contractile apparatus of vascular smooth muscle, were observed. The LA received a noticeable amount of chrna3-eGFP-, PGP 9.5-, TH-, NPY-, SP-, and CGRP-positive fibers in all three species. TEM revealed nerve terminals in close proximity to smooth muscle cells. The LA contacted in response to electrical field stimulation and exogenous noradrenalin.

It is fact that the LA no longer serves in its original capacity as a foetal shunt connecting the two great vessels. However, the presence of contractile smooth muscle is present long after obliteration and until senescence. These muscular elements have noticeable sympathetic and sensory innervation. It may be postulated that the contractile abilities of LA myocytes may act on the two great vessels to which it is attached causing a change in their distensibility.

T65

ROLE OF PEROXISOMES IN BODV-INDUCED NEUROINFLAMMATION UNDER MODIFIED TNF SIGNALING PARADIGMS

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Borna disease virus 1 (BoDV-1)-induced neuroinflammation can cause marked morphological and functional abnormalities of neuronal and glial cells. Peroxisomes are known as pivotal regulators of the immune system. Therefore, we thought to investigate their role in the neuropathology of BoDV-1 infection under conditions of different TNF-α signaling paradigms, such as elevated levels of TNF-α and in the absence of the (pro-apoptotic) TNF receptor (TNFR)1 and (anti-apoptotic) TNFR2 pathways. Using indirect immunofluorescence stainings, quantitative analyses of the peroxisomal biogenesis protein PEX14 (a marker for the total number of peroxisomes) and the peroxisomal matrix enzyme catalase (a marker for the redox state of cells) were performed in three different brain regions (i.e. the hippocampal formation, cerebellum and cerebral cortex) of 42-day-old wild-type mice in comparison to TNF-α transgenic mice (TNFTg; TNF-α is overproduced mainly in the hippocampus, cerebral cortex, striatum and thalamus), TNFR1- and TNFR2- knockout mice, with and without BoDV-1 infection at day 0 after birth. Our findings imply that murine neonates with: (i) prolonged exposure of brain cells to TNF-α; (ii) and BoDV-1 infection showed a reduction in the number of catalase-positive peroxisomes in the hippocampal formation, cerebral cortex and cerebellum; (iii) exposure to TNF-α plus BoDV-1-infection did not cause a further decrease in catalase abundances compared to non-infected TNFTg animals; (iv) and TNFR1, in the absence of TNFR2, appears to enhance peroxisome numbers in neurons. Going forward, we have planned for immunofluorescence stainings and subsequent quantitative analyses of mitochondria in similar brain regions of the same 4 mice variants, with and without BoDV-1 infection; and, in addition, to confirm recent data and hypotheses in hippocampal slice cultures.

T66

THE ROLE OF OXYTOCIN IN SEXUAL SENSATION SEEKING: A CANDIDATE GENE STUDY LOOKING AT RS3796863 AND RS53576

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Researchers have shown the involvement of oxytocin in sexual relationships and bonding with partners. However, some people with high sexual sensation seeking (SSS) mostly prefer to have casual sex and change partners frequently. Thus, it would be interesting to investigate the potential relationship between oxytocin and SSS levels. Participants (N=274) provided saliva samples and completed the Sexual Sensation Seeking Scale (SSSS) and the Personal Assessment of Intimacy in Relationship (PAIR). The saliva samples were analyzed to genotype the two Single Nucleotide Polymorphisms (SNPs) of rs3796863 from Cluster of Differentiation 38 gene (CD38) and rs53576 from oxytocin receptor gene (OXTR) by PCR. Data analysis indicated that the carriers of homozygous A-alleles of rs3796863 had significantly lower scores in SSSS in comparison to carriers of homozygous and heterozygous C-alleles. In addition, carriers of AA (rs3796863) + GG (rs53576) had lower SSS scores than carriers of CC (rs3796863) + AA (rs53576). The carriers of homozygous and heterozygous A-allele showed significantly higher scores in emotional, sexual and intellectual intimacy in comparison to those of CC carriers of rs3796863 SNP; but none of the intimacy scales showed any significant difference for rs53576 SNP. In conclusion, only the polymorphisms of the rs3796863 SNP could predict the level of SSS, and the emotional, sexual and intellectual intimacy. None of the polymorphisms of the rs53576 SNP could predict the level of different types of intimacy, but the interaction between the two SNPs can be a predictor for the level of SSS.

Section 6 - Reproduction in Man and Animals

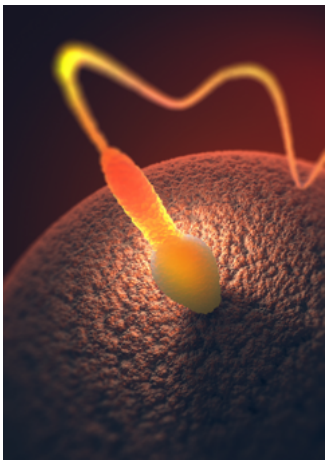
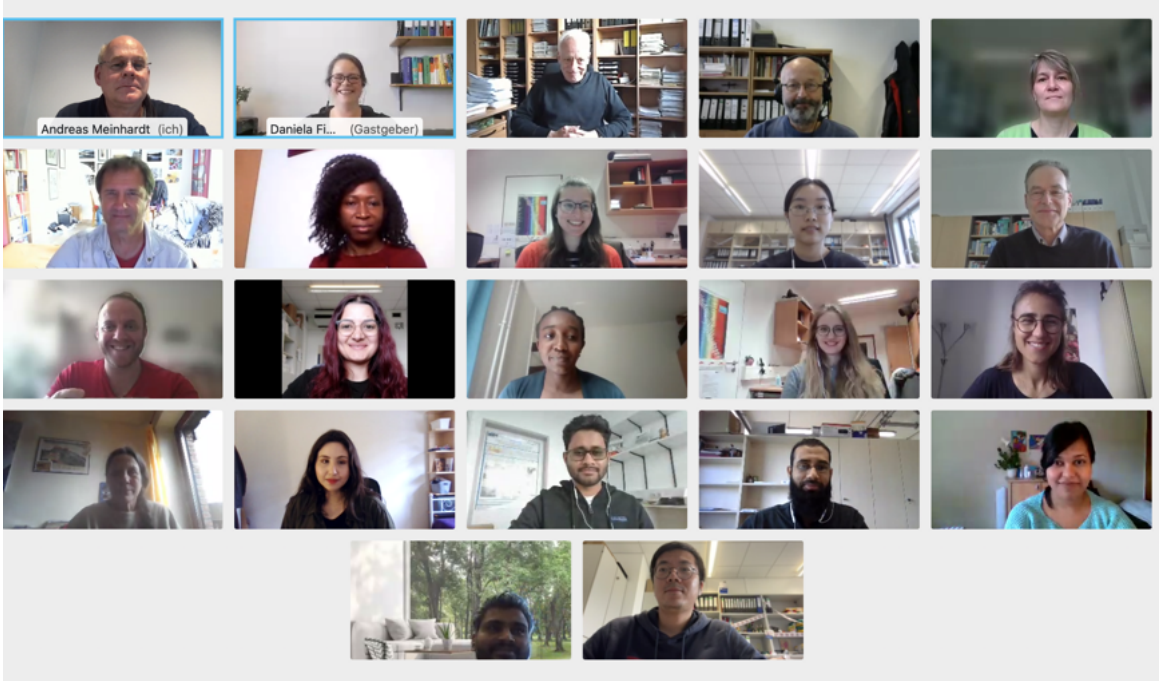


Image: colourbox.com



Day 2: Thursday, September 30th, 2021

Section 6 - Reproduction in Man and Animals — Chairpersons: Christine Rager & Shanjid Shiplu & Shashika Kothalawala & Hiba Hasan

09:00-09:30	**Prof. Robin Hobbs** (Monash University / Hudson Institute of Medical Research, Clayton, Australia) ***Maintenance and regeneration of the male germline***
11:15-11:35	**Hiba Hasan**: *Activin A, chemokines and their receptors are important players in the inflammatory response during chronic testicular inflammation in mice*
11:35-11:55	**Hang Yan**: *Epigenetic Dysregulation of Tumor Suppressor Genes in CP/CPPS: studies on liquid biopsies for biomarker development*
11:45-12:05	**Rashidul Islam**: *Tumour infiltrating T lymphocytes in human testis cancer – identification and functional analysis*
12:05-12:15	**Wei Peng**: *Loss of CCR2 inhibits testicular fibrosis caused by experimental autoimmune orchitis: possible role for activin A in fibrosis development*
13:15-13:25	**Shashika Kothalawala**: *Use of mass spectrometry to identify proteins related to impaired sperm morphology and motility in infertile men*
13:25-13:35	**Magdalena Anastazja Kuchta**: *Natriuretic peptides affect prostatic smooth muscle cells and are potential drugs for BPH*
13:35-13:45	**Jane Maoga**: *The role of the membrane-type 1 matrix metalloproteinase (MM14) in the pathophysiology of endometriosis*
13:45-13:55	**Christine Rager**: *A novel approach to visualize superficial wall movements in seminiferous tubules*
13:55-14:05	**Agnes Mwaura**: *TGF-β1/β2 mediate reduction in betaglycan shedding via Alk-5 and SMAD3 pathway in human endometrical cells*
14:30-14:40	**Vishnu Kumar**: *What's in there? Looking at immune cells in the entire male reproductive tract*
14:40-14:50	**Hassan Kabbesh**: *Formation of the Testicular Immunological Barrier through Immune Modulation by Somatic Cells*
14:50-15:00	**Dingding Ai**: *The role of different immune cell subsets in the immune response to bacterial infection of the murine epididymis*
15:00-15:10	**Shanjid Ahmed Shiplu**: *Ten-eleven-translocation enzymes (TETs), DNA methyltransferases (DNMTs) and polycomb repressive complex 2 (PRC2): links to male infertility*
15:10-15:20	**Sèyi Vanvanhossou**: *Demographic Processes and Recent Selection Signatures in Beninese Indigenous Cattle breeds*

K6

MAINTENANCE AND REGENERATION OF THE MALE GERMLINE

Prof. Robin Hobbs[1]

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Maintenance of male fertility is dependent on spermatogonial stem cells (SSCs) that self-renew and generate differentiating germ cells for production of spermatozoa. SSC function is dependent on growth factors produced within the testis microenvironment plus cellular factors that regulate gene expression within SSCs and modulate responses to growth factor stimulation. Despite the importance of SSCs for male fertility, the molecular mechanisms that regulate their function and maintenance remain incompletely understood. Importantly, SSC function and male fertility can be compromised by multiple factors including exposure to genotoxic drugs. However, cellular pathways mediating the regenerative response of SSCs following germline damage and loss of SSC function with age are poorly studied. Our research focuses on defining genetic controls and cellular pathways regulating SSC function and male fertility. We employ a range of *in vivo* and *in vitro* experimental systems allowing dissection of mammalian SSC function. We have defined essential roles for the developmental transcription factors PLZF and SALL4 in maintenance of SSC activity and the central importance of mTORC1 signalling in SSC fate regulation. In addition, our studies have characterised cellular heterogeneity within the SSC and progenitor cell pool using single cell approaches and demonstrated the dynamic nature of spermatogonial states with important clinical implications. Current studies are focused on understanding cellular machinery modulating the response of SSCs to stimuli from the niche and molecular mechanisms supporting germline regenerative capacity.

T67

THE ROLE OF DIFFERENT IMMUNE CELL SUBSETS IN THE IMMUNE RESPONSE TO BACTERIAL INFECTION OF THE MURINE EPIDIDYMIS

Ai, D[1], Winter, L[1], Chakraborty, T[2], Bhushan, S[1], Pleuger, C[1], Meinhardt, A[1]

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Acute epididymitis is a common urogenital disease often caused by infections with ascending uropathogenic *E. coli* (UPEC). Although the epididymis is composed of a single continuous duct, previous studies revealed that the different epididymal regions, namely initial segment (IS), caput, corpus and cauda, show striking differences in their immune responses. While initial segment and caput remain mostly unaffected, the cauda shows a strong immune response accompanied with fibrotic remodeling, resulting in duct obstructions and ultimately, infertility. However, the underlying reasons for the different immune responses are still unclear.
Therefore, this study is aiming at analyzing the different immune responsivenesses of the epididymal regions using a mouse experimental epididymitis model. UPEC were injected into the vas deferens and disease progression was assessed at different time points after infection (day 1, 3, 5, 7, 10, 14, 18). Histological alterations were assessed by Masson-Goldner-Trichrome staining and mRNA expression levels of inflammatory cytokines (Tnfα and *Il10*) by RT-qPCR (n=3–4). UPEC and infiltrating immune cell populations, i.e. neutrophils and macrophages, were detected by immunofluorescence (n=3).
With time progression after UPEC infection, immune responses and histological changes varied amongst regions. Cauda epididymides of UPEC-infected WT mice showed fibrosis and leukocyte infiltrations, but not caput and IS. Especially neutrophils and macrophages were highly abundant and co-localized within the corpus and cauda. This is in line with significantly increased mRNA expression levels of Tnfα and Il-10 in corpus and cauda, which was not evident in the IS and caput. These results identified the transition from the progression to resolution of inflammation at day 14 after infection as indicated by alternating expression of Tnfα and *Il10* and neutrophil clearance by macrophages (efferocytosis). Further investigations on WT and macrophage-depleted mice will concentrate on the particular function of resident macrophages in the initiation of the immune response and resolution of inflammation.

T68

ACTIVIN A, CHEMOKINES AND THEIR RECEPTORS ARE IMPORTANT PLAYERS IN THE INFLAMMATORY RESPONSE DURING CHRONIC TESTICULAR INFLAMMATION IN MICE

Hasan, H[1], Peng, W[1], Bhushan, S[1], Wahle, E[1], Pleuger, C[1], Stefan, G[2], Loveland, KL[3,4], Meinhardt, A[1,3], Hedger, M[3,4], Fijak, M[1]

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Experimental autoimmune orchitis (EAO) is a model of autoimmune-based chronic testicular inflammation. It shares features with idiopathic clinical male infertility and is characterized by production of auto-antibodies against testicular antigens as well as severe immune cell infiltration into the interstitium. Elevated levels of pro-inflammatory cytokines and chemokines, especially activin A and CCL2, as prominent inflammatory mediators during testicular inflammation in EAO mice, are hypothesized to act as pro-inflammatory and pro-fibrotic agents leading to testicular damage. To induce EAO, C57BL/6J (WT) and CCR2 (receptor for CCL2) deficient (CCR2-/-) mice were immunized with testicular homogenate. In WT EAO, testicular architecture was disturbed, while it was preserved in CCR2-/- EAO mice. The gene expression of several chemokines and their receptors was changed in WT EAO testis, but attenuated by CCR2 deficiency, particularly the mRNA expression of chemokine ligand Cxcl13 (B cell chemoattractant) was increased ∼100 - fold in WT EAO mice as measured by qRT-PCR. Moreover, B cells and plasma cells were observed in WT EAO testis using immunoflourscence and their presence was not as prominent in CCR2-/- EAO mice. Since activin A was upregulated during EAO, its influence on immune and testicular cells was assessed. Stimulating bone marrow derived macrophages (BMDM) (surrogate of testicular macrophages) with activin A (50ng/ml) led to a decrease in the gene expression of most chemokines. Co-culture of activin A treated BMDM with T cells led to a decrease in the induction of pro-inflammatory cytokines (TNF-α and IFN-γ) in T cells. Our preliminary data show that chemokines and chemokine receptor mRNA expression are altered in WT EAO testis. Mice deficient for CCR2 are protected from EAO development. Disturbances in the chemokine network point to a crucial role of CCL2/CCR2 axis in exacerbating testicular inflammation. Activin A emerges as a potential candidate in regulating chemokine network in the testis.

T69

TUMOUR INFILTRATING T LYMPHOCYTES IN HUMAN TESTIS CANCER – IDENTIFICATION AND FUNCTIONAL ANALYSIS

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T cells are critical to tumour development and associated immune surveillance. CD4+ regulatory (Treg) and follicular helper T cells (Tfh) are associated with poor prognosis of different cancers, but their involvement in human testicular germ cell tumours (TGCT) is unknown. Therefore, we aim to identify and characterise Treg and Tfh in TGCT development and progression. For this, human testis samples with seminoma (SE; n=23), or germ cell neoplasia *in situ* (GCNIS) with/without lymphocytic infiltrates (LY) (n=10, each) were compared to samples containing revealing normal spermatogenesis (NSP; n=10), or hypospermatogenesis with infiltrates (HYP+LY; n=10). Antibodies against CD20cy, CD68, CD3, CD4, CD8, CD25, FOXP3, CXCR5, and BCL6 used in immunohistochemistry (IHC) showed that T cells, including Treg and Tfh cells, are more abundant in SE compared to all other groups NSP, HYP+LY, GCNIS, and GCNIS+LY samples. Furthermore, flow cytometry was performed on sam-

ples collected from different localisations of TGCT-containing testes: tumour (tu), tu-adjacent, tu-distant, and contralateral testis (n=16, in a range of 9.51x102–1.12x106 cells). By histopathological analysis, samples were categorised into pure SE (n=7), embryonal carcinoma (EC, ≥80%) (n=4), other tumours (n=4), and undefined (n=1). We used the same antibody panel for flow cytometry and for IHC. A high abundance of T cells was observed in tu sites of SE compared to other localisations in SE, EC (≥80%) and other tumours. Furthermore, in addition to the predominant CD4+ and CD8+ T cell subsets (6.73–60.2% and 20.32–43.3 % of CD45+ cells, respectively), Treg and Tfh cells (0.070–34.2% and 0–4.17% of CD45+ cells, respectively) were detected in all localisations of testis samples, suggesting that Treg and Tfh cells also influence TGCT biology. This study has demonstrated the immune cell complexity and indicated the possible importance of rarer T cell subtypes in the tumour immune environment; future experiments will interrogate their functions in TGCT.

T70

FORMATION OF THE TESTICULAR IMMUNOLOGICAL BARRIER THROUGH IMMUNE MODULATION BY SOMATIC CELLS

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The blood-testis-barrier (BTB), which is mainly based upon Sertoli cells (SC), divides the seminiferous epithelium into a basal and an adluminal compartment. The main role of the BTB is to form an immunological barrier in order to preserve the meiotic and post-meiotic stages of the germ cells from the immune system. The BTB is composed by a number of tight junction proteins, mainly the claudins family. Disorder of the BTB integrity caused by any internal or external factors might result in male infertility.
Our study aims at elucidating the role of different cell combinations (mainly SC and peritubular cells (PC) isolated from adult rats) on the BTB integrity and to elucidate the contribution of each cell type to the testicular immunological barrier (TIB). Furthermore, we treated rat primary SC with particular cytokines and hormones to address their effects *in vitro* on the TJ proteins and thus the BTB integrity.
Our experiments showed that primary SCs are the main constituent of the BTB *in vitro*. Co-culturing of both rat SCs and PC on Matrigel only had a negligible effect on the tightness of the barrier. Likewise, transmigration assay of polarized rat macrophages (M0-M1-M2) did not show a significant difference of the number of migrated macrophages through a monolayer of SC or through a co-culture of SC and PC. Moreover, treatment of SC with bone morphogenetic protein2 (BMP2), interleukin-6 (IL-6) or transforming growth factor beta-3 (TGF-β3) resulted in a negative effect on the barrier tightness. In contrast, treatment of SC with testosterone upregulated the tight junction proteins zonula occludens^{-1} (ZO-1), junctional adhesion molecule-3 (JAM-3) and the barrier tightness via the classical or non-classical androgen pathway. SC tight junctions are the main constituent of the BTB *in vitro*.

T71

USE OF MASS SPECTROMETRY TO IDENTIFY PROTEINS RELATED TO IMPAIRED SPERM MORPHOLOGY AND MOTILITY IN INFERTILE MEN

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Spermatogenesis requires expression of approx. 2000 genes; genetic causes are likely involved in up to 30% of male infertility cases. Nevertheless, only few causative genes have been identified. Thus, further research is required to identify causes and potential treatments for different forms of male infertility. Complementing next generation sequencing approaches, mass spectrometry is a powerful tool to identify protein expressions between control and infertility groups. This study focused on identifying proteins altered in human ejaculated spermatozoa with impaired morphology and motility to identify

proteins associated with key defects in sperm function.
Human ejaculates classified as normozoospermia (NORM, n=3) or defective morphology-motility (asthenoteratozoospermia (AT), n=3) were prepared ensuring equal amount of proteins per sample and subjected to mass spectrometry. In silico analysis of differentially expressed proteins was performed. Selected proteins were examined by immunohistochemistry (IHC) in testicular biopsies from men with normal spermatogenesis (NSP) or spermatid arrest (SDA) phenotype.
36 proteins were significantly up or downregulated in AT compared to NORM. In silico analysis identified several interesting candidate proteins of which five were selected for further investigation: ACTRT2, IPO4, CCDC105, IFT57 are testis specific proteins, and IHC revealed their localisation in human germ cells (all) with focus on spermatids (ACTRT2, IFT57, IPO4). Importantly, ACTRT2 and CCDC105 were notably reduced in spermatids in SDA. As epididymal-specific protein, ELSPBP1 was identified to be increased in AT.
In conclusion, we identified 36 proteins that show quantitative changes in AT compared to NORM human sperm; these may have important functions in spermiogenesis and alterations might be linked to abnormalities in sperm function of infertile men. Further studies are underway to characterise the localisation of selected proteins in sperm, to better understand their functional role in human spermatozoa and to validate their differential expression in a larger set of samples.
Supported by DFG GRK 1871/1

T72

NATRIURETIC PEPTIDES AFFECT PROSTATIC SMOOTH MUSCLE CELLS AND ARE POTENTIAL DRUGS FOR BPH

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Since the natriuretic peptides (NPs; ANP, BNP, CNP) were discovered between 1980–1990 their functions were well described in many organs and tissues. A high concentration of CNP was found in the male reproductive tract such as the prostate. In addition, high levels of CNP were also detected in porcine seminal plasma. However, further studies on the function of NPs in the prostate are missing. NPs act through cyclic GMP (cGMP) signaling pathways which regulate for example relaxation of smooth muscle cells or cell proliferation. This could be of interest for the treatment of benign prostatic hyperplasia (BPH) since an increase in muscle tone and cell proliferation are the main changes in this disease. For this, the hormonal influence of NPs on the cGMP signalling pathway and its significance for BPH needs to be investigated. First of all, we showed that cGMP pathway components, e.g. the receptors for natriuretic peptides GC-A and GC-B, are expressed in total human prostatic tissue and in cultured human primary prostatic smooth muscle cells (HPrSMCs). Interestingly, GC-B, the receptor for CNP, was higher expressed than the ANP and BNP receptor GC-A. In agreement, we found that CNP, more than ANP, activates the cGMP production in HPrSMCs in a dose-dependent manner by cGMP ELISA. Human primary aortic smooth muscle cells (HAoSMCs) were measured as reference cells. To show the effects of NPs on cell proliferation in a short- and long-term manner MTT assays were performed. For an *ex vivo* setup single rat prostate glands were isolated and the effects of NPs on the contractility of these glands were investigated by live-Imaging. CNP (and ANP) could significant-ly decrease contraction frequency and muscle tone. Thus, natriuretic peptides affecting muscle tone and proliferation might be promising potential drugs for BPH treatment.

T73

WHAT'S IN THERE? LOOKING AT IMMUNE CELLS IN THE ENTIRE MALE REPRODUCTIVE TRACT

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The male reproductive tract is comprised of the testes, epididymides, vasa deferentia, the accessory sex glands (seminal vesicles, prostate, ampullary, bulbourethral, and preputial glands), urethra, and penis. In men, infection and inflammation of the reproductive tract is a main etiology of fertility impairments. Infectious agents such as bacteria and viruses gain access through the urethral orifice and can ascend to the more distal parts of the genitourinary (GU) tract. Leukocytes play a major role in defense from ascending pathogens, but – as studies from other organs indicate - play also important functions in normal tissue homeostasis. Therefore, we aim to characterize the immune cell subsets and their functions longitudinally throughout the male reproductive tract during development as well

as under normal and inflammatory conditions in adult mice. In this regard, we used a combined approach of flow cytometry and immunofluorescence analyses. Data obtained thus far revealed that the penis contains the largest proportion of CD45+ immune cells (≈6%) in relation to total cell numbers, whereas the testis harbors the lowest proportion of immune cells (≈0.2%). Among leukocytes, B cells (CD19+) comprise the predominant population in the lower GU tract (penis ≈14%). In contrast, macrophages are the dominant leukocyte population in the upper GU tract (testis ≈55%). Moreover, organs are characterized by distinct macrophage subpopulations. As an example, $CX3CR^1CD^{206}MHC_{II}$ macrophages mainly reside in the interstitial space or the periphery of the organs, while $CX3Cr_1CD_{206}MCH^{II}$ macrophages present near the lumen. The differential phenotype and localization of macrophage subsets indicate a specific function of these cells in the respective organs that we wish to elucidate.

T74

THE ROLE OF MEMBRANE-TYPE 1 MATRIX METALLOPROTEINASE (MMP14) IN THE PATHOPHYSIOLOGY OF ENDOMETRIOSIS

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Endometriosis is a benign condition that occurs when tissue similar to the endometrium grows outside the uterine cavity. It is associated with chronic pelvic pain, dysmenorrhea and infertility. Recent population-based studies have indicated that it affects about 1–2% of women in the reproductive age. Matrix metalloproteinases (MMPs) are enzymes that are involved in the degradation of the extracellular matrix as well as the basement membrane. Recent studies have indicated the possible role of MMPs in the pathogenesis of endometriosis. Thus, the present study was aimed to determine the role of membrane type-1 matrix metalloproteinase (MMP14) in the pathophysiology of endometriosis. The pattern of localization of MMP14 in eutopic endometrium of patients with and without endometriosis, as well as in ectopic endometrium was determined by immunohistochemistry. ELISAs were employed to determine MMP14 levels in serum of healthy (n=52) and endometriotic (n=62) patients and in endocervical mucus of healthy (n=28) and endometriotic (n=38) patients. MMP14 was observed to be localized in the glandular epithelial cells of eutopic endometrium of healthy and endometriotic patients. Similarly, MMP14 was localized in the glandular epithelial cells of ectopic endometrium. From our preliminary results, MMP14 expression in endometriotic patients is higher during the proliferative phase compared to the secretory phase. Currently, we are analysing more samples for comparison of MMP14 expression in eutopic and ectopic endometrium. No differences were observed in MMP14 levels in serum and mucus in the proliferative and secretory phases of both healthy and endometriotic patients. MMP14 serum and endocervical mucus levels were significantly higher in endometriotic patients compared to healthy controls. Localization of MMP14 in the endometrium suggests a possible role in uterine functions that perhaps may be related to endometriosis. Additionally, increased levels of MMP14 levels in serum and mucus samples of endometriotic patients indicate that MMP14 may play a role in endometriosis and further experiments are necessary to establish its precise role.

T75

TGF-B1/B2 MEDIATE REDUCTION IN BETAGLYCAN SHEDDING VIA ALK-5 AND SMAD3 PATHWAY IN HUMAN ENDOMETRIAL CELLS

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Betaglycan (BG) is a membrane-bound co-receptor and modulator of some transforming growth factor beta (TGF-β) superfamily ligands. BG undergoes proteolytic cleavage, termed shedding, to release soluble betaglycan (sBG) which often antagonizes TGF-β signaling in several physiological and pathological processes. Endometriosis is a gynecological condition characterized by the presence of endometrial-like tissue outside the uterus. The present study aimed to investigate shedding of BG and signaling pathways involved in human endometrial cells. Endometriotic epithelial 12Z and endometrial stromal THESC cells were treated with increasing concentrations of TGF-β1/β2 (1–15 ng/ml) and sBG levels evaluated using ELISAs. An ALK-4/5 inhibitor (LY364947, 10 μM) as well as double and single SMAD2 and SMAD3

gene knockdowns using siRNAs (100 nM and 150 nM, respectively) were used to investigate the signaling pathways. Inhibition of BG shedding was analyzed using tissue inhibitors of metalloproteinases3 (TIMP3, 2.5–10 nM) besides a pan-matrix metalloproteinases (MMP) inhibitor (GM6001, 10 μM). TGF-β1 and -β2 stimulation of 12Z and THESC cells resulted in a significant dose-dependent reduction in BG shedding. The TGF-β1/2-mediated reduction in BG shedding in 12Z cells was found to be TGF-β type I receptor (ALK-5)-dependent. Additionally, the SMAD2/3 double gene silencing significantly ameliorated the reduction in BG shedding. Further analysis using single gene knockdowns revealed that TGF-β1/2-mediated reduction in BG shedding was SMAD3- but not SMAD2-dependent. Notably, in both 12Z and THESC cells, shedding of BG was significantly attenuated by TIMP3 in a dose-dependent manner and partially (∼40%) by the pan-MMP inhibitor, signifying regulation of BG shedding by MMPs. Together, our data propose involvement of MMPs in shedding of BG and demonstrate that the canonical TGF-β pathway involving TGF-β/ALK-5/SMAD3 signaling is required in TGF-β-mediated reduction in BG shedding in endometriotic cells. The exact role of the TGF-β-mediated reduction in BG shedding in endometriosis merits further investigation.

T76

LOSS OF CCR2 INHIBITS TESTICULAR FIBROSIS CAUSED BY EXPERIMENTAL AUTOIMMUNE ORCHITIS: POSSIBLE ROLE FOR ACTIVIN A IN FIBROSIS DEVELOPMENT

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Experimental autoimmune orchitis (EAO) is a mouse model of chronic testicular inflammation where fibrosis is a characteristic hallmark. Levels of pro-inflammatory and pro-fibrotic mediators such as TNFα, CCL2 (ligand for CCR2) and activin A are increased during EAO, an observation followed by the infiltration of leukocytes into the testicular interstitium. Sertoli cells are a source of activin A and increased levels are produced after challenge with inflammatory factors such as TNFα *in vitro*. Recruited fibrocytes expressing hematopoietic markers, extracellular matrix (ECM) proteins and CXCR4 contribute to the fibrogenesis. Increased expression of matrix metalloproteases (MMPs) also indicates the development of fibrosis. In this study, the interplay of CCR2 and activin A on the development of testicular fibrosis was investigated. EAO was induced by active immunization with testicular antigens in C57BL/6J (WT) and CCR2-/- mice (n=5–8/group). Flow cytometry, immunofluorescence and qRT-PCR indicated that the increased number of ECM expressing immune cells and the increased expression of MMPs caused by EAO were reduced in CCR2-/- testes compared with WT testes. Bone marrow-derived macrophages (BMDMs) were used as a surrogate of testicular macrophages to test the influence of activin A on the production of fibronectin, CXCR4 and MMPs. BMDMs cultured in the presence of 50 ng/ml activin A or in conditioned medium from cultured Sertoli cells (SCCM) stimulated with 50 ng/ml TNFα were analysed by qRT-PCR. Results showed that SCCM from TNFα-stimulated Sertoli cells induced the expression of fibronectin mRNA in BMDMs (n=5). Moreover, activin A increased the mRNA expression of fibronectin, CXCR4, MMP2 and MMP14 in BMDMs with a concomitant decrease of MMP-9 and TIMP1 (n=8). Activin A also elevated the percentage of fibronectin+ and CXCR4+ BMDMs (n=5) by flow cytometry. Follistatin, a potent activin A antagonist, inhibited these effects. Taken together, these data indicate that CCR2 and activin A are required for the development of fibrosis during testicular inflammation.

T77

A NOVEL APPROACH TO VISUALIZE SUPERFICIAL WALL MOVEMENTS IN SEMINIFEROUS TUBULES

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In the testis, smooth muscle cells (SMCs), also referred to as peritubular cells (PTCs), surround the germinal epithelium of the seminiferous tubules (STs). STs host the proliferation and maturation of male germ cells to become spermatozoa. Once shed from the epithelium, spermatozoa are still immotile hence require an active driving force propelling them to-

wards the rete testis. In this regard, the contractility of PTCs plays a pivotal role for male reproduction. In rodents the spermatogenic stages are arranged section-wise and can be distinguished by histology and transillumination.
In this study a custom-built Fiji-based code has been established to characterize spontaneous superficial wall movements of two spermatogenic stages (dark: before and pale: after spermiatio) in isolated rat STs. Besides an impressive optical break down, that enables quick differentiation between stronger and weaker moving tubular regions over time, this code transfers time-lapse movies into a dataset applicable for further statistical analysis pixel by pixel. The latter confirmed a significant difference in the spontaneous contraction patterns of dark and pale tubules.
When treating both tubule stages with a donor of nitric oxide (NO), known to relax SMCs, the code detected a significant reduction of the spontaneous contractions, independent of the respective spermatogenic stage.
In STs of human patients, spontaneous contractions and NO-induced effects could also be visualized by using the same *ex vivo* approach.
In agreement, isolated human PTCs revealed an increase of relaxation-mediating cGMP by NO and showed a reduced calcium increase by noradrenaline when inhibiting cGMP hydrolysis with sildenafil.
Different contraction patterns of the observed ST stages (before and after spermiatio) might reflect different local functions. The luminal fluid in different sections leads to a varying internal pressure on the ST wall. Since the NO-synthesis is known to be stretch-induced, modulating local NO-effects on the germinal epithelium seem likely.

T78

TEN-ELEVEN-TRANSLOCATION ENZYMES (TETS), DNA METHYLTRANSFERASES (DNMTS) AND POLYCOMB REPRESSIVE COMPLEX 2 (PRC2): LINKS TO MALE INFERTILITY

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The epigenetic modifiers TET1–3, DNMT1/3A/3B, and PRC2 regulate DNA and histone methylation, respectively. In mice, Tet1 and PRC2 (H3K27me3) dysregulation were associated with male infertility. As infertile men often exhibit epigenetic aberrations in sperm cells, we hypothesize that dysregulation of TETs, DNMTs, PRC2 may contribute to idiopathic male infertility.

Using testis sections from men and Tet1-wildtype and -*KO* mice, we analyzed TET1/Tet1, core PRC2 components (EED/Eed, EZH2/Ezh2, SUZ12/Suz12), and H3K27me3 by immunohistochemistry (IHC). Using motile spermatozoa from fertile donors and subfertile men, we compared mRNA levels and promoter methylation of TET1/2/3, DNMT1/3A/3B. Western blot (WB) analyses of PRC2 components and H3K27me3 were done using human spermatozoa.

In humans, TET1, H3K27me3, and EZH2 co-expressed in the spermatogonia B, leptotene spermatocytes, and round spermatids. In wild-type mice, Tet1 was expressed in stage X-XI elongating spermatids, whereas, in homozygous Tet1-*KO* mice, Tet1 expression was absent in germ cells. In Tet1-KO, H3K27me3 expression was shifted from stage V-VI spermatogonia B to stage VII-VIII pre-leptotene spermatocytes, and from stage IX-XI elongating spermatid to stage X-XI elongating spermatids. Ezh2 had gained expression in stage VII-VIII pre-leptotene spermatocytes and stage VI-X pachytene spermatocytes, whereas Suz12 had shown a new expression pattern in late elongating and elongated spermatids. Eed expression was completely lost. PRC2 components were not preserved in sperm except H3K27me3. TET1 mRNA levels were significantly upregulated in the sperm of subfertile men.

Thus, knocking out of Tet1 has potential impacts on Suz12 and Eed expression. However, RT-qPCR results in human sperm pointed to an aberrant upregulation of TET1. Subsequent analyses of mRNA and promoter methylation of TET2/3 and DNMT1/3A/3B, H3K27me3-ChIP-qPCR and methylation analyses of bivalent developmental promoters will be performed to determine, if there is a link between dysregulation of TETs, DNMTs, PRC2, and male infertility.

T79

DEMOGRAPHIC PROCESSES AND RECENT SELECTION SIGNATURES IN BENINESE INDIGENOUS CATTLE BREEDS

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The Dwarf (forest) Lagune and the Savana Somba cattle in Benin are typical representatives of the endangered West-African indigenous shorthorn taurine. The Lagune were previously imported to different African and Europeans countries and bred as Dahomey cattle; whereas the Somba contributed to the formation of two indigenous crossbreeds known as Borgou and Pabli cattle. These breeds are now affected by demographic, economic, environemental pressures in production systems. Considering current and historical genomic data, we applied formal test of admixture, estimation of admixture proportion and dates, and of genomic inbreeding coefficients to characterise the demographic processes in the five breeds. Subsequently, we unravelled the most recent selection signatures using the cross-population extended haplotype homozygosity (XP-EHH) approach. Our results confirm the origin of the European Dahomey cattle as derived from Lagune ancestors. These animals displayed no indicine or European taurine background but they shared on average, 40% of their genome with common ancestors, dated approximatively eight generations ago. We detected evidence of admixture in the Somba and Lagune cattle but they exhibited more than 92% of African Taurine ancestral (AFT) proportion. Moderate AFT ancestral proportion (42%) were also infered in the Pabli in contrast to the current Borgou populations ($\leq$ 34%). Irrespective to the admixture proportions, each of the investigated populations presented several known selection signatures related to adaptive traits (immune response, feed efficiency, heat stress) but also economic traits (reproduction, growth, milk). The subregion of BoLA class IIb (spanning DSB, BOLA-DYA, etc) under selection in Somba catle is interestingly uncommon in other African breeds, suggesting further investigations to understand its association with specific adaptation to endemic diseases in Benin. Overall, our study provides insights to recent evolutionary processes in the Beninese indigenous cattle and their ability for conservation and genetic improvement.

T80

EPIGENETIC DYSREGULATION OF TUMOR SUPPRESSOR GENES IN CP/CPPS: STUDIES ON LIQUID BIOPSIES FOR BIOMARKER DEVELOPMENT

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About 90% of patients with prostatitis syndrome are classified with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). CP/CPPS may potentially lead to prostate cancer (PCa) in elder age, but the molecular background of this disease remains unclear. Tumor suppressor genes (TSGs) prevent malignant transformation of cells and their epigenetic dysregulation was often observed in cancer. We hypothesize that epigenetic aberrations in TSGs may also happen in CP/CPPS and contribute to development of PCa.

Using liquid biopsies, i.e. somatic cells from exprimate urine (urine after prostate massage) and semen samples, we aimed to analyze the promoter methylation and mRNA-expression of TSGs in CP/CPPS patients in comparison to healthy donors. Our study included seven PCa-associated TSGs (BMP4, EDNRB, BMP7, PTGS2, PITX2, CDKN2A and GSTP1). Moreover, somatic cells from exprimate urine and semen were separated in leukocytes and epithelial cells using CD45- and EpCAM-antibody-coupled magnetic beads, and each cell type was confirmed by immunofluorescence staining.

Promoters of CDKN2A, BMP4, BMP7, EDNRB, and PTGS2 were significantly higher methylated in semen of CP/CPPS patients in comparison to controls. Promoters of CDKN2A and EDNRB were also significantly higher methylated in urine samples of CP/CPPS patients in comparison to controls. Accordingly, expression of CDKN2A and PTGS2 was significantly downregulated in CP/CPPS patients' semen and urine. Epithelial cells isolated from urine showed a lower methylation of CDKN2A-promoter in comparison to urine-leukocytes. However, leucocytes isolated from semen showed a lower CDKN2A-promoter methylation than epithelial cells.

Overall, our results point to an epigenetic dysregulation of TSGs in CP/CPPS. Liquid biopsies, particularly exprimate urine and semen, seem to be a promising source for PCa-biomarker development and should be pursued further.

Section 7 - Bioresources, Bioinformatics and Biotechnology

Image: colourbox.com

Day 1: Wednesday, September 29th, 2021		
Section 7 - Bioresources, Bioinformatics and Biotechnology		Chairpersons: Anjani Nayak & Nadine Sella
09:15-09:45	**Prof. Gisbert Schneider** (ETH Zürich, Schweiz / ETH Center Singapore) ***De novo Drug Design with Machine intelligence***	
11:30-11:40	**Fabian Jannik Tann**: *Feature selection for binary classification with focus on antiviral peptides*	
11:40-11:50	**Wendell Albuquerque**: *Cleavage of wine haze proteins by peptidases from Drosophila suzukii*	
11:50-12:00	**Beatrice Tobisch**: *Mycorrhiza and hydropriming use in early soybean development under German field conditions*	
12:00-12:10	**Nadine Sella**: *Insights in methyl anthranilate biosynthesis of Wolfiporia cocos*	
12:10-12:20	**Anjani Nayak**: *Optimizing industrial production of Hermetia illucens larvae*	

K7

DE NOVO DRUG DESIGN WITH MACHINE INTELLIGENCE

Gisbert Schneider[1]

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Drug design may be regarded as a pattern recognition process. Medicinal chemists are skilled in visual chemical structure recognition and their association with (retro)synthesis routes and pharmacological properties. In this context, various "artificial intelligence" (AI) methods have emerged as potentially enabling technology for drug discovery and automation, because these systems aim to mimic the chemist's pattern recognition process and take it to the next level by considering the available domain–specific data and associations during model development. Part of the appeal of applying AI methods in drug design lies in the potential to develop data-driven, implicit model building processes to navigate vast datasets and to prioritize alternatives. This represents at least a partial transfer of decision power to a machine intelligence, and could be viewed as synergistic with human intelligence; that is, a domain-specific implicit AI that would augment the capabilities of medicinal chemists in drug design and selection. More ambitiously, the ultimate challenge for drug design with AI is to autonomously generate new chemical entities with the desired properties from scratch (*de novo*), without the need for the often prohibitively costly experimental compound screening.
We will review the principles of AI methods for *de novo* drug design, emphasizing ligand-based approaches that have proven useful and reliable in "little-data" scenarios. Selected prospective case studies will be presented, ranging from targeted molecular design to fully automated design-make-test-analyse cycles. We provide a critical assessment of the possibilities and limitations of the individual approaches and dare forecasting the future of drug design with machine intelligence.

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T81

CLEAVAGE OF WINE HAZE PROTEINS BY PEPTIDASES FROM *DROSOPHILA SUZUKII*

Albuquerque, W[1], Gand, M[1], Zorn, H[1]

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Heat-unstable wine proteins, in particular thaumatin-like proteins (TLPs) and chitinases (CHIs), interact with wine matrix components such as polyphenols and sulfite to promote protein self-aggregation and haze. Bentonite clay is still the state-of-the-art method for the clarification of white wines, but the application of proteolytic enzymes (peptidases) is considered an alternative fining method for preventing wine protein aggregation by cleavage. Insects are interesting sources of peptidases, because they live in a broad variety of ecological niches and feed on diverse nutrient sources. In this present study, recombinant TLPs and CHIs were cleaved by peptidases from *Drosophila suzukii* and the peptides generated at different pH values were analyzed by top-down LC-MS proteomics. Cleavage spots were identified for both haze proteins after hydrolysis by peptidases in a pH range of 3 to 7. Peptidases from *D. suzukii* may be considered as a fining agent in the wine industry.

T82

OPTIMIZING INDUSTRIAL PRODUCTION OF *HERMETIA ILLUCENS* LARVAE

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World population growth and increased incomes have resulted in a higher demand for protein, usually provided by milk and meat, that is greater than its production rate. To meet this growing demand, alternative protein sources and sustainable production systems for animal proteins have been identified. As a consequence of this effort the number of insect-protein producing industries for food and feed is rising. However, these industries face many challenges that are the background of recent scientific projects.
The black soldier fly, *Hermetia illucens* (BSF; Diptera: Stratiomyidae), represents one of the potential insects that can assist as feed in a sustainable production of e.g. fish or shrimps in aquaculture. It is valued for its high quality protein, fat, and its ability to thrive on a variety of bio-wastes. However, it is still

necessary to optimize insect production, using a step-by-step approach. To this end, the first experiments in this study are planned with feed trials. Plant-based byproducts would be used to study BSF larvae performance: survival percentage, growth rate, total yield, waste reduction index, amino acid and fatty acid concentration. Individual experiments are planned to optimize feed particle size, substrate composition, density of larvae per box, feeding depth, and feeding rate. Chicken feed would act as the positive control diet for all the experiments, and further, larvae reared on different substrates would be analyzed for anti-microbial peptides. In addition, insect frass would be investigated for its chemical and physical properties to be potentially used as bio-fertilizer. At the end of the study, as a validation step, an industrial level feed trial would be conducted to check laboratory optimized diets. Thus, the project focuses on increasing the quality and quantity of larval yield, finding other uses for insect products and byproducts, while taking into account the concept of circular economy.

T83

INSIGHTS IN METHYL ANTHRANILATE BIOSYNTHESIS OF *WOLFIPORIA COCOS*

Sella, N[1], Sommer, S[1], Büttner, J[1], Rühl, M[1,2]

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Studies on the basidiomycete *Wolfiporia cocos* often focuses on anti-inflammatory or antitumor effects of compounds produced by the sclerotium of this fungus as it is traditionally used in Chinese medicine. In addition, some studies deal with the sclerotial growth of this fungus. However, *W. cocos* synthesizes a variety of aroma active compounds that are interesting for food or cosmetics industry, too. Therefore, the volatile compounds produced by mycelium of *W. cocos* during fermentation of black current pomace with or without supplementation of aspartate have been analysed by means of gas chromatography. Afterwards, a transcriptomic study has been set up with RNA extractions depending on the occurrence of the main aroma compounds to determine the respective biosynthetic pathways. Methyl anthranilate is one of those compounds, whose biosynthesis in fungi is not yet known and, thus is investigated in the present study. For this purpose, a whole genome sequencing has been conducted by a commercial sequencing provider as the already published genome lacks the required quality. The automatic annotation of the sequencing provider was improved further by manual curation to obtain a robust annotation. Genes coding for the enzymes of the shikimate pathway, which produce the necessary precursors for methyl anthranilate biosynthesis, have been identified in the genome. Then, the sequencing data of the transcriptome has been mapped to the new genome and expression levels of all relevant genes have been determined. As a result, the biosynthesis of methyl anthranilate and its precursors have been proposed based on *in silico* experiments. Further studies will address the validation of these findings *in vitro*.

T84

FEATURE SELECTION FOR BINARY CLASSIFICATION WITH FOCUS ON ANTIVIRAL PEPTIDES

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Predictions with machine learning methods have established new insights over the last years in many fields. They are promising in reducing costs in wet-lab work by *in silico* pre-selection and can reveal new information.

Due to viral threats and little or no options in their treatment, classifiers for predicting antiviral peptides are trained to find new drug possibilities. Antiviral peptides occur naturally in many organisms. They can have additional effects like antibacterial properties, reducing the chance of secondary infections of the weakened host.

Choosing the right features for classification is crucial for the prediction outcome. Hence a great variety of properties are calculated, normalised, grouped and finally drafted through a weighted vote of different feature selection strategies. Automation shall boost performance of classifiers, resulting in higher productivity of researchers.

Section 8 - Chemical Design and Analysis of Molecular Systems

Image: colourbox.com

Day 2: Thursday, September 30th, 2021

Section 8 - Chemical Design and Analysis of Molecular Systems Chairpersons: Julian Schneemann & Usman Ali & Felix Graf

17:00-17:30	**Prof. Stephan Becker** (Institute for Virology, Philipps University Marburg, Germany) ***Emerging viruses: Challenges for the Global Village***
14:30-14:40	**Darya Dudko**: *Biosynthesis of vitamin B12 with the known producing strains and novel candidates*
14:40-14:50	**Felix Graf**: *Identification of Novel Yeasts for Potential Wine Aroma Improvement*
14:50-15:00	**Parab-Jainal Haque**: *High versatility of IPP and DMAPP methyltransferases enables synthesis of C6, C7 and C8 terpenoid building blocks*
15:00-15:10	**Julia Büttner**: *Transformation of the model fungus Coprinopsis cinerea for linalool biosynthesis*
15:10-15:20	**Usman Ali**: *Hydrothermal Studies of Silica Monoliths and their Impacts on Heterogeneous Organo-catalysis*
15:20-15:30	**Katrin Wiltschka**: *Polychlorinated biphenyl (PCB) loads in mine water - congener specific analysis by SPME-GC-MS*
15:45-15:55	**Nils Holger Anschütz**: *AP-SMALDI-MSI of Cryptosporidium parvum-infected cells*
15:55-16:05	**Domenic Dreisbach**: *Spatially-resolved metabolic networks in Danaus plexippus — Combining molecular networking, on-tissue derivatization and high lateral resolution MSI*
16:05-16:15	**David Lüke**: *MALDI mass spectrometry imaging of glycoconjugates*
16:15-16:25	**Julian Schneemann**: *Low-temperature plasma (LTP) post-ionization of non-polar analytes*
16:25-16:35	**Azar Rezaei**: *Chemical topography of metal-associated allergens on non-planar everyday items*
16:35-16:45	**Michael Waletzko**: *Qualitative and quantitative analysis of cholesterol and lipids under ambient conditions using nanospray desorption electrospray ionization*
16:45-16:55	**Katja R Wiedemann**: *Imaging of Schistosoma mansoni eggs in host tissue*

T85

HYDROTHERMAL STUDIES OF SILICA MONOLITHS AND THEIR IMPACTS ON HETEROGENEOUS ORGANO-CATALYSIS

Ali, U[1], Rafael, M[1], Kevin, T[1], Mathias, T[2], Bernd, MS[1]

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In this particular research we are going to study the effects of different hydrothermal temperatures on mesoporosity of silica monoliths and their ultimate impacts on heterogeneous Organo-catalysis. Silica Monoliths were prepared by famous Nakanishi method, which is then treated at different hydrothermal temperatures. The hydrothermal step controls the mesoporosity and surface area of silica monoliths, which play an important role in immobilization of organic catalyst on silica surface. Physiosorption studies carried out to investigate porosity and BET surface area after treatment at different hydrothermal temperatures. Ar 87K and N2 77K used as adsorbates while Autosorb and Qudrasorb techniques are used as physiosorption studies tools. After interpreting data form physiosorption,the Organic catalyst will be immobilized on different silica monoliths and their performance will be analyzed in terms of yield of product per time.

T86

AP-SMALDI-MSI OF *CRYPTOSPORIDIUM PARVUM*-INFECTED CELLS

Anschütz, NH[1], Gerbig, S[1], Muñoz, JDV[2], Silva, LMR[2], Hermosilla, C[2], Taubert, A[2], Spengler, B[1]

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[2] *Institute of Parasitology, Justus Liebig University, Giessen, Germany*

Parasites and resulting diseases pose health and economic threats worldwide. To overcome these problems, the parasites need to be studied extensively. In this study, a distinct obligate intracellular apicomplexan parasite, *Cryptosporidium parvum*, was studied, using mass spectrometry (MS) and MS imaging (MSI), combined with high-performance liquid chromatography (HPLC) or matrix-assisted laser desorption/ionisation (MALDI). The aim was to identify molecular biomarkers for parasitic infections of host cells and to clarify their function. With MALDI MS(I), infected and non-infected cell pellet samples were examined for possible markers. For this purpose, a Q ExactiveTM HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in combination with an AP-SMALDI5 AF imaging ion source (TransMIT GmbH, Giessen, Germany) was used (Mass resolution R = 240,000 @ m/z 200; pixel size: $\geq$ 5 µm). The two software packages Mirion (TransMIT) and Perseus (MPI of Biochemistry, Martinsried, Germany) were used to find potential biomarkers, and HPLC-MS/MS (Dionex UltiMate 3000 RSLC-System, Thermo Fisher Scientific, Dreieich, Germany) experiments were performed for their structural identification. The software tool LipidMatch (SECIM, Gainesville, USA) was used for assignment. Highly immunoreactive primary bovine small intestine cells (BSIC) were used to authentically simulate *in vivo* conditions. Immortalized cell lines (HCT 8) were used for comparison as a simplified infection model. MSI of cell monolayers allowed for depicting marker compounds in parasite-infected single host cells in comparison to non-infected controls. *C. parvum*-infected bovine intestinal biopsy samples were additionally examined by MALDI MSI, thereby mimicking the *in vivo* situation. The current state of marker detection for *C. parvum* will be presented here.

T87

TRANSFORMATION OF THE MODEL FUNGUS COPRINOPSIS CINEREA FOR LINALOOL BIOSYNTHESIS

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Fermentation of black currant pomace with the edible basidiomycete *Wolfiporia cocos* results in the release of a wild-strawberry resembling aroma. An important component of this aroma is linalool, a monoterpene which is part of many flowery flavours and is found in the essential oils of plants like lavender, bergamot, thyme, and jasmine. [1] Linalool occurs in form of two stereoisomers. While (R)-linalool smells lavender-like and woody, (S)-linalool is perceived as citrus, floral. The plants' essential oils are often extracted to yield linalool which can be used in cosmetics and perfumes. Although it is already known that fungi can produce linalool de novo, as to date only few fungal linalool synthases have been described. [2] Therefore, the aim was to identify and verify the enzymes responsible for linalool production in *W. cocos*.

By means of transcriptome analysis, two putative

linalool synthases were identified in the genome of *W. cocos*. To verify that these enzymes are responsible for linalool formation in *W. cocos*, they were inserted into the genome of the model basidiomycete Coprinopsis cinerea. C. cinerea, with its known genome, is well studied regarding e.g. mating types and fungal development. Transformation of this fungus is also an established tool for the elucidation of biosynthetic pathways [3], therefore it can be used for the verification of results from transcriptome analysis.

References:

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[2] Zhang *et al.*, Commun Biol 2021, 4:223

[3] Kues, MMBR 2000, 64:316–353

T88

SPATIALLY-RESOLVED METABOLIC NETWORKS IN *DANAUS PLEXIPPUS* — COMBINING MOLECULAR NETWORKING, ON-TISSUE DERIVATIZATION AND HIGH LATERAL RESOLUTION MSI

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Metabolic networks are interconnected pathways, describing spatially-organized biochemical reactions and transport processes of chemical compounds within living organisms. However, decomposing complex metabolite networks into chemical compound classes and molecular annotations remains a major bottleneck due to the absence of repository-scaled databases. Additionally, using "soft-ionization" mass spectrometry imaging (MSI) methods, a broad range of metabolite classes (e.g. steroids) show low intrinsic ionization efficiencies, thus restricting the molecular characterization of metabolic networks in their spatial context of tissues and cells. Here, we describe a multi-modal mass spectrometry-based method combining computational metabolome mining tools and high-resolution MSI for the spatially-resolved analysis of metabolic networks at the low-micrometer scale. Applied to plant-toxin sequestration in *Danaus plexippus* as a model system, we first utilized liquid chromatography (LC)-MS-based molecular networking in combination with *in silico* chemical characterization to facilitate structural elucidation and molecular identification of 32 different steroidal glycosides for the host-plant Asclepias curassavica. These comprehensive metabolite annotations guided subsequent matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) analysis of cardiac glycoside sequestration in *D. plexippus*. We developed a spatial context preserving on-tissue chemical derivatization protocol (OTCD), which substantially improved cardiac glycoside ion yields (by at least one order of magnitude) by selective covalent charge-tagging using Girard's reagent T (GirT). To illustrate the potential of this methodology relative to conventional MALDI MSI, we visualized previously inaccessible (sub)-cellular distributions (2 µm and 5 µm pixel size) of steroidal glycosides in *D. plexippus* – thereby providing novel insight into sequestration of toxic metabolites.

T89

BIOSYNTHESIS OF VITAMIN B12 WITH THE KNOWN PRODUCING STRAINS AND NOVEL CANDIDATES

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[2] *Institute of food chemistry and food biotechnology, Justus Liebig University Giessen*

Vegetarian and vegan diets have developed into modern nutritional trends in recent years and only in Germany the number of vegetarians has increased more than tenfold over the last 20 years1. Nevertheless, vegans are at a particular risk of vitamin B12 deficiency since this vital nutrient is not present in the foods of plant origin2. A lack of vitamin B12 can cause severe health problems, which is why there is an urgent need for vegan vitamin B12 sources. Since many bacteria are able to synthesize vitamin B12, microbial biosynthesis is nowadays applied for the commercial production of vitamin B123. Thus, we aim to search for known and novel vitamin B12 -producing strains and to analyze them for vitamin B12 production with different analytical techniques. For this purpose, microbiological assay, vitamin B12 riboswitch-based biosensor and LC-MS/MS were applied for the vitamin B12 analysis in this work. Since the microbiological assay and the riboswitch biosensor turned out to be sensitive to inactive B12-analogues and to have very high detection limit, respectively, we have chosen the LC-MS/MS analysis for the further investigation. Using the LC-MS/MS-based method developed in this study we were able to identify vitamin B12 in the cell extracts of three producing strains and also in the cell extracts of five newly discovered candidates for vitamin B12 production. Moreover, quantification results revealed that the amounts of vitamin B12 produced by the candidate strains I, II, IV and V were

even higher than those in the cell extracts of the previously described producer E.meliloti.
The developed LC-MS/MS-based method proved to be reliable for fast and sensitive determination and quantification of vitamin B12. Identification of vitamin B12 in the cell extracts of the candidate strains opens a new perspective in the search for more vegan vitamin B12 sources.

T90

IDENTIFICATION OF NOVEL YEASTS FOR POTENTIAL WINE AROMA IMPROVEMENT

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High amounts of aroma compounds in fruits occur in form of glycosides. These glycosides are not directly accessible for the human aroma perception due to the high polarity (and thus low volatility) of the sugar residues. However, they can contribute to beverage aroma improvement after hydrolysis of the precursors during the processing or fermentation of fruit juices. This liberation is either a result of acidic hydrolysis or an enzymatic process catalyzed by glycosidases. While acidic hydrolysis can be accompanied by rearrangements of the molecules, enzymatic hydrolysis preserves the native structure of the compounds.

Enzymes used commercially for wine aroma improvement are often limited in their enzymatic capacity due to the harsh fermentation parameters. Moreover, they can also mediate hydrolysis of unstable pigments and the release of unpleasant flavours. Therefore, yeast strains with selected hydrolytic activities for specific aroma glycosides can be a great opportunity to improve fruit juice and wine aroma complexity.

2100 non-Saccharomyces yeasts isolated from grapes and black currants at the Hochschule Geisenheim University were cultivated and screened for their β-glycosidic activities using the model substrate 4-methylumbelliferyl-β-D-glucopyranoside. β-glycosidase specificity was measured by gas chromatography/solid phase micro extraction (GC-SPME) in samples of glycoside extracts from grape must incubated with whole cells of promising strains.

Several isolates with high β-glycosidase activities were identified in the collection of autochthonous non-Saccharomyces yeasts. Measurements under various conditions revealed differences in the β-glycosidase pH-optimum, while subsequent GC-SPME analyses indicated a high selectivity of some of the identified glycosidase activities for specific aroma compounds.

The isolates identified in this screening are potential candidates for the optimization of fruit processing leading to the production of beverages with an improved aroma composition. Additionally, further investigations can help to understand the mechanisms of aroma liberation specificities of the selected yeasts.

T91

HIGH VERSATILITY OF IPP AND DMAPP METHYLTRANSFERASES ENABLES SYNTHESIS OF C6, C7 AND C8 TERPENOID BUILDING BLOCKS

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The natural substance class of terpenoids covers an extremely wide range of different structures, although their building block repertoire is strongly limited. Nearly all terpenes discovered so far are made up of the two terpene precursors DMAPP and IPP, both having five carbon atoms. Recently, S-adenosylmethionin-dependent IPP MTases were described, which can transfer one or two methyl groups to this C5 prenyl pyrophosphate. This study aims at the characterization of novel MTases that modify terpene precursors and the demonstration of their suitability for biotechnological purposes. After selecting putative candidate genes from different bacterial operon contexts, the substrate and product spectra of four so far undescribed and three known MTases were analyzed and compared. All seven enzymes accepted IPP as substrate and altogether five C6 compounds and six C7 compounds were formed within the reactions. A high deprotonation site selectivity as well as high stereoselectivity could be observed for most of the biocatalysts. Only the enzyme from Micromonospora humi also accepted DMAPP as substrate, converting it into 2-methyl-IPP *in vitro*. To test the applicability of the MTases *in vivo*, respective *E. coli* strains were investigated with regard to release of corresponding terpene alcohols. Within these experiments the strain expressing the M. humi MTase turned out to produce different C8 terpenoids, which were further investigated with isotopic labelling studies. They revealed the occurrence of a hydride shift

step within the MTase-catalyzed reaction and enabled structure clarification for this compound. Our study demonstrates the occurrence of C5 prenyl pyrophosphate MTases with very different catalytic properties in bacteria, which provide biosynthetic access to many novel terpene-derived structures.

T92

MALDI MASS SPECTROMETRY IMAGING OF GLYCOCONJUGATES

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MALDI mass spectrometry imaging (MSI) is a powerful tool to obtain spatial distribution information for biomolecules in complex samples. The distributions of lipids, especially phospholipids, are easily accessible with this method. Biomolecules that require more elaborate workflows for visualization with MALDI MSI are glycoconjugates. Glycoconjugates are formed by glycosylation of biomolecules resulting in, for example, glycoproteins or glycolipids. These molecular species are known to play crucial roles in cell-cell interactions. In schistosomiasis, for example, it appears that interactions between the parasite and the host are mediated by glycoconjugates, causing the survival of the parasite. To study metabolomic pathways between parasites and hosts, the detection of biomolecules at the contact surface is of special interest. Therefore, we aim to establish MALDI MSI workflows for glycolipids and N-glycans to track glycoconjugates at the highest possible lateral resolution. As an interesting application, livers of infected hamsters were analyzed. Upon infection with schistosomes, hundreds of eggs are produced daily. Many eggs reach the environment via the feces, however, the remaining eggs get trapped in organs such as spleen or liver. Here, these eggs release enzymes into the host tissue. As a reaction of the tissue, granuloma surrounding those eggs are formed. Whereas lipid analysis of this sample type was already performed, no data is available for larger glycolipids and/or N-glycans. Presenting the workflow and first examples of MALDI MSI measurements, differences between infected hamster liver with granuloma and control samples are shown with respect to glycoconjugates. The preliminary results demonstrate the usability of our setup for glycoconjugate analysis. The combination of high lateral resolution and high mass resolution as well as high mass accuracy allows for reliable annotation of possible glycoconjugate species. Future validation of the results by nano liquid chromatography tandem mass spectrometry will hopefully help to investigate metabolomic pathways/interactions during schistosome infection.

T93

CHEMICAL TOPOGRAPHY OF METAL-ASSOCIATED ALLERGENS ON NON-PLANAR EVERYDAY ITEMS

Rezaei, A[1], Bhandari, D Heiles, S[1], Schindler, S[1], Spengler, B[1]

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It is well-known that some metals/alloys are sensitizing the skin of susceptible individuals. Trace amounts of metals and chemical compounds containing metal ions can affect human health, i.e., cause allergy or inflammation. However, details of this metal-induced allergic reaction are not well understood. Complexes of nickel, copper and zinc are known to be easily formed through contact of metal alloys with ubiquitously available organic compounds such as triglycerides, amino acids and fatty acids found in skin secretions. Thus, an analytical technique is required to characterize and identify metal complexes formed on metallic everyday products, ideally offering a chemically specific, laterally resolved view of allergy-causing processes. We here present an analytical workflow to characterize metal-organic compounds formed on the surface of jewelry or coins. As a first step, the composition of a 1-Euro coin was investigated. Subsequently, different kinds of lipids present in fingerprints were evaluated at different time points with an AP-SMALDI5 AF ion source coupled to a Q Exactive HF instrument, using 3D-surface autofocusing LDI mass spectrometry imaging.
Moreover, regarding observing the possible structures of metal-lipid complexes formed in skin allergy, formed complexes with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) as standard and $NiCl_2$ were investigated by electrospray ionization (ESI-FT-ICR). In our study, we could not observe significant residue or complex ion in synthetic sweat solution in presence of 1 Euro coin and, nickel foil impressed by fingerprint.
Future measurements will investigate how fatty acids change on the surface of coins.
Acknowledgement
Financial support by DAAD-GSSP "Chemistry for Life" and the Giessen Graduate Center for the Life Sciences GGL is gratefully acknowledged.

T94

LOW-TEMPERATURE PLASMA (LTP) POST-IONIZATION OF NON-POLAR ANALYTES

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Non-polar compounds including metabolites and lipids are challenging to detect using ionization techniques like matrix-assisted laser desorption/ionization (MALDI) because they suffer from low ionization efficiencies. Efficient ionization techniques for non-polar analytes are plasma-based ionization methods, such as low-temperature plasma (LTP), which, however, cannot efficiently desorb compounds from biological tissue. To overcome these obstacles, we combined infrared (IR)-MALDI with a plasma-based post-ionization technique, aiming to investigate the abundance and distribution of non-polar analytes from complex biological samples.

To combine these two ion source technologies, a plasma generator was added to an atmospheric-pressure IR-MALDI source. The transfer capillary that directs the ions and neutral particles from the sample surface to the vacuum of the mass spectrometer was upgraded with a T-piece. The active species from the plasma stream are introduced trough the T-piece and interact with the analyte molecules. Operation parameters like the helium flow rate and the voltage of the LTP were tuned to optimize signal intensities of authentic standards.

IR-MALDI mass spectrometry imaging (MSI) in combination with LTP post-ionization was used to visualize sterols, fatty acids and alkaloids in different biological tissues with a spatial resolution down to 20 µm. No sample preparation steps, other than cryosectioning and thawing of the sample in a desiccator, were necessary. The water content of the tissue was sufficient to act as a matrix for IR-MALDI.

The combination of IR-MALDI and LTP post-ionization increased signal intensities up to thousandfold compared to IR-MALDI and made it possible to detect substances that were not detectable without post-ionization.

Here, we present a technique that makes non-polar analytes accessible by IR-MALDI without sample preparation. The potential of this methodology for MALDI MSI will be demonstrated. In the future, we plan to investigate the influence of the sample's water content on the ionization behaviour in detail.

T95

QUALITATIVE AND QUANTITATIVE ANALYSIS OF CHOLESTEROL AND LIPIDS UNDER AMBIENT CONDITIONS USING NANOSPRAY DESORPTION ELECTROSPRAY IONIZATION

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During the last decade, ambient ionization has become a prominent method for identification and characterization of molecules in mass spectrometry (MS) and mass spectrometry imaging (MSI). Nanospray desorption electrospray ionization (nano-DESI) is a soft and less destructive ionization method that combines locally defined liquid extraction with electrospray ionization in front of the MS inlet capillary.

The focus in this study was put on the detection of cholesterol and its distribution across different regions in mouse brain (Mus musculus), because cholesterol is one of the most abundant individual molecular species in plasma membranes of animals, making up to 15% of dry weight of the white matter (WM). To understand the importance of biochemical and physiological roles of cholesterol, it is necessary to quantify and localize the lipid within the sample section.

Tissue sections of mouse brain were used as a standard reference system for nano-DESI MSI investigations, in comparison to MALDI MSI. Cholesterol was targeted as the molecule of interest due to its biological importance. Calibration curves were acquired with cholesterol and cholesterol-d7 standard solutions with ESI and nano-DESI. Additionally, tissue sections were measured by using cholesterol-d7 with a given concentration in the solvent as internal standard or pre-sprayed in a defined amount and used for acquisition of ion images.

The investigation with nano-DESI MSI showed molecular distributions in agreement with reference data given in literature. The achieved improvements establish nano-DESI as a reproducible extraction-based method for soft and less destructive ambient MSI. The resulting lateral resolution suits for qualitative and qualitative identification of lipids and metabolites especially for cholesterol.

T96

IMAGING OF *SCHISTOSOMA MANSONI* EGGS IN HOST TISSUE

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Schistosoma mansoni is a blood fluke, causing schistosomiasis in humans. During acute infection, patients typically suffer from non-specific symptoms such as fever and cough. Main symptoms of chronic infection are abdominal pain and diarrhoea. Infected humans secrete eggs with their faeces or urine, but some straying eggs are trapped in other organs of the host and secrete enzymes. Due to the reaction of the tissue, granuloma enclose the eggs within a few days. Currently, only one drug, Praziquantel, is available. Praziquantel is widely used since the 1970s, which justifies the fear of upcoming resistance. For finding new drug targets, it is crucial to get a better understanding of the interaction between host and parasite. MALDI MSI (matrix-assisted laser desorption/ionization mass spectrometry imaging) is a powerful tool to visualize the topological distribution of substances of interest.

Cryosections (20 µm thick) of infected hamster liver were prepared and coated with DHB (2,5-dihydroxybenzoic acid) matrix for positive-ion mode and DAN (1,5-diaminonaphthalene) matrix for negative-ion mode, using a pneumatic sprayer. MS images were recorded using an AP-SMALDI5 AF ion source coupled to a Q Exactive high-resolution orbital-trapping mass spectrometer with 10 µm pixel size and a mass resolution of R = 240,000 at m/z 200.

By combining MALDI MSI with additional LC-MS/MS (liquid chromatography-tandem mass spectrometry) measurements of lipid extracts and statistical analysis, it was possible to characterize the spatioregional distribution of hepatic lipids and to identify marker signals for *S. mansoni* infection. Some markers were assigned to specific structures within the tissue. For example, only ether-PE were detected in the outer part of granuloma. Other markers were detected in higher intensities due to infection without any special regional accumulation.

The discovered alterations in hepatic lipid distribution caused by infection encourage us to further analyse the parasite-induced metabolic changes in the host's parenchyma.

T97

POLYCHLORINATED BIPHENYL (PCB) LOADS IN MINE WATER - CONGENER SPECIFIC ANALYSIS BY SPME-GC-MS

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Polychlorinated biphenyls (PCBs) are among the persistent organic pollutants (POPs) that have been banned worldwide since 2001 and can exert chronic toxicity even in very low concentrations. Despite the small water concentrations, especially the higher chlorinated PCBs can accumulate in food webs due to their physicochemical properties. Produced as industrial chemicals, PCBs were used as insulating oil in transformers and capacitors, including underground mining. As a legacy of mining, numerous (closed) mines still contain relevant quantities of PCBs. Mine water must be pumped out regularly and discharged into rivers accompanied by qualitative and quantitative monitoring. Due to high salt and metal concentrations, mine water represents one of the largest pollutant streams by volume in the world and has the potential to contaminate water bodies, conservation areas, and aquifers. However, until today especially inorganic pollutants are considered and treated. If monitoring of PCB takes place, it mainly aims at six indicator PCBs. In the present study, a solid-phase micro extraction (SPME) method coupled to gas chromatography-mass spectrometry (GC-MS) was optimised for the sensitive extraction of numerous PCB congeners from mine water. As a result, a large number of PCB congeners was detected in water from different mines in Germany showing a varying quantity and composition of PCB congeners. The presented method allows a comprehensive and labour saving analysis of PCBs in matrix rich mine waters. The presentation concludes with an outlook on the intended nanoparticle-catalysed dechlorination of PCBs in the "AntiPOP" project, presenting a promising elimination process.

Section 9 - Ecology and Global Change

Image: colourbox.com

Day 2: Thursday, September 30th, 2021

Section 9 - Ecology and Global Change — Chairpersons: Michael Hausschild & Leonhard Sommer & Alexander Konrad

10:30-11:00	**Prof. Lars Tranvik** (Uppsala University, Sweden) ***The fate and role of terrestrial organic matter in inland waters, from molecular to global scale***
111:15-11:25	**Kai Jansen**: *Role of clay mineral aggregation on sorption of benzyldimethyldodecylalkylammonium chloride*
11:25-11:35	**Alexander Konrad**: *Species Composition and Land Use Shape Soil Dissolved Organic Matter Quality and Quantity*
11:35-11:45	**Marigona Morina Gashi**: *Phytoremediation of pesticide contaminated soils and the transformation of chlorinates pesticides in plants*
11:45-11:55	**Leonhard Sommer**: *Ecological restoration of species-poor grassland – long-term success of mown material transfer in floodplain meadows at the Northern Upper Rhine*
11:55-12:05	**Niklas Janis Schnepel**: *Remote sensing based approaches for monitoring traditional orchards in Hesse, Germany*
12:05-12:15	**Philipp Koellmann**: *Innovative combine harvesting as an approach for integrated non-chemical weed control in central Hessen/ Germany*
13:15-13:25	**Ferdinando Binacchi**: *How bad are grain legumes for climate and agriculture?*
13:25-13:35	**Michael Hauschild**: *The impact of organic agriculture and specific agricultural practices on climate change mitigation*
13:35-13:45	**Eva Völker**: *Innovative approach for enhancing the productivity of organic wheat and soybean varieties by optimizing the growing space*
13:45-13:55	**Marvin Rades**: *Effects of long-term microplastic exposure on energy reserves and symbiosis of corals*
13:55-14:05	**Vanessa Tirpitz**: *Does the dose make the poison? The effects of different microdebris concentrations on reef-building corals*
14:05-14:15	**Annalena Barth**: *Effects of different protein sources on the nutrient profile and growth of whiteleg shrimp (Litopenaeus vannamei)*
14:15-14:25	**Catarina Martins**: *Water flow modulates the effect of ocean acidification on respiration and photosynthesis of reef-building corals*

K9

THE FATE AND ROLE OF TERRESTRIAL ORGANIC MATTER IN INLAND WATERS, FROM MOLECULAR TO GLOBAL SCALE

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Dissolved organic matter (DOM) in inland waters plays a substantial role in the global carbon cycle, and thus potentially affect climate as well. This presentation is an overview of the dynamics and fluxes of carbon involving DOM, from micro-scale to global scale. DOM is a heterogeneous mixture of decomposition products, and the reactivity and controls of the molecular composition are a central topic in aquatic biogeochemistry. Furthermore, DOM contributes substantially to evasion of carbon dioxide and methane to the atmosphere, but is also an important precursor of carbon that is buried in sediments. The loss of DOM from the water column is mediated via microbial and photochemical mineralization, as well as sedimentation upon formation of particles by flocculation or by sorption to minerals. The factors that constrain and promote loss of DOM from the water column will be discussed, and compared across different habitats, from soil to sea.

T98

EFFECTS OF DIFFERENT PROTEIN SOURCES ON THE NUTRIENT PROFILE AND GROWTH OF WHITELEG SHRIMP (*LITOPENAEUS VANNAMEI*)

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Shrimps are globally one of the most important aquaculture species with an estimated annual production volume of about 6 million tonnes, dominated by the whiteleg shrimp *Litopenaeus vannamei*. However, even when produced in sustainably managed aquaculture systems, the utilized feed is often highly unsustainable. The most common protein source for feed is fishmeal, mainly obtained by processing large quantities of small pelagic fish into fish meal and fish oil. With global overfishing, alternative protein sources must be exploited to sustain the expected future growth of aquaculture. These protein sources must meet essential requirements, such as an adequate amino acid profile, high digestibility and good palatability. In order to determine these requirements for *L. vannamei*, a feeding trial was conducted with three different feeds. Accordingly, three groups of shrimps were fed with: 1) natural fresh feed (squid, mussels and krill), 2) artificial dry feed commonly used in the shrimp industry, and 3) sustainable dry feed supplemented with fresh larvae of the black soldier fly (*Hermetia illucens*). The trial was performed in a closed recirculating system. The shrimps were assessed for size and mass and photographed at the beginning and end of the trial. It was found that the shrimps with the artificial feed had the highest growth rates, the sustainable feed had slightly lower growth rates, but the mass was clearly more homogeneous compared to the other two groups. To determine the physiological parameters causing these effects, an amino and fatty acid profile will be established after the trial to compare the nutrient efficiency of the different diets. This study is expected to provide deeper insights into the metabolization of nutrients by whiteleg shrimp, enabling further work on sustainable insect-based diets to conserve the natural resources of the oceans in the future.

T99

HOW BAD ARE GRAIN LEGUMES FOR CLIMATE AND AGRICULTURE?

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The EU aims at reducing green house gases (GHG) emissions by 55% by 2030 to limit anthropogenic impacts on climatic shifts. Crops belonging to the Fabaceae family may play an important role within this shift, by providing a more environmentally friendly source of food and feed protein while enhancing soil nitrogen (N), possibly replacing chemical fertilizers. As nutrient inputs to the soil increase, so do questions on fate of N which may end up in below ground water flows, or lost in the atmosphere, raising knowledge gaps about tracing fate of nutrients. We report one season of nitrate (NO_3^-) leaching and nitrous oxide (N_2O) volatilization, originating from 2 winter (pea – Pisum sativum- and faba –Vicia fava-) and 4 summer (pea, faba, lupine - Lupinus albus- and soya – Glycine max-) grain legumes, grown on a silty clay loam in central Germany under organic farming. 12 cylindrical cartridges of 10 cm height 10 cm diameter were installed for each crop variety at 1 meter depth before the sowing of winter varieties and excavated back ten months later. The cartilageswere filled with a silica gel which would bind to nitrate as water passed through and accumulated N over the crop-

ping season. Nitrous oxide emissions instead were assessed every Wednesday through automated cavity ring down spectroscopy and monitored peak times of N volatilization. We report the first study on annual nitrate leaching and nitrous oxide emissions of the most commonly cultivated grain legumes in Germany.

T100

THE IMPACT OF ORGANIC AGRICULTURE AND SPECIFIC AGRICULTURAL PRACTICES ON CLIMATE CHANGE MITIGATION

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Organic agriculture has a high potential for climate change mitigation. Several studies showed that organic agriculture reduces greenhouse gas emissions and enhances carbon sequestration in the soils. There are, however, few studies looking at all climate-relevant factors and little is known about the organic practices that hold the most potential to reduce agriculture's impact on the climate. In this study, we will perform a meta-analysis, evaluating published scientific data on the effect of organic agriculture, crop rotation, tillage and fertilization on soil organic carbon (SOC), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3) emissions. Over 3500 studies were identified via literature search in Web of Science and filtered for eligibility, resulting in around 300 studies investigating different agricultural systems worldwide that will be included in the final analysis. This research aims at providing a holistic review of the impact of organic agriculture and specific agricultural practices on the climate. The results from the meta-analysis will be utilised to inform a merit-based remuneration system, in order to incentivise farmers to apply the best possible management practices and thus reduce the negative impact of agriculture on the environment.

T101

ROLE OF CLAY MINERAL AGGREGATION ON SORPTION OF BENZYLDIMETHYLDODECYLALKYLAMMONIUM CHLORIDE

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Quaternary alkylammonium compounds (QAACs) are cationic organic surfactants and disinfectants that are frequently detected in aquatic and terrestrial systems due to their numerous applications and large production volumes. Several studies suggest that exposure to QAACs may promote resistance against different antibiotics, and exposure at sub-inhibitory concentrations, as influenced by sorption and desorption processes, might regulate resistance formation in the environment. In soils, QAACs mainly bind to negatively charged constituents, such as organic matter and clay minerals. At environmentally relevant concentrations, cation exchange is the major mechanism for QAAC sorption in soils. Furthermore, the degree of aggregation of mineral phases might contribute to interparticle spaces that could influence QAAC sorption, which has not yet been a research focus. Goal of our study was therefore, to discern the role of aggregation in QAAC sorption experiments. The sorption of benzyldimethyldodecylalkylammonium chloride (BAC-C12, as model substance for QAACs) up to 1000 µg/L on two different clay minerals (montmorillonite and illite) was examined in deionised water and electrolyte solutions, representing systems of well-dispersed and more aggregated clay particles, respectively. Calcium chloride (0.01 M) and sodium chloride (0.02 M) solutions were used to induce aggregation. Additionally, the influence of interlayer cations in montmorillonite was studied. The order of sorption capacity of clay minerals and the degree of aggregation depended on the background solution. Calcium chloride caused a clearly more visible aggregation than sodium chloride. Unexpectedly, the sorption of BAC-C12 was higher for illite than for montmorillonites in deionised water. Calcium-exchanged montmorillonite (Ca-Mt) was a stronger sorbent than sodium montmorillonite (Na-Mt). In the presence of electrolytes, however, both montmorillonites sorbed BAC-C12 stronger than illite. These results show that aggregation of clay minerals has a marked effect on the sorption capacity for QAACs.

T102

INNOVATIVE COMBINE HARVESTING AS AN APPROACH FOR INTEGRATED NON-CHEMICAL WEED CONTROL IN CENTRAL HESSEN/ GERMANY

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Standard combine harvesters release weed seeds that are taken up during the threshing process on the fields. Thus weed seeds can be spread over vast areas on the fields as studies have shown. Standard cereal harvest is though potentially forcing up current challenges of weed control that already exist.
This issue is picked up in the context of developing an innovative combine harvesting technique as an alternative non-chemical approach for weed regulation. The new technique bases on a gadget collecting weed seeds during the threshing process. In this study, the effectivity of innovative combine harvesting with regards to picking up weed seeds during threshing process has been analyzed.
Retained material from threshing, consisting of straw, chaff and weed seeds was transferred into trays for the seed germination. Emerged seedlings were indentified as species and individuals per species were counted. It was figured out that the new technique has the potential to reduce weed seed inputs to the soil seedbank.⌢
As an example for collecting grass seeds with the innovative combine harvesting method, the collection rates were estimated for seeds of the grass species Bromus secalinus. In the field trial a major proportion of 60% of the current seed potential of the field could be retained. Another 31% of Bromus-seeds were released on the field and 9% were retained by the grain tank of the combine harvester.
Rates for collecting weed seeds were usually mostly double as high in organic cropping systems as in the conventional alternative. Over both years of the field trial in 2019 and 2020, 25 different arable plant species were picked up by innovative combine harvesting. Among these 19 herbal species and six grass species.

T103

SPECIES COMPOSITION AND LAND USE SHAPE SOIL DISSOLVED ORGANIC MATTER QUALITY AND QUANTITY

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Soils serve as the largest terrestrial organic carbon reservoir. While most soil carbon is bound to minerals as mineral-associated organic matter, dissolved organic matter (DOM) transports carbon, nutrients, and pollutants in soils and between ecosystem boundaries. DOM can be described as the product of the incomplete decomposition of organic matter that has escaped complete mineralization to CO_2. It plays a key role in biogeochemical cycles because organic substances containing energy, carbon, nitrogen, phosphorus, and many other elements are more mobile and more accessible to microorganisms in dissolved form than in undissolved form. Understanding the controls of the composition and concentration of DOM as a link between biogeochemical cycles of different elements and between terrestrial and aquatic ecosystems is therefore essential for sustainable land use. Land use intensity and species composition of terrestrial ecosystems influence DOM quantity and quality, while the controlling mechanisms are largely unknown.
The forest and grassland sites of the Biodiversity Exploratories offer the perfect basis to explore these mechanisms due to their number and diversity as well as the amount of information regarding their biocenoses and abiotic site conditions.
We hypothesize that the composition and productivity of plant species communities and associated soil microbial communities shape the concentration and composition of DOM. Additionally, plant communities producing litter with low nutrient content and soil microbial communities dominated by fungi will increase the concentration of DOM with low nitrogen content and low oxidation state.

T104

WATER FLOW MODULATES THE EFFECT OF OCEAN ACIDIFICATION ON RESPIRATION AND PHOTOSYNTHESIS OF REEF-BUILDING CORALS

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Ocean acidification (OA) constitutes a major threat to reef-building corals, affecting their physiology and leading to reduced growth. Yet, OA effects on respiration and photosynthesis, key processes of coral metabolism, differ considerably among species. This may be due to species-specific effects of water flow, an environmental factor that modulates these processes under normal conditions. However, its effects under OA conditions remain poorly understood. Therefore, we performed a three-month aquarium experiment to test whether water flow modified the effect of OA on coral respiration and photosynthesis. For this, we exposed the reef-building coral species *Acropora cytherea*, *Pocillopora verrucosa*, and *Porites cylindrica* to control and OA conditions (500 and 1000 µatm pCO_2, respectively) and assessed respiration and photosynthesis rates in two flow regimes (2 cm s^{-1} and 6 cm s^{-1}). We found that under OA conditions calcification and surface growth decreased, while respiration increased in all species. Yet, OA had a variable effect on photosynthesis, which decreased in *A. cytherea*, was unaffected in P. verrucosa, and increased in *P. cylindrica*. Water flow only changed the metabolic response in *A. cytherea* and P. verrucosa. Moderate flow (6 cm s^{-1}) exacerbated the respiratory increase and the photosynthetic decrease of *A. cytherea* in OA, but low flow (2 cm s^{-1}) exacerbated the OA-increase in respiration of P. verrucosa. Overall our results indicate that water flow aggravates the effect of OA on reef-building corals and that slow-flow habitats may shelter only some species from this stressor. These findings help understand the role of hydrodynamics in coral physiology and highlight the importance of considering environmental variables in the design of conservation strategies.

T105

USING THE TRANSECT DESIGN TO STUDY INTERFACES OF HETEROGENEOUS AGROFORESTRY SYSTEMS

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Agroforestry systems – the combination of trees/shrubs and crops/grassland on the same land - are complex and diverse agroecosystems that can deliver multiple ecosystem services. Perennial vegetation and the lack of soil tillage in the tree lines have direct and indirect influences on soil quality indicators and related ecosystem services including carbon sequestration, nutrient cycling, water storage and soil biodiversity. Especially silvoarable systems (perennials on cropland) are expected to show differences in soil characteristics and associated services between the tree rows and the adjacent arable field. An important question is how we can study this internal complexity of an agroforestry system.
Experimental studies on agroforestry systems often use a transect sampling design, in which samples are taken in the tree row and at various defined distances from it into the centre of the adjacent arable field. This transect sampling is a well-established method to document spatial differences in the soil and to study the heterogeneity of the soil ecosystem under agroforestry practices. However, designs of agroforestry systems differ widely in terms of trees and field crops, the distance between tree rows, planting densities and study designs. Randomized block designs and non-agroforestry control plots are often not available.
Therefore, we developed a standardized transect sampling design for the newly established silvoarable agroforestry site at Gladbacherhof, Villmar Aumenau. The design was primarily used for soil analyses but further adapted to other research topics like crop health and yield as well as insect and weed abundance. This design can be adapted to other agroforestry sites. The resulting data comparability can support researchers, farmers and decision makers in taking informed decisions at a time of increasing recognition of agroforestry practices in the German agricultural sector.

T106

PHYTOREMEDIATION OF PESTICIDE CONTAMINATED SOILS AND THE TRANSFORMATION OF CHLORINATED PESTICIDES IN PLANTS

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Organochlorine pesticides (OCPs) have been widely used for the control of human and animal diseases by treating transmitting insects and pests that caused significant damages in crops. They belong to the persistent organic pollutants (POPs) and are known for their slow degradation and accumulation in living organisms causing health problems in animals, fish and humans. As OCP uptake can lead to accumulation in primary products, their removal from soil is crucial.
Considering the big natural concern of agricultural pollution, the assessment of phytoremediation is presented as a very good alternative to conventional treatments of soil due to advantages such as low cost, large application areas and the possibility of in-situ treatment. The identified fundamental processes of the plants used for remediation make this environmental friendly method a very good approach for the OCP contaminated soil.
In greenhouse experiments, the optimisation of the uptake of OCPs from the contaminated soil into the plant will be carried out using mobilising agents. Studies suggest that *Miscanthus x giganteus* and *Ricinus communis L.* have shown good results in the ability for OCP uptake.
The fate of OCPs accumulated in plants will be thoroughly studied in laboratory experiments with sophisticated analytical methods (GC – MS and LC – HRMS) with regard to emerging transformation products and evaluate the efficiency of phytoremediation.

T107

EFFECTS OF LONG-TERM MICROPLASTIC EXPOSURE ON ENERGY RESERVES AND SYMBIOSIS OF CORALS

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The increasing pollution of the world's oceans with plastic waste, especially microplastics, poses considerable challenges to marine faunas and ecosystems. Corals as suspension feeders are particularly vulnerable, and adverse effects, such as reduced growth or reduced feeding rates, have already been observed. However, the consequences of microplastics on the complex "coral holobiont" (i.e., coral host organism and associated microorganisms) remain poorly understood. Many of the impairments could be due to changes in symbiotic interactions between the coral and the unicellular dinoflagellates, so-called zooxanthellae, as well as reduced energy reserves. We, therefore, determine the long-term effects of microplastic pollution on the energy reserves of corals and their associated photosymbionts. We conduct a controlled fifteen-month microcosm experiment in which we exposed the reef-building coral *Acropora muricata* to polyethylene microplastics at a concentration of 200 particles per litre and used a microplastic-free control group for comparison. We analyse the energy reserves (lipids, proteins, and carbohydrates) using colourimetric methods. Additionally, we assess zooxanthellae density by haemocytometer counts and chlorophyll content of the harboured zooxanthellae by photospectrometric measurements. The analyses of energy reserves in the coral tissue could reveal physiological shifts due to the long-term exposure to microplastics, thus providing a more comprehensive picture of the effects of microplastics on corals. With this study, we aim to unveil essential aspects of the impacts of microplastics on coral physiology and infer the possible consequences for reef-building corals.

T108

REMOTE SENSING BASED APPROACHES FOR MONITORING TRADITIONAL ORCHARDS IN HESSE, GERMANY

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Traditional orchards (Ger.: "Streuobstbestände") are a type of land use in Germany and other Central European Countries with ecological, cultural and economic importance. They form special habitats for a multitude of different species because they combine features of open landscapes and forests. Due to this, they have been in the focus of natural conservation efforts in the last decades. They are protected biotopes in Hesse (as well as other federal states of

Germany) and agricultural aid programs aim to support their preservation. Therefore, there is a need to monitor traditional orchards to ensure that the goals of nature conservation and agricultural aid programs are achieved. However, current on-site monitoring is time consuming and has limitations related to area size, distance and available personnel. Remotely sensed data and associated methods have already been successfully applied in the monitoring of forests and fruit plantations and show promise to be used for traditional orchards. Thus, the aim of this project is to use remotely sensed data, such as multitemporal light detection and ranging (LiDAR) and multispectral data, to develop transferable monitoring methods for traditional orchards. Here, the focus lies on data that is available for the entirety of Hesse and that is already collected at regular intervals. With these data, quantitative and qualitative parameters (e.g. tree height, crown base height, pruning condition, species composition, tree age class as well as the number and density of the trees and their respective temporal changes) will be mapped. For this, tree crown segmentation as well as machine and deep learning algorithms will be used. Additionally, different sources, types and resolutions of remotely sensed data (e.g. imagery from UAVs, aircrafts and satellites) will be tested to identify possible minimum requirements for detecting the different parameters and to give recommendations for future monitoring efforts.

T109

ECOLOGICAL RESTORATION OF SPECIES-POOR GRASSLAND – LONG-TERM SUCCESS OF MOWN MATERIAL TRANSFER IN FLOODPLAIN MEADOWS AT THE NORTHERN UPPER RHINE

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Species-rich floodplain grassland is one of the most species-rich habitats in Central Europe, but likewise endangered. An important method to restore species-rich floodplain meadows is the transfer of mown material from valuable donor grassland sites to restoration sites in order to introduce seeds of plant species that had receded before. The long-term success of mown material transfer on species-poor grassland has rarely been investigated so far. In this study, 20 mown material stripes on such grassland at the Northern Upper Rhine in Hesse, Germany, were investigated for vascular plant species 13 to 16 years after material transfer. Each mown material stripe contained three different soil preparation treatments. Additionally, the corresponding donor sites and untreated zones of the species-poor grassland were assessed. Biomass samples were taken from all sites. During the following months, these samples will be analysed concerning yield potential and forage quality of the different grassland stocks. Corresponding soil samples will be analysed for pH value, nutrient content, and grain size. The soil data and soil preparation will be used together with a hydrological model as predictors of the success of mown material transfer with regard to species establishment and fodder value. We expect a link between the abiotic similarity of corresponding donor and restoration sites and target species establishment, whereas the differences between soil preparation treatments may have diminished over time. If this holds true, results will support the importance of abiotic factors for the choice of suitable donor sites and restoration sites in further restoration projects, enabling more efficient resource use in ecological restoration of species-rich grassland.

T110

DOES THE DOSE MAKE THE POISON? THE EFFECTS OF DIFFERENT MICRODEBRIS CONCENTRATIONS ON REEF-BUILDING CORALS

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The pollution of the environment with anthropogenic debris is increasing and considered to be a major threat to marine ecosystems. Particularly microplastic (1–1000 µm) is gaining attention in public and science and has been shown to affect, for example, reef-building corals. Most studies on the effects of microplastics focus on a single polymer type. However, the effects of naturally occurring mixtures of microplastics, known as â€˜marine microdebris', are still poorly understood. Thus, the goal of our study was to assess the influence of â€˜typical' marine microdebris on reef-building corals. Therefore, we exposed two common reef-building corals species (*Stylophora pistillata* and *Pocillopora verrucosa*) for twelve weeks to four different concentrations (0.1, 1, 10, and 100 mg/l) of an assortment of microdebris. The microdebris mixture was composed of both fi-

bres (polyamide, polyester, polypropylene) and particles (polyethylene, polyvinylchloride, polystyrene) and compared to a microdebris free control treatment. We studied coral growth rates (i.e., changes in surface area, volume, and calcification) using 3D scanning and buoyant weighing to infer on effects on the coral host. We assessed the photosynthetic activity of the associated photosymbionts using Pulse Amplitude Modulation (PAM) Fluorometry to conclude on the performance of the coral symbiont community. As an additional indicator of stress, we recorded coral polyp activities. While the experiments are still running, we expect our findings to contribute to a better understanding of the effects of microdebris mixtures on reef-building corals. This will allow for a better assessment of what measures can be taken to reduce this anthropogenic pressure on coral reefs.

T111

MYCORRHIZA AND HYDROPRIMING USE IN EARLY SOYBEAN DEVELOPMENT UNDER GERMAN FIELD CONDITIONS

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Soybean production in Europe is spreading northwards towards colder areas with shorter vegetation periods. Plant breeding and pre-sowing techniques, like hydropriming, are used to speed up the plant development. Mycorrhiza are known to improve nutrient uptake and may improve plant development. In our study, we tested the impact of hydropriming and a mycorrhiza product on three varieties of soybeans in German field conditions in a two-year study. We analysed plant and root development (e.g. emergence, plant hight, duration of development) as well as yield parameters. We found no improvements in yields, but in contrast to previous studies, germination was reduced with hydropriming. In most of the tested characteristics hydropriming did not show an effect or had a negative effect, while mycorrhiza had no effect. The use of a principal component analysis, which considers the different properties in one graph, showed that the factors year and soybean variety have a greater influence than the treatments (hydropriming or mycorrhiza). Both techniques, hydropriming and inoculation with mycorrhiza did not show the expected results and are therefore no strategy of adapting soybean cultivation to shorter vegetation periods. Breeding for early development might be the more successful way.

T112

INNOVATIVE APPROACH FOR ENHANCING THE PRODUCTIVITY OF ORGANIC WHEAT AND SOYBEAN VARIETIES BY OPTIMIZING THE GROWING SPACE

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Wheat produced by organic farming stagnates at a low yield level compared with wheat produced by conventional farming. While breeding approaches have not yet achieved significant advances in minimising this yield gap, alternative crop management strategies have been proven suitable to increase crop performance. Alternative crop management strategies such as either optimal row spacing as well as optimal plant densities improve growth conditions compared to conventional drill sowing by minimising intraspecific competition for water, light and nutrition. However, optimizing the growing space by equalling row spacing and within-row spacing with different cultivars has not yet been studied sufficiently.
The aim of this study is to quantify to which extent organic wheat and soybean yields can be increased and to elucidate the main factors being responsible for the expected yield increase. To meet the goals, three wheat varieties soybean varieties will be planted with stepwise rising row and within-row spacings by means of a precision seeding technique. The highest yield per area will be by plotting a yield curve as a function of the area per plant. To elucidate the main factors of the yield increase, yield parameters such as ears vs. pods per plant, grain per ear vs. pod, seed weight and yield per plant will be assessed. The trial is arranged as a split plot including three varieties, seven density patterns, and four replications and will be replicated for three years.

Section 10 - Clinical Sciences

Image: colourbox.com/Kiyoshi Takahase Segundo

Day 2: Thursday, September 30th, 2021

Section 10 - Clinical Sciences Chairpersons: Hendrik Lehmann & Michael Cekay & N.N. & Jiawen Yojng & Anna-Lena Proksch

17:45-18:15	**Prof. Stefan Hippenstiel** (3R Centre, Charité, Berlin, Germany) ***Charité 3R – 3R implementation at a university hospital — How can a medical faculty enhance application of the 3Rs?***
11:15-11:25	**Rebecca Hasseli**: *Prevalence of Neuropathies in Rheumatic and Musculoskeletal Diseases*
11:25-11:35	**Reem Jamous**: *Ex vivo bone organ culture mimicking defect healing in vivo*
11:35-11:45	**Michaela Melzer**: *Rho/ROCK inhibition promotes TGF-β3-induced tenogenic differentiation in mesenchymal stromal cells*
11:45-11:55	**Veronika Lehner**: *When structure matters and matter structures: Nano-3D-printed biphasic polymer scaffolds in a 3D cell culture model*
11:55-12:05	**Carla Doll**: *Comparative study of equine chronic tendon lesion appearance in low- and high-field magnetic resonance imaging*
12:05-12:15	**Alina Hagen**: *Platelet lysate in the cultivation of mesenchymal stromal cells: Are there differences between horses and dogs?*
13:15-13:25	**Hendrik Lehmann**: *Canine whole blood stored at 4°C provides hemostatic activity for 15 days.*
13:25-13:35	**Anna-Lena Proksch**: *Role of bile acid profiling and cobalamin status as non-invasive biomarkers in dogs with chronic liver disease*
13:35-13:45	**Sebastian Stricker**: *Regulation of human and microbial transglutaminases in the context of coeliac disease*
13:45-13:55	**Jiawen Yong**: *Influence of leptin and compression in GAS-6 mediated homeostasis of periodontal ligament cell*
13:55-14:05	**Fabian Edinger**: *Investigation of the time course of Des-Arginin9-Bradykinin, Angiotensin II, and mitochondrial DNA in critically ill COVID-19 patients*
14:05-14:15	**Wenjie Sheng**: *Evaluation of Anti-mouse and Anti-rabbit IgG Nanobodies Conjugates with SNAP-tag as Secondary Antibodies in Immunofluorescence*
14:30-14:40	**Paul Brunk**: *Novel methods of severity assessment in a murine model of chronic obstructive pulmonary disease (COPD)*
14:40-14:50	**Ruth Charlotte Dartsch**: *Alveolar regeneration in Idiopathic pulmonary fibrosis is not impaired by aberrantly activated Notch1 signalling*
14:50-15:00	**Manuel Richter**: *Longitudinal Right Atrial Function in Incident Pulmonary Arterial Hypertension*
15:00-15:10	**Michael John Cekay**: *Lung cancer and its comorbidities: Comparing surrogate parameters for pulmonary hypertension and chronic obstructive pulmonary disease for their prognostic relevance.*
15:10-15:20	**Philipp Arndt**: *Metabolic reprogramming of tumor associated macrophages and ist impact on lung cancer progression*
15:20-15:30	**Nazli Salik**: *Microenvironmental control of lung cancer metastasis*
15:45-15:55	**Salisa Kruijning**: *Crosstalk between cellular hypoxia sensing and histone methylation systems and its effect on cancer progression*
15:55-16:05	**Chaoyu Zhang**: *SNAP-tag based antibody drug conjugates in precise treatment of triple-negative breast cancer*
16:05-16:15	**Lucas Kimmig**: *Tissue-Resident Alveolar Macrophages are Characterized by Substrate Flexibility and a Subordinate Role of Metabolism for Inflammation*
16:15-16:25	**Anca-Laura Amati**: *C-reactive protein-mediated inhibition of ATP-induced inflammasome activation*
16:25-16:35	**Martin Reichert**: *Influence of preoperative functional status on perioperative immune response after major visceral surgery – What the surgeon has to do with mitochondria*

K10

CHARITÉ 3^R – 3R IMPLEMENTATION AT A UNIVERSITY HOSPITAL - HOW CAN A MEDICAL FACULTY ENHANCE APPLICATION OF THE 3RS?

Prof. Stefan Hippenstiel[1]

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Charité, the university hospital in Berlin, is the joint medical faculty of the Freie Universität Berlin and the Humboldt-Universität zu Berlin, and conducts about 50.000 animal experiments per year for biomedical research. Since 2018, it actively strengthens the in-house implementation of the 3Rs by the foundation of Charité 3^R. Charité 3^R is a body of the faculty and supports 3R research and education by funding research projects, workshops and courses. These measures are accompanied by communication and outreach activities.
The vision of Charité 3^R is to better understand, diagnose and treat human diseases by fostering the principles of the 3Rs, which comes along with a cultural change within the field of biomedical research. Three strategic aims support this vision: 1. Ensure the principles of the 3Rs are clearly reflected within the areas of teaching and learning at Charité. 2. Ensure Charité 3^R initiates Berlin-wide research collaborations aimed at promoting the principles of the 3Rs. 3. Ensure Charité 3^R secures new strategic partners across national and international borders in order to develop joint concepts for the rapid and rigorous implementation of the 3Rs. The strategic aims are implemented by numerous measures in the three pillars communication, education and research. Collabortion with strong partners is an essential part of Charité 3^Rs work. Together with FU, HU and TU Berlin as well as BfR, RKI and MDC, Charité 3^R has raised funding for the Einstein Center 3R (EC3R), a Berlin-wide collaboration for alternative methods in biomedical research funded with up to € 5.3 million until the year 2026 by the Einstein Foundation Berlin.

T113

MICRORNA ANALYSIS IN MONOCYTE SUBSETS OF PATIENTS WITH VASCULAR DISEASE

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Monocytes/macrophages play an important role in the development of athereosclerotic lesions, since they lodge in the intima and subintima of arteries, thereby contributing to the formation of obstructive atherosclerotic plaques that are prone to rupture, leading to thrombosis. The aim of this study is the analysis of expression patterns of specific miRs, which was observed recently in our lab to be differentially regulated in human infarcted monocytes and atherosclerotic plaques, in different subtypes of circulating monocytes, as well as in plasma of patients suffering for the first time from stable angina pectoris or acute coronary syndrome .and in the monocytic cell line THP1 . The correlation between cellular and plasma expression of the specific miRs, and the overall plaque burden and plaque phenotypes will be assessed.in the patient probes, THP1 cells will be stimulated by IFNγ /LPS for M1 and IL-4 for M2. differantiat miRs will be analyzed also under this condition using AgomiRs /AntagomiRs we can then determine involvement of miRs in the differantieted process of monocytes Finally, this study will identify miRs in monocytes subsets in correlation to atherosclerosis progression and function.

T114

C-REACTIVE PROTEIN-MEDIATED INHIBITION OF ATP-INDUCED INFLAMMASOME ACTIVATION

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Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine. While IL-1β is involved in host defense against infections, it also plays a central role in the pathogenesis of life-threatening systemic inflammation triggered in about a third of the patients undergoing major open surgery. Hence, controlling the systemic inflammation induced by surgical trauma is of outstanding biomedical relevance.
IL-1β release by mononuclear phagocytes can be induced by Toll-like receptor agonists, followed by stimulation with extracellular ATP, an important danger signal in sterile inflammation. Increased systemic IL-1β stimulates the hepatic expression of C-reactive protein (CRP), a pentameric acute phase protein known to have both pro- and anti-inflammatory effects.
We previously demonstrated that native CRP inhibits the ATP-mediated release of IL-1β from human monocytic cells, in a mechanism involving nicotinic acetylcholine receptors (nAChRs). The activity

of CRP depends on the presence of soluble endogenous ligands, that thus far haven't been characterized. Our project aims to identify endogenous CRP ligands and to analyze the inhibitory effect of CRP/ligand complexes in patients plasma after major abdominal surgery. We developed an immunoprecipitation method for CRP depletion from patients plasma. The extracted CRP is being analyzed for its soluble ligands via mass spectrometry. The effect of serial plasma dilutions before and after CRP-depletion on the IL-1β release from LPS and ATP-stimulated autologous blood cells as well from isolated peripheral blood mononuclear cells (PBMCs) was analyzed before, as well as on days 2 and 5 after major abdominal surgery. We were able to demonstrate that CRP-depleted plasma has a diminished anti-inflammatory capacity when compared to CRP-containing plasma, though further patients need to be recruited to validate these results.
Based on these findings, we intend to develop modified CRP that is devoid of pro-inflammatory activity but prevents ATP-induced IL-1β release, therefore dampening ATP-mediated sterile inflammation without increasing the risk of sepsis.

T115

METABOLIC REPROGRAMMING OF TUMOR ASSOCIATED MACROPHAGES AND ITS IMPACT ON LUNG CANCER PROGRESSION

Arndt, PF[1,2], Mansouri, S[2], Cekay, M[1,2], Eul, B[1], Pullamsetti, SS[1,2,3], Grimminger, F[1,2,3], Seeger, W[1,2,3], Savai, R[1,2,3,4]

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Lung cancer is the leading cause for cancer related death worldwide. Even though early-stage lung cancer is treatable with intense regiments, around 50% of all lung cancer patients are diagnosed in stage IV, resulting in poor overall prognosis. Although novel therapy options have been developed, only a fraction of lung cancer patients profit from targeted therapy and checkpoint inhibitors. Therefore, further research regarding the molecular mechanisms causing lung cancer progression is paramount. In this context, the tumor microenvironment (TME) has been identified as a hallmark of lung carcinogenesis. The TME includes fibroblasts, endothelial cells and immune cells. Previous data from our lab shows that a majority of those immune cells comprise macrophages, so called tumor-associated macrophages (TAMs). Macrophages are characterized by an exceptional flexibility, meaning they can adapt different phenotypes following specific stimuli. We showed that around 70% of all TAMs adapt an anti-inflammatory, pro-tumoral phenotype. Furthermore, we were able to demonstrate that macrophage – cancer cell crosstalk plays an important role in lung cancer progression, angiogenesis and metastasis. As metabolome plays a crucial role in regulating cellular functions and carcinogenesis. It has been demonstrated, that differently polarized macrophages show different metabolic states, However, it is currently unknown how the metabolome regulates lung cancer progression in the TME. The aims of this project are to identify cell and secretory metabolome in TAMs and cancer cells and tumor-cell crosstalk using mass spectrometry-based metabolome analysis. Secondly, to verify functional and phenotypic capacities of identified metabolite *in vitro*. For this purpose, macrophages will be treated with the candidate metabolite and analyzed via qPCR, western blot and functional assays. Thirdly, the identified metabolite will be further validated in different in animal models. Finally, we aim to validate the identified metabolite as a prognostic factor in all stages of lung cancer.

T116

NOVEL METHODS OF SEVERITY ASSESSMENT IN A MURINE MODEL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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The aim of the 3R-principle, the basic principle to promote animal welfare in animal-based research, is to reduce the number of animals used, replace animal-

models wherever possible and to refine models and procedures so they impose the least possible harm, stress, pain and suffering on the animals.
Identification and assessment of the burden imposed is the very first step of refinement and the basis for every measure to follow. Therefore, it is of the utmost importance to have evidence-based and objective methods of severity assessment at hand. Unfortunately, methods that meet these requirements are scarce and the methods that are available focus on physiological parameters and rarely assess mental impairments themselves.
In this project, voluntary wheel running (VWR) in mice, a novel method that has been the subject of recent refinement-research, as well as a holistic approach that includes the assessment of the mental state of the animals are integrated in a murine model used in cardiopulmonary-research, in order to address these questions.
In a side-by-side comparison, established methods of severity assessment (scoring, faecal corticosteroids, monitoring of homecage-behaviour), a system to detect VWR, methods to detect mental impairments (behavioural analysis such as the open-field-test, elevated-plus-maze and light-dark-box), the analysis of serotine and dopamine-concentrations in certain brain areas and histological analysis of the olfactory epithelium are integrated into an animal-model of chronic obstructive pulmonary disease (COPD).
Although results have not been obtained, it is expected that VWR as a method of severity assessment can be extended into the field of animal-based cardiopulmonary-research and that potential mental impairments can be assessed with the used methods. Furthermore, the results make it possible to completely characterized the animal-model regarding its severity and potential refinement-aspects can be identified. The holistic approach implemented can then be applied to further models and fields of animal-based research.

T117

LUNG CANCER AND ITS COMORBIDITIES: COMPARING SURROGATE PARAMETERS FOR PULMONARY HYPERTENSION AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE FOR THEIR PROGNOSTIC RELEVANCE.

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Lung cancer is the leading cause of cancer related deaths worldwide. Furthermore, this disease is often accompanied by various comorbidities. Pulmonary hypertension in the context of lung cancer has been described very recently as a major poor prognostic factor. In this study, we compared surrogate parameters for COPD and pulmonary hypertension for their prognostic relevance in lung cancer. For this purpose, we calculated the impact of COPD, specifically FEV1 and FEV1/FVC on the survival of patients with lung cancer using the cox proportional hazard survival model. Notably, no significant impact of FEV1 and FEV1/FVC on progression free survival (PFS) and overall survival (OS) was observed in our lung cancer patient cohort. Although the data may be insufficient to claim the absence of any effect of FEV1 or FEV1/FVC on PFS or OS, this finding remarkably differs from the strong correlation between PFS/OS and PA/PA/A ratio, we noted in the same patient cohort. In addition, we calculated a cox proportional hazard survival model after adjusting for FEV1 and FEV1/FVC. Importantly, even after adjusting for FEV1 and FEV1/FVC our dataset is sufficient to conclude that median overall survival is significantly reduced in patients with a PA size$\geq$28mm ($p = 0.023$) and PA/A ratio$\geq$1 ($p < 0.001$). In conclusion, we believe that our dataset provides strong evidence that an increased PA/PA/AA ratio, probably indicating PH, is the main predictor of survival in this lung cancer cohort.

T118

ALVEOLAR REGENERATION IN IDIOPATHIC PULMONARY FIBROSIS IS NOT IMPAIRED BY ABERRANTLY ACTIVATED NOTCH1 SIGNALLING

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Idiopathic pulmonary fibrosis (IPF) is the most abundant idiopathic interstitial pneumonia with a fatal median survival worse than many solid cancers. Chronic repetitive type II alveolar epithelial cell (AECII) injury as the major initiating disease mechanism facilitates a defective alveolar regeneration and AECII cell death. Until today there is no treatment available improving this disturbed epithelial regeneration process. Notch signalling, a highly conserved developmental pathway is persistently activated after major lung injury and promotes an aberrant alveolar regeneration and alveolar cyst formation reminiscent of microscopic honeycombing in IPF. We previously identified Notch signalling in AECII in the Bleomycin model of lung fibrosis and hypothesized that aberrantly activated Notch1 signaling facilitates hyperproliferation in IPF AECII subpopulations.
Histological analysis of explanted IPF lungs revealed no signature of an overwhelming Notch1 activation in hyperplastic human IPF AECII. Instead, IPF AECII only rarely showed signs of Notch activity, as indicated by the Notch target gene Hes1. We investigated the proliferative potential of alveolar epithelial derived cells in a human alveolospheres assay. Rather than being hyperproliferative, IPF AECIIs subpopulations showed a significantly reduced proliferative capacity compared to AECIIs isolated from healthy organ donors. Interestingly, a specific Notch1 receptor blockade showed no impact on the proliferating human IPF AECII, whereas global Notch inhibition by γ-Secretase inhibition did. Instead, activation of Wnt signalling by GSK3 inhibition promoted AECII growth, as evidenced by significantly larger sphere sizes of Wnt-activated AECII. To further clarify the Notch activation status in IPF AECII protein analysis of sorted IPF AECII showed no upregulated Hes1 as well as Notch1 level compared to donor organ AECII. Finally, induction of Endoplasmatic Reticulum Stress as a major hallmark of chronic IPF AECII injury and apoptosis in a human Notch positive lung adenocarcinoma cell line revealed a dampened Notch activity represented by a Notch target gene downregulation.

T119

COMPARATIVE STUDY OF EQUINE CHRONIC TENDON LESION APPEARANCE IN LOW- AND HIGH-FIELD MAGNETIC RESONANCE IMAGING

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For the diagnosis of equine tendon lesions, magnetic resonance imaging (MRI) is increasingly important. In MRI, acute tendon lesions appear hyperintense in T2- and T1-weighted sequences, whereas the signal intensity decreases especially in T2 sequences over time. In recent years, equine MRI examination became more feasible through a low-field MRI system (0.27 Tesla (T) MRI; Hallmarq Veterinary Imaging) that is suitable for standing, sedated horses. The examination in high-field MRI would offer an even more detailed insight, however, for 3 T MRI investigations, appropriate settings for equine tendons are not published yet.
In this study, equine superficial digital flexor tendons were dissected from limbs obtained from an abattoir. Based on macroscopic evaluation, tendons were chosen to be examined in the equine-dedicated low-field MRI system. Subsequently, 6 healthy and 6 chronically damaged tendons were also examined in a 3T MRI (Magnetom Verio; Siemens Healthcare GmbH) in horizontal position as usual for the high-field MRI, and in vertical position corresponding to the procedure in the equine system. Cross-sectional areas (CSAs) and signal intensities (SIs) were compared for tendons and lesions, respectively. For CSA and SI measurements of tendons and lesions, high correlations were evident between both T1 sequences used in high-field (T1 spin echo (SE) and T1 fast low angle shot 2d (fl2d)) and the T1 gradient echo sequence used in low-field MRI. Differences were shown in the detection of tendon lesions, which was better in T1 fl2d than in T1 SE sequence and better in vertical than in horizontal position, with sensitivities of 88.79% and 51.72% in the horizontal position and 100% and 91.38% in vertical position, respectively. Consequently, the orientation to the magnetic field is of relevance as well, suggesting that not only the different composition of lesions compared to healthy

tissue causes hyperintensity, but also the altered fibre orientation.

T120

INVESTIGATION OF THE TIME COURSE OF DES-ARGININ9-BRADYKININ, ANGIOTENSIN II, AND MITOCHONDRIAL DNA IN CRITICALLY ILL COVID-19 PATIENTS

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The severe acute respiratory syndrome coronavirus (SARS-CoV-2)-induced pandemic is affecting health care systems worldwide with the corona virus disease 2019 (COVID-19). Bradykinin and its metabolite des-arginin9-bradykinin (DA9B) induce vasodilatation and increased vascular permeability. DA9B is demounted by ACE2, which is downregulated by SARS-CoV-2. Therefore, elevated levels of DA9B could augment the course of COVID-19. Thus, the time course of DA9B of COVID-19 patients was analysed after the admission to the intensive care unit (ICU).
This single-centre, prospective, observational, proof-of-concept study included 29 critically ill patients with COVID-19 and 29 control patients, matched by age, gender and pre-existing conditions. DA9B and angiotensin 2 (AT-II) levels were analysed by enzyme-linked immunosorbent assays, and NADH-ubiquinone oxidoreductase chain 1 (ND1) mitochondrial DNA (mtDNA) was evaluated by quantitative PCR. While blood withdrawal was performed during the first 24h after admission to the ICU, 24h and 72h thereafter, respectively, blood was collected from control patients only once.
After admission to the ICU significant elevated levels of DA9B, AT-II and ND1 mtDNA were measured compared to the control group (DA9B: 4,158 ± 2,761 vs. 1,206 ± 1,180 pg/ml, $p < 0.001$; AT-II: 302 ± 281 vs. 122 ± 50 pg/ml, $p = 0.002$; ND1 mtDNA: 1,099 ± 1,993 vs. 73 ± 54 copies/µl, $p = 0.010$). Further, no significant changes between the different time points were analysed within the COVID-19 group regarding DA9B, AT-II and ND1 mtDNA.
The increased levels of ND1 mtDNA may reflect the acute pro-inflammatory state of the COVID-19 patients. Since elevated concentrations of DA9B were measured, blockade of the DA9B B1 receptor will offer new therapeutic strategies for these patients.

T121

PLATELET LYSATE IN THE CULTIVATION OF MESENCHYMAL STROMAL CELLS: ARE THERE DIFFERENCES BETWEEN HORSES AND DOGS?

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Orthopedic diseases are very common in equine and canine medicine. However, their treatment remains a clinical challenge. The treatment of equine tendon diseases and canine osteoarthritis is currently in the focus, because the conventional therapy is very protracted and the reinjury rates are high. For these relevant orthopedic problems, regenerative therapy has gained attention and especially the application of cell-based therapeutics has obtained tremendous interest. Clinical application of multipotent mesenchymal stromal cells (MSC) requires prior *in vitro* cultivation. Fetal bovine serum (FBS), which is the current gold standard as a basal medium supplement for the cultivation of many cell types, including equine and canine MSC, is afflicted with several problems. Different alternatives are being tested, with promising results using platelet lysate in human MSC culture. The aim of this study was to establish a standardized protocol for equine and canine platelet lysate (ePL and cPL) production and to test the ePL and cPL in equine and canine MSC culture. For ePL production, whole blood was collected from 20 horses and for cPL production from 12 dogs and processed into platelet concentrates using a buffy coat method. In these concentrates, the cells were lysed by freeze/thaw cycles. The pooled ePL and cPL from all donors were evaluated as culture medium supplement in comparison with FBS, using equine and canine adipose-derived MSC. The ePL was suitable for equine MSC culture. In passage 5 equine MSC, karyotype analyses by GTG-banding suggested a higher genetic stability in the MSC cultured with ePL. However, canine MSC showed an altered phenotype with cPL compared with MSC in FBS-supplemented medium, and differentiation of canine MSC also displayed differences to equine MSC. Further analyses into MSC functionality, viability, senescence and genetic stability will reveal whether cPL is still a viable alternative to FBS for canine MSC culture.

T122

PREVALENCE OF NEUROPATHIES IN RHEUMATIC AND MUSCULOSKELETAL DISEASES

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Introduction:
In rheumatic and musculoskeletal diseases (RMDs), peripheral neurons can be affected, which can result in sensory symptoms like pain, burning, tingling, numbness and motor symptoms like muscle-atrophy or even paresis. More detailed knowledge about the prevalence and the cause of neuropathy (NP) in RMD are urgently needed, especially as RMD patients may develop different subtypes of NP. The aim of this project was to assess the prevalence and the individual types of NP in rheumatoid arthritis (RA), spondyloarthritis (SpA) and systemic sclerosis (SSc) patients, and to elucidate the clinical, neurophysiological and neuropathologic features of associated NP.
Methods:
Baseline questionnaires and neurological and physical examination were used to elucidate the presence of neuropathic pain and autonomic dysfunction. Laboratory tests were performed to exclude other causes for NP. Electrophysiological tests were performed to differentiate demyelinating from axonal large fiber (LF)NPs. Additionally, skin biopsies were used to detect an involvement of small fibres (SF). We plan to include 100 patients and 15 healthy persons in this study.
Results:
A total of 64 patients and 4 healthy persons were already included (median age 60 years (range 22–88)). The majority of the patients were female (78%). The mean disease duration was 9 years (1–46 years). More than 50% of the patients were diagnosed with RA, 17 with SpA and 10 with SSc. Data of 51 patients were already evaluated. Neurophysiological and neurological examination showed 24 axonal, 2 demyelinating, 6 mixed types and 12 patients with only clinical signs of NP. A combined LFNP and SFNP was present in 21 of the patients. In 5 patients, only a SFNP was detectable, and in only two patients, no NP was detectable.
Conclusions:
NP was detectable in 96% (49/51) of the RMD patients, with LFNP predominating. This high proportion of NP in RMD suggests a surprisingly high coincidence of both diseases.

T123

EX VIVO BONE ORGAN CULTURE MIMICKING DEFECT HEALING *IN VIVO*

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Bone healing is a complicated process that goes through subsequent overlapping phases. Bone research is most commonly conducted through preclinical animal models and *in vitro* studies. However, *in vitro* systems fail to preserve bone integrity as seen in living bone. *In vivo* methods cause significant pain and distress in the animals. This study aimed to establish a whole bone organ culture to allow investigation of bone healing in a controlled manner that closely mimics the *in vivo* processes.

Long bones were collected from rats under sterile environment. Drill hole in the metaphyseal region was created in all samples. Additionally, a drill hole defect filled with anti-microbial paste was used to investigate the changes. *Ex vivo* culture was maintained in an incubator at 37 °C and 5% CO_2. Culture medium and cell proliferation around the bones were monitored. Bones were harvested at day0 and day7. Histology and chemical analysis using Time of flight-Secondary Ion Mass Spectrometry (Tof-SIMS) was carried out.

The daily monitoring of *ex vivo* cell culture revealed the presence of numerous inflammatory cells during the early time points. Mesenchymal stem cells were seen in the culture medium around the whole bone organ. Whole bone organ was alive until the end

of day7. Histology showed the presence of erythrocytes in the bone marrow around the drill hole defect. Moreover, more tissue were formed within the hole as a response to the fracture healing process. Tof-SIMS showed the diffusion of anti-microbial paste in the bone marrow.

The preliminary results showed the survival of the whole bone organ in the *ex vivo* condition until day7. Further trials are going on to keep bone alive after day7 through the supply of required nutrients. Moreover, the project is also developing to find alternatives through 3D bioprinting.

T124

TISSUE-RESIDENT ALVEOLAR MACROPHAGES ARE CHARACTERIZED BY SUBSTRATE FLEXIBILITY AND A SUBORDINATE ROLE OF METABOLISM FOR INFLAMMATION

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Macrophages are key effector cells of the immune response and form a crucial line of defence against pathogens. Recently, novel insights into cell metabolism have demonstrated a strong link between cellular metabolic pathways and immune effector function. In particular, the breakdown of glucose by way of glycolysis has been tied to a pro-inflammatory state. However, much of the data have been derived from murine bone marrow-derived macrophage (BMDM) models and the applicability to other macrophage populations, in particular tissue-resident ones, is not clear. Many tissue-resident macrophage populations exist in vastly different metabolic environments, suggesting that cellular metabolic regulation of inflammation may be diverge from bone-marrow derived counterparts.

Tissue-resident alveolar macrophages (TR-AMs) are the most abundant immune cell in the lungs and are an important component of a regulated and dysregulated response to infection and inflammation. TR-AM dysfunction is implicated in the pathogenesis of lung infections and inflammatory disorders such as the acute respiratory distress syndrome (ARDS). We analysed TR-AM metabolism in detail to understand its role for the energetic and immunologic phenotype of the cell with the ultimate hope of therapeutically targeting cell metabolism and augmenting inflammation. We were able to demonstrate that TR-AMs possess a highly unique metabolic profile that differs significantly from BMDM metabolism. Most importantly, TR-AMs show a high degree of substrate flexibility and readily adapt to pathway inhibition. The close link between metabolic phenotype and cellular inflammatory function seen in BMDMs was not evident in TR-AMs. This may result from the unique metabolic niche TR-AMs occupy (rich in oxygen, lacking in glucose) or the different cell lineage compared with circulating monocytes and BMDMs. Our results question the link between macrophage metabolism and effector function observed in BMDMs for tissue niche macrophages and caution against the overly broad application of findings across macrophage populations.

T125

CROSSTALK BETWEEN CELLULAR HYPOXIA SENSING AND HISTONE METHYLATION SYSTEMS AND ITS EFFECT ON CANCER PROGRESSION

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A key driver of cancer progression is the ability of tumour cells to adapt to different microenvironments,

such as reduction of oxygen availability (hypoxia), which is an intrinsic consequence of the rapid proliferation of tumour cells. Adaptive responses to hypoxia activate key hallmarks of cancer, including tumour cell plasticity and proliferative and survival signalling, which are closely linked to the regulation of gene expression through epigenetic mechanisms. Currently, many details remain unknown about how the tumour microenvironment and cell-intrinsic mechanisms control epigenetic regulatory pathways in different cancer settings that promote tumour invasion and metastasis.

A previous small loss-of-function screening study of histone methyltransferases and histone demethylases revealed a novel regulatory link between the H3K27-specific histone methyltransferase enhancer of zeste homolog 2 (EZH2) and hypoxia-inducible factors (HIFs). Recent evidence suggested that regulation occurs, at least in part, at the gene expression level and exhibits variation between breast and lung carcinoma and glioblastoma cell lines. Moreover, we observed that the enzymatic activity of EZH2 might have been dispensable regarding its HIF regulatory function upon chemical inhibition of EZH2. In addition to the *in vitro* EZH2-HIF experiments, immunohistochemical staining of H3K27me3 in glioblastoma patient-derived sections showed local imbalances of H3K27me3 in hypoxic tumour areas, similar to previously reported EZH2 patterns. Collectively, these initial observations strongly suggest a connection between hypoxia and EZH2-dependent gene regulatory mechanisms in cancer cells.

This project will identify how EZH2 regulates HIF under hypoxia and elucidate the crosstalk between cellular hypoxia sensing and histone methylation systems and its effect on tumour progression *in vitro* and *in vivo*.

T126

CANINE WHOLE BLOOD STORED AT 4°C PROVIDES HEMOSTATIC ACTIVITY FOR 15 DAYS.

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Administration of blood products is an essential part of resuscitation in dogs with severe trauma to ensure oxygen delivery to the tissue. In the past, whole blood (WB) stored at 4°C was assumed to considerably lose its hemostatic activity compared to fresh whole blood immediately. However, recent data in people suggest adequate coagulation activity of WB stored 21 days at 4°C. Thus, our hypothesis was that canine WB stored in in Citrate Dextrose phosphate (CPD) as anticoagulant (WB-CPD) and WB-CPD with the addition of a commercial solution containing phosphate, adenine, glucose, guanosine, saline and mannitol (PAGGS-M) would equally provide adequate hemostatic activity for 21 days. We collected 450ml WB from 21 canine donors. Blood of three dogs donating at the same day was pooled to overcome inter-individual heterogeneity in platelet (PLT) function. The pooled WB was divided into 2 aliquots. Aliquot 1 (WB) was left unchanged and assigned to group 1 (n=7 aliquots) and in aliquot 2, PAGGS-M was added and assigned to group WB-PAGGS (n=7 aliquots). In both groups, aliquots were stored at 4°C in vertical position and analyzed for the following 28 days. On day 0, 1, 3, 5, 10, 15, 21, 28, samples were drawn aseptically and analyzed to assess PLT number and function as reflected by the area under the aggregation curve (AUC) obtained with impedance aggregometry. Global coagulation activity was assessed with whole blood thrombelastography (TEG). Further analyses regarding storage lesion were instituted including microbiological culture testing. In both groups was a significant decline in AUC within the first 5 days (mean -885 and - 33%, $p < 0.001$). After that, PLT function remained stable until day 15. TEG parameters were stable for 15 days. All microbiological cultures were negative. In conclusion, canine whole blood can be stored for 15 days for patients with acute hemorrhage.

T127

WHEN STRUCTURE MATTERS AND MATTER STRUCTURES: NANO-3D-PRINTED BIPHASIC POLYMER SCAFFOLDS IN A 3D CELL CULTURE MODEL

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Osteochondral lesions (OCLs) can occur due to trauma, or can be caused by chronic degenerative processes, such as osteoarthritis, which is the most common joint disease [1] and affects more than 242 million people worldwide [2]. According to the Robert Koch Institute, 17.9% of adults in Germany over 18

are afflicted. For those aged 65 and over, the prevalence is considerably higher, at 48.1% in women and 31.2% in men [1].
Within the framework of the "Poly-Implant-Druck" project, a collaboration of five research institutes, a novel therapeutic approach for OCL has been defined. In particular, biphasic but yet monolithic nano-3D-printed polymer scaffolds were designed to mimic the ideal geometric and mechanic properties of bone and cartilage tissue respectively. Additionally, bioactive fillings create favourable conditions for the ingrowth of local stem cells and, thereby, the integration of an implant into the surrounding healthy tissue.
Preceding *in vitro* experiments, carried out within the scope of another doctoral thesis, indicated a good cytocompatibility of the osteo-phases in these scaffolds with human mesenchymal stem cells. However, since cartilaginous tissue exhibits unique properties and demands, a specialised and innovative 3D-cell culture system was designed for this study. Micro pellets obtained by this process were cultivated under particular conditions, meeting the hypoxic needs of cartilage, by applying the chemical component Dimethyloxaloylglycine. Results of these experiments, which employ cutting-edge techniques, are of key importance for the animal study, embedded within this project. Preliminary findings in a 2D-cell culture system suggest promising results in the 3D-cell culture model. First data will be available by the time of the 2021 annual GGL conference taking place.
In conclusion, the "Poly-Implant-Druck" project offers an innovative and promising therapeutic approach for treating one of the major health issues in our growing and aging society.

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T128

RHO/ROCK INHIBITION PROMOTES TGF-B3-INDUCED TENOGENIC DIFFERENTIATION IN MESENCHYMAL STROMAL CELLS

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Tendon injuries are frequently occurring diseases in horses and humans. Due to hypocellular and hypovascular properties, the tendon has only a very low regenerative capacity. Therefore, mesenchymal stromal cells (MSC) represent a promising therapeutic tool for tendon regeneration. Their tenogenic differentiation is crucial for tissue engineering approaches and may support their beneficial effects after cell transplantation *in vivo*. The transforming growth factor (TGF)-β, which is signalling through intracellular smad molecules, is a potent paracrine mediator of tenogenic induction. Moreover, scaffold topography or tendon matrix components induced tenogenesis via activation of the Rho/ROCK cascade. The aim of this study was to investigate the interplay of Rho/ROCK and TGF-β3/smad signalling in tenogenic differentiation in both human and equine MSC.
Primary equine and human MSC isolated from adipose tissue were cultured as monolayers or on tendon-derived decellularized scaffolds to evaluate the influence of the ROCK inhibitor Y-27632 on TGF-β3-induced tenogenic differentiation. The MSC were incubated with and without TGF-β3 (10 ng/ml), Y-27632 (10 μM), or both. On day 1 and day 3, the signalling pathway of TGF-β and the actin cytoskeleton were visualized by smad 2/3 and phalloidin stainings, and gene expression of signalling molecules and tendon markers was assessed.
ROCK inhibition was confirmed by disruption of the actin cytoskeleton. Activation of smad 2/3 with nuclear translocation was evident upon TGF-β3 stimulation. Interestingly, this effect was most pronounced with additional ROCK inhibition in both species ($p < 0.05$ in equine MSC). In line with that, the tendon marker scleraxis showed the strongest upregulation when TGF-β3 and ROCK inhibition were combined ($p < 0.05$ in human MSC). The regulation pattern of tendon extracellular matrix components and the signalling molecules TGF-β3 and smad 8 showed differences between human and equine MSC.
The obtained results showed that ROCK inhibition promotes the TGF-β3/smad 2/3 axis, with possible implications for future MSC priming regimes in tendon therapy.

T129

ROLE OF BILE ACID PROFILING AND COBALAMIN STATUS AS NON-INVASIVE BIOMARKERS IN DOGS WITH CHRONIC LIVER DISEASE

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Acknowledgement of chronic liver disease in dogs by non-invasive measures is simple, but determination of its aetiology usually requires invasive methods such as liver biopsy. In humans, bile acid and faecal microbiota profiling are promising not only to allow differentiation between health and liver disease but more importantly to discriminate different liver diseases. Thus, they could function as non-invasive diagnostic tools to identify the underlying aetiology of a diagnosed chronic liver disease. Liver disease can be accompanied by elevated vitamin B12 (cobalamin) levels (hypercobalaminaemia). Whilst clinical signs of cobalamin deficiency are well-known, signs of hypercobalaminaemia are insufficiently characterised. Among them, paradoxical signs of hypocobalaminaemia are described in patients with evidence of cellular cobalamin deficiency despite hypercobalaminaemia. In dogs, little is known about hypercobalaminaemia and its role in liver disease. Along with bile acid profiling, cobalamin status might play a role as non-invasive biomarker to distinguish different types of liver disease. Hypothesis is that dogs with chronic liver disease have derangements in their cobalamin status and that different types of liver disease cause different clusters of bile acid and faecal microbiota profiles.
Aims of this project are to determine cobalamin status, bile acid, and faecal microbiota profiles in dogs with different types of liver disease and to assess their role in canine chronic liver disease as diagnostic tools. Thirty dogs with chronic liver disease and 30 dogs with portosystemic shunt will be included. For cobalamin metabolites (measured in blood and urine), reference values in 120 healthy dogs will be established beforehand. Bile acids of dogs with liver disease are examined in blood, urine, and faeces using liquid chromatography mass spectrometry. Faecal microbiota profiling will be examined by 16 sDNA sequencing using QIIME2 software.

T130

INFLUENCE OF PREOPERATIVE FUNCTIONAL STATUS ON PERIOPERATIVE IMMUNE RESPONSE AFTER MAJOR VISCERAL SURGERY – WHAT THE SURGEON HAS TO DO WITH MITOCHONDRIA

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Preoperative physical condition, especially frailty and sarcopenia are well known contributors to poor postoperative outcome of patients following major abdominal or thoracic surgery. Enhanced physical status correlates with mitochondrial amount in muscle cells and function and it modulates the immune response to adopt a more anti-inflammatory phenotype. Along this line mitochondrial malfunction was related to sepsis-induced immune dysfunction. However, the impact of steril surgical trauma on the perioperative mitochondrial profile of peripheral blood mononuclear cells (PBMCs) and correlation with markers of systemic inflammation has been poorly investigated by now.

PBMCs were collected perioperatively from 15 patients admitted to major abdominal surgery. Maximal respiratory capacity was increased specifically in the five patients who experienced postoperative complications already 0–2 h after surgery. Along this line also the pro-inflammatory cytokines Interleukin (IL)-6 and IL-8 were increased 0–2 h after surgery in these patients with complications compared to pre-surgery and patients without complications, as were the values for peripheral blood leukocytes and the C-reactive protein at later time points. Proteins for mitochondrial fission and fusion processes were decreased in western blot analyses at 0–2 h after surgery, indicating impaired mitochondrial dynamics postoperatively.

These data suggest an increased mitochondrial respiration as an early marker of inflammation as well as for the development of postoperative complications after major abdominal surgery. In contrast, mitochondrial dynamics is impaired following major surgical trauma. As next step it will be investigated if preoperative physical status of our patients subjected to major abdominal surgery can influence perioperative

mitochondrial function, systemic inflammation and clinical outcome.

T131

MICROENVIRONMENTAL CONTROL OF LUNG CANCER METASTASIS

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Lung Cancer (LC) is the overall leading cause of cancer death in humans and the main cause of mortality is metastasis. Non-small cell lung cancer (NSCLC) accounts for 85% of LC and 25–30% of NSCLC patients develop brain metastases.
Increasing evidence has linked LC with other chronic lung diseases such as chronic obstructive pulmonary disease (COPD), which is an independent risk factor for LC and the mortality is correlated with the co-existence of COPD. The high prevalence of LC with COPD may related with premature ageing in the lungs, genetic predispositions to either disease, or common pathogenic factors, such as hypoxia or inflammation. On the other hand, the connection between Pulmonary Hypertension (PH) and LC is less well understood. Hypoxia and inflammation in local tumour microenviroment (TME) influence pre-metastatic niche formation and tumour plasticity for the adaptation to the metastatic organ microenvironment. However, the link between systemic hypoxia and inflammation-induced microenvironmental changes and the severity of LC and metastasis is unclear. Therefore, we aim to discover the mechanisms by which chronic lung diseases that frequently co-occur with LC promote a (pre)metastatic niche permissive for lung and brain colonization through intra- and inter-organ communication with using PH and COPD disease models.
As a preliminary study design, we established an *ex vivo* model to investigate the mechanisms of different brain microenvironments on metastatic growth. For this, we optimized the conditions for tumour sphere generation and brain slice co-culture of various LC cell lines. Furthermore, we have started to generate TME mapping tools for cellular and molecular characterization of the early and pre-metastatic niches in experimental PH/COPD settings.
This project will reveal the contribution and mechanistic link of chronic lung diseases to the metastasizing capacity of LC to the brain and within the lung.

T132

EVALUATION OF ANTI-MOUSE AND ANTI-RABBIT IGG NANOBODIES CONJUGATES WITH SNAP-TAG AS SECONDARY ANTIBODIES IN IMMUNOFLUORESCENCE

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As a part of diagnostics or immunotherapeutic, antibodies and antibody fragments have several important functions, including their specificity, serum half-life, immunogenicity, folding-stability, and especially the binding affinity. In immunoassays, Immunoglobulin G(IgG) can detect the primary antibodies which bind to target molecules, then amplify the primary signals. Thus, IgG is the common secondary antibody. Because of difficult recombinantly, IgG modified antibodies must be obtained from living animals or mammalian cell culture systems, high manufacturing costs and time consumption are also challenging. Heavy-chain antibodies (HCAbs), have been described in species derived from camelid. Since they are composed of two identical heavy chains and are devoid of light chains, their antigen-binding part is composed of only one single immunoglobulin (Ig) variable region (VHH or Nanobody), this makes nanobodies are merely 1.5 ×2.5 nm and 13 kD in size. Moreover, nanobodies also can be easily expressed functional and high yields in bacteria and mammalian cells, even easily modified at the genetic level for labeling purposes in many ways. Additionally, our group has established an innovative enzymatic site-specific conjugation strategy based on the SNAP-tag technology, so we are aiming to extend the application of SNAP-tag technology and nanobodies which have properties of anti-mouse and anti-rabbit IgG to develop a sustainable alternative secondary antibody for immunofluorescence assays.
We generated anti-IgG-SNAP fusion proteins as secondary antibodies (nano-anti Mouse IgG1 Fab-SNAP, nano-anti Mouse IgG1 Fc-SNAP, nano-anti Mouse IgG2a Fc-SNAP, nano-anti Mouse IgG kappa chain-SNAP, nano-anti Rabbit IgG Fc-SNAP), then labeled them with fluorescence dyes. To validate their specific binding as secondary antibodies by flow cytometric analysis, from our current results, nano-anti Mouse IgG kappa chain-SNAP showed good specificity with EGFR antibody and CD326 antibody.

T133

INFLUENCE OF LEPTIN AND COMPRESSION IN GAS-6 MEDIATED HOMEOSTASIS OF PERIODONTAL LIGAMENT CELL

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Growth arrest-specific protein 6 (GAS-6) regulates several immunomodulatory and inflammatory mechanisms in different tissues including bone. Clinical studies suggest that GAS-6 levels may be directly involved in obesity predisposition. This study aimed to determine whether GAS-6 is associated with the homeostasis of periodontal ligament (SV-PDL) cells in the presence of adipokines or compressive forces.
The SV-PDL cell line (M. Somerman, NIH, USA) was used. Western blots were employed for TAM receptors detection. Cells were stimulated using different concentrations of GAS-6. The migration and proliferation were measured by a standard scratch test and MTS assay. The mRNA expression was analyzed by RT-PCR. Release of TGF-β1, GAS-6, Axl were verified by ELISA.
Western blot showed that TAM receptors are expressed in SV-PDL cells. GAS-6 has a promoting effect on cell migration and proliferation. RT-PCR analysis revealed that GAS-6 induces Collagen-1, -3, Periostin and TGF-β1 mRNA expression whereas it reduces Caspase-3, -8, -9 and IL-6 mRNA expression. Furthermore, secreted GAS-6 in SV-PDL is reduced in response to both compressive forces and leptin and upregulated by IL-6. Additionally, ADAM-10 inhibition reduces GAS-6 and Axl release on SV-PDL cells. Periodontal ligament cells express TAM receptors and release GAS-6 protein *in vitro*. The GAS-6 feedback mechanism on SV-PDL cells is influenced by leptin as well as by compressive forces. ADAM-10 metalloprotease blockade can effectively reduce GAS-6 and Axl release. GAS-6/TAM interactions contribute to periodontal ligament cells homeostasis. Leptin inhibits the GAS-6 release independently of ADAM-10 metalloprotease.

T134

SNAP-TAG BASED ANTIBODY DRUG CONJUGATES IN PRECISE TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER

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Female breast cancer is the fifth leading cause of cancer mortality, and has now surpassed lung cancer as the leading cause of global cancer incidence in 2020. Triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2), which causes the poor efficiency of targeted therapy based on these antigens. Antibody-drug conjugates (ADCs) are a novel antitumor therapy, which combines specificity of monoclonal antibody and chemotherapy. Unfortunately, the generation of current ADCs are relying on using random conjugation methods. These methods generate heterogeneous products, resulting in inconsistent pharmacokinetic, efficacy and safety profiles.
To overcome these limitations, we took the advantages of enzymatic site-specific conjugation strategy based on the SNAP-tag technology to produce homogeneous ADCs.
The epidermal growth factor receptor (EGFR), the epithelial cell adhesion molecule (EpCAM) and chondroitin sulfate proteoglycan 4 (CSPG4) were overexpressed in TNBC. Therefore, we generated three single chain antibodies which are linked with SNAP-tag genetically, namely scFv-425 (anti-EGFR)-SNAP, scFv-anti-EpCAM-SNAP and scFv-anti-CSPG4-SNAP. SNAP-fused proteins were expressed by HEK293T cells, and then purified from the supernatant using a Ni-NTA Superflow cartridge. The targeting activity of the single chain antibodies was confirmed by flow cytometry and confocal microscopy. To determine its therapeutic properties, antibodies were conjugated with cytotoxic agent Amino-PEG4-Val-Cit-PAB-MMA. The cell viability and induction of programed cell apoptosis were determined using XTT cell viability assay and caspase assay.

Index of GGL Supervisors

Section 1

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