Kompetenznetzwerk NRW

2nd Annual Meeting

December 5th - 6th, 2024

Kasteel Bloemendal, Vaals, Netherlands

Abstract Book

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Welcome

Dear Attendees,

We are delighted to welcome you to the 2nd Annual Meeting of the 3R Competence Network NRW, taking place on December 5th and 6th, 2024, at Kasteel Bloemendal, Vaals, Netherlands. Following the success of our inaugural event last year at Kasteel Bloemendal, we are excited to gather once again under the motto "**Science for Tomorrow: Redefining Research with the 3Rs**" to continue our shared mission of advancing medical progress with the highest standards of animal welfare.

Over the past year, our network has grown and made significant strides in our four main areas of focus: science management, networking, education, and communication. This meeting provides an invaluable opportunity to reflect on these achievements, explore new developments in the field, and strengthen the connections that are the foundation of our network.

This year's program is designed to inspire and engage you. Over the course of 1.5 days, you will have the chance to hear from distinguished experts in 3R research, participate in thought-provoking sessions, and share your own work with peers. Besides a keynote lecture and sessions on Replacement, Reduction, Refinement and New Thinking in 3Rs, we will dedicate a session for short talks from selected abstracts.

Our event will also feature a poster session, where you can present your research and exchange ideas in a collaborative setting. On the evening of December 5th, we invite you to join us for a special Get Together at the Tivoli Business & Event in Aachen. This evening promises to be a highlight, celebrating excellence in our field while providing an opportunity to network in a more informal setting.

We are truly excited to convene with you all, to share knowledge, foster collaborations, and continue building a strong, impactful network. There are many reasons to be part of this event, and we are very much looking forward to seeing you there!

Best regards,

3R Competence Network NRW Organizing Team

About

3R Kompetenznetzwerk NRW

Our goal: medical progress in line with the best possible animal welfare

The eight faculties of medicine of the universities in North Rhine-Westphalia have formed a 3R Competence Network. In line with the 3R principle, the network aims to enable (bio-) medical progress in NRW in line with best animal welfare and to specifically support research, innovation and training in the field.

Our vision for the state of North Rhine-Westphalia is that the structural and spatial integration of animal experimentation units in 3R centers will become a catalyst for innovation to reconcile urgently needed medical progress with the best possible animal welfare. For experimental research, direct and constant face-to-face exchange among all stakeholders is of key importance. In our view, the creation and implementation as well as consequent improvement of integrative 3R concepts is a central key to success.

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3r-netzwerk-nrw@ukbonn.de

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Network Manager
Michael To Vinh

michael.tovinh@ukbonn.de

Team Assistant
Antonia Henderson

antonia.henderson@ukbonn.de

Network Coordinator
Lena Kistermann

lkistermann@ukaachen.de

Public Relations
Burak Bali

burak.bali@ukbonn.de

Program

Day 1 | Thursday, December 5th, 2024

08:00 **Registration**

09:00 **Welcome Ceremony**

09:30 **Session 1:** Refinement chaired by Branko Zevnik (Cologne)

Stephanie Krämer (Giessen) - "Expanding the Culture of Care in animal research"

Daniel Segelcke (Münster) - "Innovative Approaches to Identifying Behavioral Markers of Animal Welfare in Rodent Models: Advancing 3R Principles through AI and Machine Learning"

Susanne Wiemann (Bielefeld) - "Oral Application of Tamoxifen in Transgenice Mice"

Dirk Isbrandt (Cologne) - "3R in neurophysiology and experimental epilepsy research"

10:50 -Coffee Break-

11:10 **Session 2:** New thinking in 3Rs chaired by Marieta Toma (Bonn)

Kim Wever (Nijmegen) - "Dealing with image duplications in animal research publications"

Steven Talbot (Hannover) - "Improving Laboratory Animal Welfare with Advanced Data Science Methods"

Fernando Gonzalez Uarquin (Mainz) - "Internal communication in animal facilities for 3R progress"

Elisabeth Jurack (Bonn) - "Does understanding the 3R principle help to improve the acceptance of animal experiments? A representative survey across Germany"

13:00 -Lunch Break-

14:00 **Session 3:** Selected short talks chaired by Christine Hartmann (Münster)

Inês Cabrita (Cologne) - Kidney Organoids: Remarkable Tissues, but Not True Mini-Kidneys

Johanna Maria Hölken (Düsseldorf) - Incorporating immune cell surrogates into a full-thickness tissue equivalent of human skin to characterize dendritic cell activation

Katrin Becker (Bonn) - Application of structure equation models to reduce animal numbers in animal experiments

Day 1 | Thursday, December 5th, 2024

14:40 **Session 3:** Selected short talks (continued)

Sven Fengler (Bonn) - Human iPSC-derived brain endothelial microvessels in a standardized microphysiological system as blood-brain barrier model for drug permeability screens

Nora Rautenberg (Cologne) - Transcranial Direct Current Stimulation (tDCS) in Awake Mice: a flexible model to foster regeneration in various models of neurological disorders

Leonie Tix (Aachen) - Matching suitable lab animals to private pet owners - a key to success

15:20 **Poster Session 1** (Odd Numbers) & Coffee Break

16:20 **Session 4:** Reduction chaired by Matthias Schmidt (Bochum)

Kalliopi Pitarokoili (Bochum) - "Marker of neurodegeneration, neuroprotection and neuroinflammation in cell cultures"

Anna Stettner (Bonn) - "Using the CAM Assay to investigate the behavior of endothelial-like cells in a 3D tumor model"

Barbara Grüner (Essen) - "DNA Barcoding as a means to reduce and refine in vivo drug testing in cancer"

Julia Steitz (Aachen) & Alexandra Bannach-Brown (Berlin) - "Development of a Systematic Online Living Evidence Summary (SOLES) for Animal Models testing Targeted Therapies against Cancer"

17:40 -Wrap-up Day 1-

17:50 End of scientific program of Day 1

19:30 **Social Evening Program**

19:30 Arrival at the Tivoli Business and Event

20:00 5th Aachen Animal Welfare Award Ceremony

20:45 Rejoin, Refresh and Rest

Program

Day 2 | Friday, December 6th, 2024

08:30 **Registration**

09:00 **Session 5:** Keynote Lecture chaired by René Tolba (Aachen)

Peter Bollen (Copenhagen) - "Trends and developments within the 3Rs in Denmark"

10:30 **Poster Session 2** (Even Numbers) & Coffee Break

11:40 **Session 6:** Replacement chaired by Björn Spittau (Bielefeld)

Saif-Eldin Abedellatif (Bonn) - “Melanoma Brain Metastases Patient-Derived Organoids: An In Vitro Platform for Drug Screening”

Henner Koch (Aachen) - "Human Organotypic Brain Slice Cultures: A Versatile Model System For Neurological Disease"

Florian Groeber-Becker (Düsseldorf) - "In vitro epithelial Models: At the crossroads between Biology - Engineering and Material Science"

Lena Thiebes (Aachen) - "Modelling the Human Airway Mucosa – A sophisticated In Vitro System for Airway Research"

13:00 - Wrap-up Day 2 & Closing Remarks -

13:15 End of scientific program Day 2

13:30 3R-Network Meeting (only for 3R centers)

15:00 End of the 2nd Annual Meeting of the 3R-Competence Network NRW

Keynote lecture

Keynote lecture

Peter Bollen

University of Copenhagen

Trends and Developments within the 3Rs in Denmark

The Danish 3R Center has existed since 2013. It was established by the minister of Food and Fishery, as a cooperation between the Danish Veterinary and Food Administration, Industry and Animal Protection organizations. Its aim was to disseminate the 3Rs to the research community and society, and to stimulate research within the 3Rs by making research grants available and assigning them to relevant high-quality projects. Even though the board of the 3R Center was equally composed of experts within all three areas of the 3Rs, initially a focus was placed on Refinement and Reduction, whereas Replacement was considered as replacing animals where they otherwise would have been used. Today, the focus of the 3R Center has shifted towards Replacement in a broader sense, and also animal free research has become an area of attention. The new vision of the Danish 3R Center is to work towards a future without animal research, bearing in mind that this is not immediately achievable. During the realization of this vision, continuous focus should be placed on Reduction and Refinement. The developments within the 3Rs in Denmark will be discussed and placed in an international context.

Abstracts | Talks

Expanding the Culture of Care in animal research

Stephanie Krämer

Justus Liebig University, ICAR3R-Interdisciplinary Centre for Animal Welfare Research and 3R, Gießen, Germany

The term Culture of Care (CoC) in animal-based research is understood as an appropriate behavior and an appreciative attitude towards all animals and employees. To successfully implement a CoC, all levels of an institution (management, science, care) should be involved through personal responsibility and a proactive attitude. However, an in-depth look at the different levels shows that the full implementation of a CoC has not yet been achieved to a sufficient extent.

Following on from the differentiated derivation of the historical anchoring and reception of the concept of CoC, the project concentrates on analyzing the characteristics of CoC in Germany. Qualitative social research was used to analyze the management level involved in animal experimentation, the scientific level and the care level.

The results show that the individual knowledge about the concept of a CoC results from the decisions of an actor at the respective level and from the given legal and organizational structures. As a result, CoC is understood as a complex mosaic of different categories: Organizational level, personality, science and animal welfare are main categories. These categories are underpinned by sub-categories such as implementation of the 3Rs concept, but also ethical concerns.

The investigations implies that “simple commitment” is not enough to achieve sustainable changes in the culture of animal-based research. A CoC can only be implemented if all levels involved break down old structures and rules and interact with each other. The CoC is therefore a prerequsite for all research involving animals.

Innovative Approaches to Identifying Behavioral Markers of Animal Welfare in Rodent Models: Advancing 3R Principles through AI and Machine Learning

Daniel Segelcke

Clinic for Anaesthesiology, Surgical Critical Care Medicine and Pain Therapy, University of Münster, Münster, Germany

This presentation explores innovative approaches to improving animal welfare in biomedical research through the identification of behavioral markers that indicate well-being. One of the key ethical responsibilities in animal research is to protect and enhance welfare, yet defining and measuring welfare remains a challenge. Various definitions focus on different aspects, including physical health, psychological well-being, and ethical treatment. Current tools for assessing animal welfare in laboratory rodents in real time are limited in number, lack consistency, and may cause stress to animals. Furthermore, species-specific differences and contextual factors, such as life stages or environmental conditions, complicate the standardization of welfare assessments.

Advanced video-monitoring systems, when combined with machine learning (ML) and artificial intelligence (AI), provide a promising solution for capturing and analyzing behavior in laboratory animals. This approach enables the identification of behavior outcome signatures linked to welfare. A severity-scoring system for surgical interventions and behavioral tests, such as the Forced Swim Test, will be discussed.

By utilizing ML and AI to monitor animal behavior, this methodology aligns with the 3R principles (Replace, Reduce, Refine), aiming to minimize animal suffering and reduce the number of animals used in research. The presentation emphasizes the potential of these innovative tools to transform welfare monitoring, improve animal care practices, and advance 3R research by providing accurate, real-time insights into animal well-being. These advancements should be integrated into routine experimental procedures, setting the stage for future welfare improvements in animal research.

Comparison of Tamoxifen Administration Routes in a Cre/loxP Mouse Model: A Comparative Study of Oral and Intraperitoneal Application

Susanne Wiemann

Bielefeld University, Medical School OWL, Anatomy and Cell Biology, 33615 Bielefeld, Germany

Introduction

Cre/loxP mouse models are essential tools for conditional gene manipulation, relying on tamoxifen to activate CreER recombinase for cell-specific gene recombination. Traditionally, tamoxifen is applicated via intraperitoneal (IP) injections or oral gavage, but tamoxifen administration via chow has emerged as an effective and less stressful alternative. Our comparative study explores the efficacy, advantages, and drawbacks of both routes — oral tamoxifen versus IP injections. Moreover, we are focusing on factors such as recombination efficiency and animal welfare.

Methods

In this study, we conducted a comparative analysis of tamoxifen administration routes using a Cre/loxP mouse model. Two groups of mice were used: one group received tamoxifen through two consecutive IP injections, and the other was provided with tamoxifen chow permanently. Further, experiment-specific criteria such as weight progression was closely monitored. After brain tissue preparation, recombination rate was determined to ensure that comparable recombination occurred between both application-routes.

Results

An initial weight reduction was observed in the mice that received tamoxifen chow, but their weight normalized after one week of treatment. Further, our analyses demonstrated that significant recombination could be achieved through chow-based tamoxifen administration, comparable to the results obtained with intraperitoneal injection.

Conclusion

In summary, tamoxifen administration via chow in Cre/loxP models provides more animal welfare and practical alternative to traditional IP injections, with comparable efficacy for inducing gene recombination.

3R in neurophysiology and experimental epilepsy research

Dirk Isbrandt

Institute for Molecular and Behavioral Neuroscience , University of Cologne & Head of DZNE Senior Research Group, Bonn

Developmental and epileptic encephalopathies (DEEs) are a genetically heterogeneous group of diseases with early-age disease onset and with limited treatment options, frequently resulting in a lifelong burden from developmental delay, intellectual disability (ID), behavioral abnormalities, and seizures. About one quarter of genes associated with DEEs encode ion channels, some of which, such as SCN2A, are also associated with neuropsychiatric disorders, suggesting common or overlapping pathophysiological mechanisms. Using mice hetero- or homozygous for a patient-derived gain-of-function Scn2a variant on two different genetic backgrounds, we recapitulated the clinical variability and obtained a gene dose-dependent phenotypic spectrum ranging from transient neonatal epilepsy without persistent sequelae to a chronic epilepsy phenotype with high mortality. To understand the pathophysiology underlying the complex phenotype, our multidisciplinary experimental strategy includes the quantification of neonatal, infantile, and adult in-vivo network activities correlated to the developmental transcriptomic trajectory of hippocampal maturation to identify the hippocampal CA3 hyperexcitability as an early driver of epileptogenesis in SCN2A-linked DEE.

I will detail the methods we implemented in my team to refine the permanent labeling of mouse neonates for early genotyping, describe refined cold anesthesia methods for acute depth recordings of mouse neonates, and explain how we use advanced recording technologies to reduce the number of mice needed to characterize their brain network patterns.

Dealing with image duplications in animal research publications

Kim Wever

Systematic Review Center for Laboratory Animal Experimentation (SYRCLE), Radboud University Medical Center, Nijmegen, Netherlands

Systematic reviews of animal studies are a powerful tool to support implementation of the 3R's. They are used to identify knowledge gaps, increase researchers' awareness regarding the design and quality of animal studies, and generate new insights without additional animal use (Menon et al., 2021). However, the reliability of systematic reviews is threatened by an increasing number of potentially fraudulent publications entering the scientific literature. Simultaneously, systematic reviews are uniquely positioned to detect particular warning signs of research misconduct, such as inappropriate image duplication, in a particular research field.

In a recent systematic review of animal models of early brain injury, we identified unexpected anomalies in the included studies' characteristics, which subsequently led to the detection of inappropriate image duplication in a large proportion of the studies. This investigation has so far resulted in several corrections and retractions. The planned review of the animal studies' outcomes could not be completed, as the compromised data prevented reliable evidence synthesis. A strategy for detecting an mitigating (suspected) fraudulent publications in the systematic review process is under development, to support the systematic review community in upholding systematic reviews as a valuable tool for 3R implementation.

Improving Laboratory Animal Welfare with Advanced Data Science Methods

Steven R. Talbot

Institute for Laboratory Animal Science,Hannover Medical School (MHH) Hannover, Germany

This presentation will explore key aspects of integrating data science methodologies into animal welfare assessment, emphasizing improving precision and objectivity in severity classification. The approach is grounded in analyzing, estimating, and predicting humane endpoints through a robust quantitative framework for relative severity assessment. This framework leverages multidimensional input data, such as physiological, behavioral, and clinical indicators, incorporating statistical techniques like multiple logistic regression and algorithms such as RELSA (Relative Severity Assessment). By utilizing these advanced analytical tools, the framework aims to categorize input data into data-driven severity classes, enabling more refined severity assessments. A central focus of this work is to enhance decision-making processes in laboratory animal science by fostering an evidence-based approach. This shift is crucial for ensuring that welfare assessments are more accurate, reproducible, and objective. The framework enables severity classifications to be conducted prospectively, in near real-time, and retrospectively, thereby supporting more dynamic and responsive welfare interventions. The proposed methods have practical applications in automated home cage monitoring systems, allowing continuous monitoring and rapid assessment without human intervention. Beyond laboratory settings, these approaches hold substantial translational potential, offering insights for broader preclinical and clinical research applications. Ultimately, integrating data science in animal welfare assessment represents a significant advancement toward more humane, scientifically rigorous, and ethically sound practices in laboratory animal science.

Internal communication in animal facilities for 3R progress

Fernando Gonzalez Uarquin

TARCforce3R, University Medical Center Mainz, Mainz, Germany

The reproducibility crisis refers to the problem of researchers to reproduce scientific studies. When animal experiments are performed, the responsibility for reproducibility is shared by different parties, which implies effective internal communication and collaborative approaches. Animal care staff, laboratory technicians, and early career researchers are fundamental cornerstones in this discussion. They are relevant drivers of reproducibility of scientific results, such as in the discussion on planning and conducting experiments. In this interactive presentation, I will discuss communicational gaps and hits with these three fundamental parties. I will end the talk by encouraging reflection on how communicating with these parties can improve reproducibility and mutual appreciation.

Does understanding the 3R principle help to improve the acceptance of animal experiments? A representative survey across Germany

Elisabeth Jurack

Medical Faculty, University of Bonn

Does knowledge of the 3R principle change attitudes towards animal testing? This question was the basis of a representative, Germany-wide survey conducted in summer 2024. This study investigated the extent to which information on the 3R principles in the form of a text improved attitudes towards animal testing. The control group read a general text on the topic of mice during the same period. A quiz was used to check whether the text on the 3R principles was understood. The results show that there are significant differences between the sexes with regard to animal experiments. It was also shown that there are significant differences between different age groups. The age group effect was purely due to the intervention, whereas the gender effect was also found in the control group. This study makes it clear that target groups have and develop different attitudes towards animal experiments after reading a text on the 3R principles. This makes it clear that the use of communication measures on the 3R principles should be applied in a target group-specific manner.

Kidney Organoids: Remarkable Tissues, but Not True Mini-Kidneys (POSTER ID 106)

Inês Cabrita[1,2], Vandit Shah[1,2], Thomas Benzing[1,2], Bernhard Schermer[1,2]

[1]Department II of Internal Medicine and Center for Molecular Medicine Cologne (CMMC), University Hospital Cologne, Germany; [2]CECAD, University of Cologne, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany

In recent years, significant advancements have been made in the field of 3D cell culture and so-called organoids. These are often referred to as "mini-organs," leading to the impression that they could replace animal experiments. Unfortunately, this impression does not yet reflect reality, and organoid technology represents a challenge in science communication.

In the field of kidney disease research, we use kidney organoids as the cell culture system that exhibits the highest degree of differentiation among cultured kidney cells. While these advancements can dramatically improve in vitro experiments and research, they unfortunately cannot serve as a complete replacement for animal experiments. In the case of the kidney, the complexity of the organ, with its perfusion-dependent filtration and tubule urine flow, is too high and these essential physiological features are missing in organoids. Moreover, the relevant vasculature for proper regulation of renal function, was not yet achieved. Nevertheless, kidney organoids significantly contribute to the reduction of animal testing and help refine and sharpen experimental questions.

Here, we present ongoing projects in kidney research to exemplify and discuss both the high value and the clear limitations of kidney organoids at this point in time.

Incorporating immune cell surrogates into a full-thickness tissue equivalent of human skin to characterize dendritic cell activation (POSTER ID 122)

Johanna Maria Hölken[1], Anna-Lena Wurz[1], Katja Friedrich[1], Patricia Böttcher[2], Dounia Asskali[1], Lars Vierkotten[2], Karsten Rüdiger Mewes[2], Nicole Teusch[1]

[1]Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University Düsseldorf; [2]Henkel AG & Co. KGaA

In the past decades studies investigating dendric cell (DC) activation have been conducted almost exclusively in animal models. However, due to species-specific differences in the DC subsets, there is an urgent need for alternative in vitro models allowing the investigation of Langerhans cell (LC) and dermal dendritic cell (DDC) activation in human tissue. We have engineered a full-thickness (FT) human skin tissue equivalent with incorporated LC surrogates derived from the human myeloid leukemia-derived cell line Mutz-3, and DDC surrogates generated from the human leukemia monocytic cell line THP-1. Topical treatment of the skin models encompassing Mutz-LCs only with nickel sulfate (NiSO4) or 1-chloro-2,4-dinitrobenzene (DNCB) for 24 h resulted in significant higher numbers of CD1a positive cells in the dermal compartment, suggesting a sensitizer-induced migration of LCs. Remarkably, exposure of the skin models encompassing both LC and DDC surrogates, revealed an early sensitizer-induced response reflected by increased numbers of CD1a positive cells in the epidermis and dermis after 8 h of treatment. Our human skin tissue equivalent encompassing incorporated LC and DDC surrogates allows the investigation of DC activation, subsequent sensitizer identification and drug discovery according to the principles of 3R.

Application of structure equation models to reduce animal numbers in animal experiments (POSTER ID 114)

Katrin Becker[1,2], Christian Arnold[2]

[1]University Hospital Bonn, Germany; [2]IST University of Applied Sciences, Germany

In animal experiments, causal relationships in physiological or disease processes are, e. g. analyzed by pharmacological interventions, surgeries or genetic manipulation of animals. For each intervention, an experimental group is needed. To reduce the required number of animals in line with 3R, we suggest that causal connections between variables can be calculated by structure equation modeling (SEM).

SEM is frequently used in social sciences, while in the field of veterinary medicine, this method has previously been used in breeding management of farm animals and in an ecotoxicity fish study. SEM is a second-generation statistical technique which allows comprehensive and flexible modeling to simultaneously calculate causal connections between constructs on different layers. Observed variables serve to define (formative model) or measure (reflective model) latent variables, which are processes that cannot be directly observed.

Limitations of SEM are strict requirements on the data sets and the theory-driven assessment of causal relationships. With the development of new approaches of the existing SEM technique, these limitations today can at least in part be circumvented.

SEM appears as a promising statistical tool for experimental animal research in three respects: First, pilot studies with low animal numbers could be used to identify important pathways, allowing their systematic investigation in a subsequent study. Second, more information could be obtained from a single animal; and third, the number of experimental groups could be reduced. This technique therefore might be an important contribution to reduction in line with 3R.

Human iPSC-derived brain endothelial microvessels in a standardized microphysiological system as blood-brain barrier model for drug permeability screens (POSTER ID 120)

Sven Fengler

German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Optimizing drug candidates for blood-brain barrier (BBB) penetration remains one of the key challenges in the field of drug discovery to finally target brain disorders including neurodegenerative diseases. Due to limitations of animal models in preclinical research which often poorly resembles human conditions, microphysiological systems (MPS) have been developed recently to mimic required physiological properties of human tissues in vitro to support drug development. However, it has been difficult to bioengineer human brain vessels which resemble physiological barrier properties and a perfused microvasculature in a format that is scalable to screen drugs for BBB penetration. To address this challenge, we combined MPS with human iPSC-derived brain microvascular endothelial cells (iBMECs) to establish a scalable brain microvessel in vitro model (1). In our process, iBMECs were differentiated in a standardized and automated workflow to facilitate a robust cell quality required for larger screens. After 10 days of culturing, perfused self-organized microvessels show typical BBB endothelial protein expression, tight-junctions and polarized localization of efflux transporter. Microvessels exhibited physiological relevant trans-endothelial electrical resistance (TEER), were leak-tight for 10 kDa dextran-Alexa 647 and strongly block the permeability of sodium fluorescein (NaF). Permeability tests with known reference compounds confirmed the suitability of our model as platform to identify potential BBB penetrating anti-inflammatory drugs. In summary, the here presented human in vitro model recapitulates relevant physiological properties and could thereby support brain permeability predictions of novel drugs. By using our approach, we will be able to preselect the most promising drug candidates already in the early pre-clinical phase which would help to significantly reduce the number of animal experiments or even replace them in future.

Ref.: (1) Fengler et al., Biomaterials 2022 (DOI: 10.1016/j.biomaterials.2022.121525)

Transcranial Direct Current Stimulation (tDCS) in Awake Mice: a flexible model to foster regeneration in various models of neurological disorders (POSTER ID 124)

N. Rautenberg[1,2], S. J. Blaschke[1,2], S. Vlachakis[1], M. Schroeter[1], G. R. Fink[1,2], M. A. Rueger[1]

[1]Department of Neurology, Faculty of Medicine, University of Cologne, Germany; [2]Cognitive Neuroscience, Institute of Neuroscience and Medicine (INM-3), Research Centre Juelich, Juelich, Germany

Transcranial direct current stimulation (tDCS) is a promising approach to accelerate regeneration and recovery in stroke, and preliminary data suggest beneficial effects after concussive brain injury (CBI) as well. However, the precise mechanisms of tDCS remain unclear, necessitating further research. Over recent years, we have refined a tDCS model originally developed for use in anesthetized mice, adapting it for its application in awake mice. This refinement eliminates anesthesia as a confounding factor to the underlying mechanisms, and therefore not only represents a key innovation in animal modeling, but also significantly enhances the model's potential for translational applications in human neurological research and therapy. In this refined model, a tube attached to the skull holds the stimulation electrode, while a cable connection from the neck provides a site for the reference electrode. The tube's positioning can be adapted according to the target brain region or specific setup requirements of the experiment, allowing stimulation of the fully conscious mouse in its home cage. We suggest using this model of awake tDCS in all future experimental studies of neurological disorders.

Matching suitable lab animals to private pet owners - a key to success (POSTER ID 111)

Leonie Tix, Lisa Ernst, Kira Scherer, Michaela Moss, René H. Tolba

RWTH Aachen, Germany

In accordance with legal regulations, the death of an animal should, whenever possible, not serve as the objective or endpoint of an experiment. If the continuation of life is intended, appropriate measures must be implemented to ensure that the animals receive optimal care and wellbeing. One approach is rehoming of the animals to private pet owners. In Germany, rehoming of laboratory animals by private persons is regulated in § 10 of the Animal Welfare Experimental Animals Ordinance (TierSchVersV).
However, the extent to which an animal is suitable for rehoming must be assessed individually. The Institute for Laboratory Animal Science at the RWTH Aachen University has established its own rehoming concept for this purpose. Over the past three years, more than 100 animals of various species (e.g.: mice, guinea pigs, frogs and zebrafish) have been successfully rehomed. This presentation offers an overview of the rehoming concept, detailed criteria for the transfer of laboratory animals to private pet owners with a particular focus on feedback from private owners.
This presentation aims to inspire other institutions to similarly implement rehoming programs of suitable animals in line with the 3Rs and to integrate this into their institutions' public outreach strategies.

Marker of neurodegeneration, neuroprotection and neuroinflammation in cell cultures

Kalliopi Pitarakoili
Neuroimmunology, Ruhr University Bochum, Bochum, Germany

The diseases of the peripheral nervous system (PNS) involve multiple mechanisms of inflammation and degeneration. Typical inflammatory neuropathies are the acute inflammatory and the chronic inflammatory demyelinating polyneuropathy (CIDP) but they also involve distinct mechanisms of neurodegeneration. An established animal model to examine inflammation in the PNS is the experimental autoimmune neuritis (EAN), which is induced after immunization of Lewis rats with P2 myelin peptide. A minimum of 36 rats are needed for one concentration of therapeutic substance to evaluate its effects on inflammation and regeneration.

Mechanisms of PNS inflammation, degeneration and regeneration can be effectively investigated in cell cultures such as Schwann cells (SC) cultures or cultures of dorsal root ganglia (DRG) isolated from a maximum of 12 Lewis rats. Our group used such cultures to investigate the effects of the small fatty acid propionate in the PNS. We could reproduce oxidative stress in vitro via cultivation of SC or DRG with S-nitroso-N-acetylpenicillamine (SNAP) and have shown positive effects of propionate. Neuroinflammation was induced through induction of the expression of the antigen-presenting molecule major histodompatibility complex (MHC) II in SC cultures. The destruction and regeneration of axons (neurodegeration and -regeneration) was investigated in DRG cultures after oxidative stress. Concluding, the use of cells cultures allowed us to reproduce the basic characteristics of PNS pathology and investigate the relevant cell pathways without involving the model of EAN.

Using the CAM Assay to investigate the behavior of endothelial-like cells in a 3D tumor model

Anna Stettner

Institute of Experimental Oncology, University Hospital Bonn, Bonn, Germany

The chick chorioallantoic membrane (CAM) assay is a widely used in-vivo model in cancer research, offering a valuable alternative that aligns with the 3R principles (Replacement, Reduction, Refinement). This model utilizes fertilized chicken eggs, allowing for the transplantation of cancer cell lines or patient-derived tumor tissues onto the highly vascularized CAM of a developing chicken embryo. The chorioallantoic membrane (CAM) functions as the primitive respiratory organ of the developing embryo and consists of extra-embryonic tissue. Consequently, only extra-embryonic tissue is manipulated during the course of the experiment.

In this study, the CAM assay is employed as a 3D tumor model to investigate the behavior of endothelial-like cells within the tumor microenvironment (TME). Tumors grown on the CAM from a co-culture of tumor cells, particularly small cell lung cancer (SCLC) and urothelial carcinoma cell lines, along with the endothelial-like cell line b.End5, exhibited significantly enhanced vascularization. Furthermore, the formation of new vessel-like structures by these endothelial-like cells was observed, confirmed through Hematoxylin-Eosin (HE) and immunohistochemistry (IHC) stainings. Advanced imaging techniques, such as 2-Photon Microscopy, were employed to further analyze the functionality of these new vessel-structures.

In addition to assessing vascularization, this study seeks to explore the potential for increased immune cell infiltration into these tumors, enabled by the expanded vascular network. This enhanced vascularization could facilitate the intravenous testing of cellular immunotherapies like CAR-T-cells and immune cell engagers in the CAM-Assay, a process that has proven challenging up to this point.

DNA Barcoding as a means to reduce and refine in vivo drug testing in cancer

Barbara M. Grüner
Dept. of Medical Oncology, University Hospital Essen, Essen, Germany

In vitro high- throughput drug testing often fails to translate into clinical settings as it does not represent the complex interaction of the cancer cell with its environment, particular in advanced cancers and during metastasis. Therefore, in vivo drug testing remains an essential tool in cancer research, yet it is often associated with high costs, extensive time requirements, and ethical concerns. DNA barcoding offers a promising alternative to refine and reduce the need for such testing. By tagging individual cancer cell lines or patient-derived tumor xenografts with unique DNA barcodes, multiple treatments can be evaluated in a single animal model. This multiplexing approach allows for the simultaneous assessment of the efficacy of various drugs or drug combinations, significantly reducing the number of animals needed while preserving experimental integrity.

Furthermore, DNA barcoding facilitates more accurate and efficient monitoring of tumor response, enabling researchers to track tumor evolution and resistance mechanisms in real-time. This method allows for the identification of more personalized and effective therapeutic regimens in preclinical cancer models. As a result, the adoption of DNA barcoding in preclinical cancer studies could lead to a paradigm shift, aligning with the 3Rs (Replacement, Reduction, Refinement) framework by minimizing animal usage and improving the relevance of in vivo experiments. This talk will focus on the technical aspects of DNA barcoding, its applications in cancer drug testing, and the potential for reducing animal models while enhancing the precision and scalability of preclinical research.

Development of a Systematic Online Living Evidence Summary (SOLES) for Animal Models testing Targeted Therapies against Cancer (POSTER ID 107)

Julia Steitz [1] & Alexandra Bannach-Brown [2]

[1] Institute for Laboratory Animal Science, University Hospital RWTH Aachen, Aachen, Germany;
[2] QUEST Center for Responsible Research, Berlin Institute of Health (BIH) at Charité - Universitätsmedizin, Berlin, Germany

Cancer is a leading cause of death worldwide and new therapies are needed, necessitating testing in animal models. Although a wide variety of tumor models have been described in the literature, their limitations, as well as unsuccessful translation attempts are often not reported. Yet, choosing the right animal model for the drug to be tested and successfully translated into the clinic is critical.

We systematically reviewed studies testing targeted therapies with the goal to find animal models which have been successfully tested in cancer research and to identify variables that contribute to successful translation and markers of high external validity.

Given the substantial number of studies potentially relevant to this topic, we are developing and validating an automated approach to categorize and prioritize studies prior to in-depth review of disease domains with potential for greatest impact. We will establish and validate the approach with a manageable number of publications, to demonstrate the feasibility of extension to other tumor entities.

Results will be implemented in a systematic online living evidence summary (SOLES), an interrogatable database accessed through a user-friendly online dashboard. The dashboard will be used to filter studies based on study design features of interest, supporting the conduct of specific systematic reviews and highlight gaps in the literature for future research. The Cancer-SOLES platform will provide novel opportunities to identify drug and model combinations that show concordance with human outcomes, optimize the model selection process for preclinical cancer studies and to reduce the number of animals used in cancer research.

Melanoma Brain Metastases Patient-Derived Organoids: An In Vitro Platform for Drug Screening (POSTER ID 116)

Saif-Eldin Abedellatif
Uniklinik Bonn, Bonn, Germany

Background and aims: Brain metastases are prevalent in the late stages of malignant melanoma. Multimodal therapy remains challenging. Patient-derived organoids (PDOs) represent a valuable pre-clinical model, faithfully recapitulating key aspects of the original tumor, including the heterogeneity and the mutational status. This study aimed to establish PDOs from melanoma brain metastases (MBM-PDOs) and to test the feasibility of using them as a model for in vitro targeted-therapy drug testing. Methods: Surgical resection samples from eight patients with melanoma brain metastases were used to establish MBM-PDOs. The samples were enzymatically dissociated followed by seeding into low-attachment plates to generate floating organoids. The MBM-PDOs were characterized genetically, histologically, and immunohistologically and compared with the parental tissue. The MBM-PDO cultures were exposed to dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) followed by a cell viability assessment. Results: Seven out of eight cases were successfully cultivated, maintaining the histological, immunohistological phenotype, and the mutational status of the parental tumors. Five out of seven cases harbored BRAF V600E mutations and were responsive to BRAF and MEK inhibitors in vitro. Two out of seven cases were BRAF wild type: one case harboring an NRAS mutation and the other harboring a KIT mutation, and both were resistant to BRAF and MEK inhibitor therapy. Conclusions: We successfully established PDOs from melanoma brain metastases surgical specimens, which exhibited a consistent histological and mutational profile with the parental tissue. Using FDA-approved BRAF and MEK inhibitors, our data demonstrate the feasibility of employing MBM-PDOs for targeted-therapy in vitro testing.

Human Organotypic Brain Slice Cultures: A Versatile Model System For Neurological Disease

Henner Koch

Dept. of Neurology and Epileptology, RWTH Uniklinik, Aachen, Germany

The investigation of the human brain at cellular and microcircuit level remains challenging due to the fragile viability of neuronal tissue, inter- and intra-variability of the samples and limited availability of human brain material. Especially brain slice cultures have proven to be an excellent source to investigate brain physiology and disease at cellular and small network level, overcoming the temporal limits of acute slices. We gathered in-depth knowledge of long-term culturing of such human organotypic brain slice cultures for research purposes. We highlight the critical pitfalls of the culturing process of the human brain tissue and present exemplary results on viral expression, single-cell Patch-Clamp recordings, as well as multi-electrode array recordings as readouts for culture viability, enabling the use of organotypic brain slice cultures of these valuable tissue samples for basic neuroscience and disease modeling. Moreover, we established a co-culturing model of human glioblastoma cells in cultured human cortex to model glioblastoma multiforme, a devastating type of brain tumor currently not curable, where therapeutic interventions only serve as life-prolonging measures. Hence, new insights into its pathogenesis are urgently needed to define and explore new therapeutic targets and this co-culturing model, with its potential to delineate electrophysiological alterations in the peritumoral zone and glioblastoma pathogenesis in general, holds promise for developing new therapeutic strategies.

In vitro epithelial Models: At the crossroads between Biology - Engineering and Material Science

Florian Groeber-Becker
Experimental Ophthalmology, University Clinic Düsseldorf, Düsseldorf, Germany; Translational Center for Regenerative Therapies at the Fraunhofer ISC, Würzburg, Germany

This presentation explores the development of in vitro 3R models as alternatives to animal testing, focusing on novel biomaterials used as scaffolds to support tissue engineering and disease modeling. We highlight innovations in generating functional vasculature and disease-specific models, offering enhanced physiological relevance. Additionally, we discuss the scale-up of model production using automation for high-throughput applications.

Modelling the Human Airway Mucosa – A sophisticated In Vitro System for Airway Research

Anja Lena Thiebes

Respiratory Tissue Engineering, Institute for Applied Medical Engineering, RWTH Aachen University, Aachen, Germany

Respiratory diseases affect millions of people worldwide. Although animal models have provided valuable insights, they do not fully mimic the human airway and its pathologies. Thus, complex multicellular systems are required as in vitro models for translational research. As in other tissues, vascularization is an essential feature of the airway mucosa. Next to providing perfusion to exchange oxygen and nutrients, endothelial and mesenchymal cells play a crucial role in cell-cell interactions and tissue remodeling in diseases.

We have created a 3D model of the airway based on primary human cells. Three cell types are incorporated in fibrin hydrogels: airway epithelial, endothelial, and mesenchymal cells and matured in specific differentiation medium to result in both epithelial differentiation and tissue vascularization. After 28 day of culture, endothelial cells form hollow capillary-like structures supported by mesenchymal cells. A mature epithelium develops including ciliated, secretory, and basal stem cells. The model can be used to study various diseases, and we have found it to be suitable for investigating the pathophysiology of viral infections such as SARS-CoV-2 with both active and inactivated viruses. By variation of scaffold properties and application of mechanical stimuli, different disease states are mimicked to study tissue remodeling and cell response. We developed an innovative tool modeling the respiratory mucosa combining mature epithelium and a vascularized lamina propria. This platform can be utilized for complex disease modeling and personalized medicine, supporting the progressive reduction and replacement of animal studies.

Abstracts | Posters

ID 105 - ShaRing is CaRing – Die 2 R der behördenkonformen Nutzung von tierischem Gewebe zu wissenschaftlichen Zwecken

Bungardt Britta, Wollgarten Andrea, Schulz Mreike, Tolba René, Ernst Lisa

RWTH Aachen University, Uniklinikum, Aachen, Germany

Im Sinne des „3R-Prinzips", bietet die (Weiter-)Verwendung von tierischem Gewebe gerade bei der Nutzung von Großtieren eine nachhaltige Methode und unterstützt die Idee von Reduction und Replacement: Im Anschluss an einen Finalversuch, können Körperteile und Organe weiteren Forschungsgruppen zu wissenschaftlichen Zwecken zur Verfügung gestellt werden, sodass in Summe weniger bzw. keine Versuchstiere eingesetzt werden müssen.

Allerdings unterliegt die Nutzung einigen gesetzlichen Vorgaben. Organe/Gewebe von Tieren sind sogenannte „Tierische Nebenprodukte" (TNP) im Sinne des Gesetzes. TNP sind definiert als „ganze Tierkörper oder Teile von Tieren […] die nicht für den menschlichen Verzehr bestimmt sind" und in drei Kategorien unterteilt. Ganze Tierkörper oder Teile von Tieren aus der Versuchstierkunde werden dabei (soweit von der Behörde nicht anderweitig eingestuft) der Kategorie 1 zugeordnet, was der höchsten Gefährdungskategorie entspricht.

Der Umgang mit TNP ist in der EU in der Verordnung (EG) Nr. 1069/2009 mit Hygienevorschriften für nicht für den menschlichen Verzehr bestimmter tierische Nebenprodukte, ihrer Durchführungsverordnung (EU) Nr. 142/2011 und in Deutschland durch das Tierische Nebenprodukte-Beseitigungsgesetz (TierNebG 2004/2016) und ihre Durchführungsverordnung, der Tierische Nebenprodukte-Beseitigungsverordnung (TierNebV) 2006/2018 geregelt.

Dies umfasst u.a. die Registrierung der Einrichtung nach Art. 23 der Verordnung (EG) Nr. 1069/2009 bei der zuständigen Behörde und die lückenlose Dokumentation des Gewebeverkehrs. Dabei unterliegen Transport, Kennzeichnung und Verkehr strengen Vorgaben. In enger Zusammenarbeit mit dem Veterinäramt haben wir ein System eingeführt, das eine maximale Nutzung von TNP für wissenschaftliche Zwecke ermöglicht und gleichzeitig den gesetzlichen Vorgaben entspricht.

Beispielweise wurde ein Formular erstellt, das die Organisation und Bereitstellung von Organen erleichtert, sowie die behördliche Dokumentation (Handelspapiere, -register) sicherstellt. Mithilfe dieses Formulars können Forscherinnen und Forscher organspezifische Anfragen für Studien sowie für Aus-, Fort- und Weiterbildungszwecke stellen. In den vergangenen drei Jahren wurden insgesamt 32 verschiedene Organgruppen von 8 verschiedenen Tierspezies (v.a. Schweine, Schafe, Kaninchen) bereitgestellt. Über ca. 46 Arbeitsgruppen haben wir von 100 Tieren TNP als zusätzlichen Versuchszweck gespendet, wobei Gewebe von einem Tier an bis zu 9 Arbeitsgruppen weitergegeben werden konnte.

ID 108 - Advancing Laboratory Animal Science Education: Virtual Reality for Practical Skill Training in a FELASA Function A and D course

Yalda Mirzaei[1], Daniel Fink[2], Kerstin Hagemeister[3], Sebastian Fedrowitz[2], Martina Hüffel-Geuenich[1], Alexander Theissen[4], Laura Bell[2], Rene Tolba[1], Christian Bleilevens[4], Martin Lemos[2], Julia Steitz[1]

[1]Uniklinik Aachen, Institut für Versuchstierkunde, Germany; [2]Audiovisual Media Center (AVMZ), Faculty of Medicine, RWTH Aachen University, Aachen, Germany; [3]Haus für Experimentelle Therapie (HET), Universitätsklinikum Bonn, Bonn, Germany; [4]Department of Anesthesiology, Faculty of Medicine, RWTH Aachen University, Aachen, Germany

Virtual reality (VR) has emerged as a transformative technology with promising applications in laboratory animal science (LAS) education. Traditional training in LAS includes theoretical transfer of knowledge and practical skills training using live animals. Skill training is typically species-specific, rather than solely task-specific to meet participants' experimental needs. VR teaching/learning can reduce animal use in education by integrating virtual practical skills into existing courses before live animal use.. In consideration of the 3Rs, VR-teaching/learning units could help to reduce animal numbers used for education by implementing them in existing courses conveying practical skills virtually before using live animals. A shift from a species-specific to more task-specific concept could further reduce the numbers of animals used in education.

VR-teaching/learning modules were implemented into the practical skill training, including live animal training. 360° videos for mouse skill training were recorded, and 16 VR teaching/learning modules were provided to the participants before or after the skill training on live animals. An online evaluation tool was used to assess the quality, usability, and potential compliance with the 3Rs of the VR modules. The effectiveness and acceptance of virtual reality modules in our laboratory animal science education courses are promising, but their ability to fully replace hands-on training with live animals requires further evaluation.

With the VR teaching and learning modules, the 3Rs have been further integrated into our teaching and learning concept for the laboratory animal science courses. This integration aims to provide a more animal and task-specific qualification for participants in the future.

ID 109 - Evaluation of Tissue-Engineered Blood Vessels as Three-Dimensional In Vitro Testing System in Cardiovascular Research and Device Approval

Anne Glitz[1], Diana Rojas-González[2], Frederic Wolf[2], Nicole Schaaps[1], Roberta Florescu[1], Carolina Neu[1], Rahma Shahin[1], Pakhwan Nilcham[1], Felix Vogt[1], Petra Mela[2,3], Stefan Jockenhoevel[2]

[1]Uniklinik Aachen, Germany; [2]AME-Institute of Applied Medical Engineering, RWTH Aachen University, Germany; [3]Department of Mechanical Engineering and Munich School of Bioengineering, Technical University of Munich, Garching, Germany

Background: Disturbed crosstalk between endothelial cells (ECs) and vascular smooth muscle cells (SMCs) increases the risk for atherosclerosis and restenosis, however, the exact pathomechanisms are incompletely understood. Current preclinical testing models do not adequately recapitulate the complexity of human arteries. Here, we present tissue-engineered blood vessels (TEBVs) as a novel in vitro model for intimal hyperplasia.

Methods: TEBVs fabricated from SMC suspended in fibrin gel, supported by a textile mesh, were seeded with ECs at various concentrations. Furthermore, TEBVs underwent implantation of bare metal stents (BMS) and drug-eluting stents (DES) at 7 days after fabrication. TEBVs were conditioned in a bioreactor for 21 days in total.

Results: Compared with completely endothelialized TEBVs, vessels with absent or incomplete endothelium exhibited thicker vascular walls, reduced lumen diameter, higher collagen content, and more proliferating cells. Immunofluorescence imaging revealed endothelial strut coverage was nearly complete in BMS-treated but rudimental in DES-treated TEBVs. At 14 days after vascular intervention, levels of IL-6, IL-8, and MCP-1 were higher in medium samples from BMS-treated TEBVs compared to DES-treated TEBVs and untreated controls. Conversely, TNF-alpha levels at 14 days were highest in culture medium obtained from DES-treated TEBVs, although the difference was not significant compared with medium from BMS-treated and control TEBVs.

Conclusions: TEBVs are a promising approach towards an in-vitro system for the study of intimal hyperplasia. Given their similarity in size and wall thickness to human coronary arteries, TEBVs may also serve as a platform to test new stent designs.

ID 110 - Bundesnetzwerk 3R - transforming the dialogue together

Julia Engert[1], Sandra Paschkowsky[2], Carina Graf[2]

[1]Kontaktstelle Bundesnetzwerk 3R, BMBF; [2]Kontaktstelle Bundesnetzwerk 3R, VDI/VDE Innovation + Technik GmbH

To date, animals continue to be an indispensable model for research. Animal testing delivers important data about how effective novel drugs are and whether certain substances are toxic for humans. An ethical dilemma emerges – the striving for knowledge and the safety of humans on the one hand and animal welfare on the other. In this regard, the principle of the 3Rs (replace, reduce, refine) provide the current framework for doing research that is as best in line with animal welfare requirements as possible.

In 2022 the German Federal Ministry of Education and Research (BMBF) thus launched the "Bundesnetzwerk 3R" as a national platform for exchange about the 3R principles and possibilities for their implementation. The mission of the network is to facilitate an inter- and transdisciplinary dialogue between science, industry, politics, regulatory authorities and other stakeholders, thus building a strong community that jointly advances 3R research and its transfer into practical application.

The new digital platform www.bundesnetzwerk-3R.de, which launched in September 2023, forms a key element of the networks activities. All stakeholders of the German 3R landscape, each with their own research focuses and interests, are invited to become part of the interactive map and the network. Once registered, members are invited to browse through peoples expertise's and use the provided contact information to connect.

Furthermore, members are invited to several events and frequently get informed through the Newsletter. The participatory approach allows all interested stakeholders to get involved in the discourse and help shape the Bundesnetzwerk 3R.

ID 112 - Communities for Open Research Synthesis – accelerating the translation of evidence by integrating preclinical systematic reviews into the research pipeline

Alexandra Bannach-Brown, Friederike Elisabeth Kohrs, Sofija Vojvodic, Ayesha Amin Tariq, Mala Karmakar, Sarah McCann

QUEST Center for Responsible Research, Berlin Institute of Health @ Charité – Universitätsmedizin Berlin, Germany;

Communities for Open Research Synthesis (COReS) develops a targeted framework to initiate systemic change in how evidence from animal and in vitro studies is translated into improved biomedical and health outcomes. Systematic review and meta-analysis are research synthesis tools that advance high-quality biomedical research by clearly evaluating how reliable evidence is and identifying knowledge gaps, highlighting where future research is needed.

We employ a three-pillar approach to integrate preclinical systematic reviews into the research pipeline; i) building capacity with open-access education and training opportunities and comprehensive online resources for stakeholders at all experience levels, ii) scaling capacity for conduct by developing infrastructure, and iii) forging networks through interdisciplinary learning, collaboration, and peer support.

We present our freely available educational and infrastructure resources which are collated in an online digital hub. We highlight our eLearning modules and Train-the-Trainer programme to enable sustained capacity building. Our digital hub also contains access to our digital discussion forum which supports community communication and networking.

Further, we present results from our survey and interview study to examine attitudes and current practices towards systematic reviews at Charité – Universitätsmedizin Berlin. Our findings highlight specific barriers and enablers to conducting systematic reviews identified by multiple stakeholders. These local findings, together with our ongoing national survey, inform our nationwide implementation strategy.

CORes aims to enact long-lasting systemic change in how preclinical research is designed, conducted, and disseminated considering up-to-date evidence. We leverage existing community expertise and develop sustainable implementation strategies, thereby advancing high-quality research in Germany and beyond.

ID 113 - Advancing Pre-clinical Drug Testing for Ovarian Cancer Using the 3D In-Ovo Chick Chorioallantoic Membrane (CAM) Model

Anna Hartel[1], Anna Stettner[1], Eduard Below[1], Alexander Mass[2], Damian Ralser[1], Michael Hölzel[1]

[1]University Hospital Bonn, Germany, Institute of Experimental Oncology; [2]Universital Hospital Bonn, Department of Otorhinolaryngology

The chick chorioallantoic membrane (CAM) assay is a frequently used in vivo model in cancer research that aligns with the 3R principles (Replace, Reduce, Refine) by minimizing reliance on rodent models. It is based on the use of fertilized chicken eggs and complies with guidelines of the European Directive, which do not classify the model as an animal experiment.
Our established CAM model allows for the transplantation of human cancer cell lines and patient-derived tumor tissue onto the extraembryonic membrane, preserving individual tumor characteristics. Successfully transplanted tumors access the chicken blood circulatory system and therefore offer a unique platform to preclinically evaluate novel immunotherapeutic approaches in ways that cannot be conducted with conventional 2D or 3D in vitro techniques.
We are further expanding this platform to assess cellular immunotherapies by injecting human T-cells with or without immune cell engagers to evaluate tumor infiltration and anti-tumor responses. Advanced techniques such as flow cytometry, multiphoton imaging and histopathological analyses are used to thoroughly assess treatment efficacy. Additionally, we aim to refine the model for patient-derived material, addressing limitations of cancer cell line models. This includes incorporation of spatial-omic approaches and testing of novel biologicals predicted and generates by Artificial Intelligence (AI) algorithms to target ovarian cancer cells.

ID 115 - Veterinary patients in translational pain research - Adipokines and cytokines in canine lumbosacral stenosis

Stephan Leisengang[1,2,3], Dennis Gluding[4], Daniela Ott[1], Christoph Rummel[1,2,3], Martin J. Schmidt[5]

[1]Institute of Veterinary Physiology and Biochemistry, Justus Liebig University Giessen; [2]Translational Neuroscience Network Giessen (TNNG), Justus Liebig University Giessen; [3]Center for Mind, Brain and Behavior (CMBB), Philipps University Marburg & Justus Liebig University Giessen; [4]Department of Veterinary Clinical Sciences, Clinic for Small Animals (Surgery), Justus Liebig University Giessen; [5]Department of Veterinary Clinical Sciences, Small Animal Clinic - Neurosurgery, Neuroradiology and Clinical Neurology, Justus Liebig University Giessen

Translational research is about the bilateral relationship of basic and clinical sciences and focuses on the link between animal models and human diseases. In this context, veterinary science provides important links between the two fields for example by natural occurring disease models, which, however, have not been sufficiently investigated.

In the present study, we aimed to examine the role of local fat depots within the spinal canal (epidural adipose tissue; EAT) in the context of stenotic lesions at the lumbosacral junction of dogs. Compressive forces on spinal nerves of the Cauda equina result in symptoms of pain, sensory deficits and motor impairments. Moreover, locally produced inflammatory mediators (e.g., cytokines) activate or sensitize sensory neurons and thereby modulate nociception. The contribution of EAT to the inflammatory milieu has been poorly investigated. Here, we show elevated expression of the damage-associated molecular pattern HMGB1 (high-mobility group box 1) as well as its receptors RAGE and toll-like receptor (TLR-) 4 in EAT when compared to subcutaneous adipose tissue (SAT). Interestingly, dogs with Cauda equina syndrome (CES) demonstrate an altered mRNA expression profile of cytokines and adipokines with enhanced levels of tumor necrosis factor (TNF)-α and interleukin (IL)-10 and a reduction in IL-6 and leptin. In addition, we revealed new insights into inflammation-induced production of inflammatory mediators using canine adipose tissue ex vivo cultured fat explants.

In conclusion, scientists in the field of translational research can benefit from natural occurring disease models that share pathophysiological characteristics with human diseases. A closer look on diseases of our companion animals may contribute to a more comprehensive understanding of pain-associated disorders without the need to induce diseases in experimental animal models.

ID 117 - Leigh syndrome patient-derived cortical brain organoids as a model system for the study of pathomechanisms and gene therapy approaches

Sonja Heiduschka[1], Laura Petersilie[2], Stephanie Le[1], Annika Zink[1], Karl W. Kafitz[2], Christine R. Rose[2], Alessandro Prigione[1]

[1]Department of General Paediatrics, University Hospital Düsseldorf; [2]Institute of Neurobiology, Heinrich Heine University Düsseldorf

Leigh syndrome (LS) is a severe neurodevelopmental metabolic disorder caused by mutations in genes of the nuclear or mitochondrial DNA that encode for proteins in the mitochondrial respiratory chain. The pathomechanism of LS is poorly understood due to a lack of effective model systems. Patient-derived induced pluripotent stem cells (iPSCs) were used to generate cortical brain organoids (cBO) as a promising model system to shed light on LS pathology and eventually assess treatment approaches.

cBO were differentiated from patient iPSC lines carrying mutations in the mitochondrial gene MT-ATP6. Developmental characteristics regarding gene expression and cytoarchitecture were investigated. For further maturation, cBO were sliced and cultivated as cortical brain organoid slices (cBOS) at the air-liquid interface to detect calcium signals. Finally, cBO and cBOS were transduced with mCherry-linked adeno-associated virus 9 (AAV9) expressed under the synapsin 1 promotor.

cBO showed developmental features similar to the human brain, as neurogenic zones, expression of neural progenitor and neuronal markers, and occurrence of markers typical for astrocytes, oligodendrocytes and synapses in later stages. After chemically-induced metabolic stress, patient cBOS manifested an increased calcium response. cBOS and cBO exhibited AAV9-induced transduction after reaching enough maturity. The success of transduction was dependent on viral concentration, tissue accessibility and incubation time, shown by fluorescence imaging and FACS analysis.

The results indicate some differences between control and patient cBO, and functional assays are necessary to further elucidate patient-specific disease phenotypes. The successful AAV9 transduction enables testing AAV-mediated gene therapies in cBO and cBOS.

ID 119 - Advanced Education and Training Programs to Drive the Adoption of Microphysiological Systems in Academia and Industry

Elena Kromidas[1,2,3], Alessia Moruzzi[2,3], Tanvi Shroff[2,3], Silke Riegger[2,3,4], Peter Loskill[1,2,3], Madalena Cipriano[1,2]

[1]3R-Center for In Vitro Models and Alternatives to Animal Testing, Eberhard Karls University Tübingen, 72074 Tuebingen, Germany; [2]Department for Microphysiological Systems, Institute of Biomedical Engineering, Eberhard Karls University Tübingen, 72074 Tuebingen, Germany; [3]NMI Natural and Medical Sciences Institute at the University of Tübingen, 72770 Reutlingen, Germany; [4]3R-Netzwerk Baden-Württemberg, Eberhard Karls University Tübingen, 72074 Tuebingen, Germany

Microphysiological systems (MPS), including organoid and Organ-on-Chip technologies, are sophisticated tools recapitulating human physiology and are gaining increasing attention in biomedical, pharmaceutical, and basic research. Despite their increasing availability and advantages over traditional models, broader adoption of MPS has been hindered by several factors such as the complex involvement of multiple stake holders, along with limited awareness, interdisciplinary expertise, and technical skills required to operate these novel systems.

To address these challenges, the 3R-Center Tübingen has developed targeted education and training programs for a diverse audience: from young children and students to researchers like model developers and end users in academia and industry, regulators and policymakers. Therefore, content, delivery, and training materials are tailored for various age groups, expertise levels, and professional backgrounds. Program formats include amongst others an Organ-on-Chip Summer School, a teaching module in the University of Tübingen's Master program, science days for children, student internships, and a monthly webinar series.

Equipping current and future stakeholders with knowledge and skills in MPS technology is essential to raise awareness, foster deeper understanding, and prepare qualified professionals ready to adopt and advance MPS. Ultimately, these efforts aim to drive progress in 3R-aligned research.

ID 121 - 3R Approaches of Bf3R and German National Committee

Sabine Bischoff, Tobias Wagenknecht, Céline Heinl, Daniel Butzke, Mariana Lara Neves, Susanne Fieberg, Bettina Bert, Gilbert Schönfelder, Philipp Schwedhelm

Bundesinstitut für Risikobewertung, Germany

The poster describes three areas of Bf3R's work: SMAFIRA, ASR & current National Committee issues. SMAFIRA (SMArt, Feature Interactive RAnking) is a free online tool for screening PubMed abstracts. SMAFIRA uses state-of-the-art computational linguistic methods to classify and compare texts using machine learning. The tool helps to find alternative methods based on a PubMed reference document describing the animal method to be replaced. Abstracts and methods are classified into categories such as in vivo, organs, primary cells, immortal cell lines, invertebrates, humans and in silico. [1] The animalstudyregistry.org platform facilitates the design of animal studies worldwide. The platform helps researchers plan studies in terms of design, methodology, statistics and animal use. 2 To protect researchers from competing ideas, the registered study can be embargoed for up to five years. Once registered, a trial is assigned a DOI, which allows it to be cited. Funders and journals require pre-registration for funding and author guidelines, while research authorities require it for animal protocols. 3 The National Committee (NA) advises the competent German authorities and animal welfare committees on matters relating to the acquisition, breeding, housing, care and use of animals in procedures. The NA also develops recommendations on specific issues. Examples of current issues are the need for an animal welfare permit for the capture of wild animals, the consideration of the need for analgesia in terminal anaesthesia of laboratory animals, and the handling of so-called unusable laboratory animals. 4

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ID 123 - Exploring Muscle Development and Regeneration In Vitro Using a Human iPSC-Derived Organoid Model

Lampros Mavrommatis[1], Emilia Scheidereit[1], Feng Zihao[1], Daryna Pikulska[1], Paul Kießling[1], Martin Stehling[2], Dagmar Zeuschner[3], Marie-Cecile Kienitz[4], Matthias Vorgerd[5], Holm Zaehres[6], Christoph Kuppe[1]

[1]Division of Nephrology and Clinical Immunology, Group of Quantitative Cell Dynamics and Translational Systems Biology, RWTH Uniklinik Aachen, 52074, Aachen, Germany; [2]Max Planck Institute for Molecular Biomedicine, Flow Cytometry Unit, 48149 Münster, Germany; [3]Max Planck Institute for Molecular Biomedicine, Department of Cell and Developmental Biology, 48149 Münster, Germany; [4]Ruhr University Bochum, Medical Faculty, Department of Cellular Physiology, Bochum, Germany; [5]Department of Neurology, Heimer Institute for Muscle Research, BG-University Hospital Bergmannsheil, Ruhr-University Bochum, 44789 Bochum, Germany.; [6]Ruhr-University Bochum, Medical Faculty, Institute of Anatomy, Department of Anatomy and Molecular Embryology, 44801 Bochum, Germany

To advance the principles of the 3Rs (Reduce, Refine, Replace) in animal experimentation, it is essential to develop accessible, high-throughput in vitro models that accurately mimic human biological processes in health and disease. Single-cell analyses from human heart biopsies have revealed novel cell clusters of translational interest, characterized by high WNT signalling, TNNT1, and MYH7B expression, suggestive of a unique skeletal-cardiac mosaic expression profile. Here, we present a post-gastrulation organoid model derived from human induced pluripotent stem cells (iPSCs) that facilitates the study of skeletal muscle organogenesis during development and regeneration, with potential for modeling cardiac and renal lineages following mesodermal lineage segregation during embryogenesis. This organoid model generates functional myofibers and enhances the maturation and activation of myogenic progenitors, with notable in vitro confirmation of high WNT signalling and TNNT1 expression during the activation and regeneration phases of myogenic progenitors. These findings suggest a potential regenerative role for cardiomyocytes displaying this skeletal-cardiac expression profile within the cardiac niche, warranting further investigation. Our study provides a robust alternative to animal models for studying human developmental biology and regenerative mechanisms in muscle systems.

ID 125 - Interdisciplinary Centre for Animal Welfare Research and 3R – ICAR3R

Celina Pabst, Jaqueline Krüger

ICAR3R - 3R Centre JLU Giessen, Germany

ICAR3R is an interdisciplinary cooperation with a 3R professorship for Refinement at the Department of Veterinary Medicine and a 3R professorship for Replacement at the Department of Human Medicine of Justus Liebig University. It aims to promote animal protection in biomedical research and drive forward concepts for the development of alternative methods and husbandry conditions in order to make a sustained contribution to the reduction of laboratory animals.

Here, the expertise of the departments complements each other perfectly. The 3R professorship for Replacement, led by Prof. Jedlička, focusses on computer-based modelling, while the 3R professorship for Refinement, led by Prof. Krämer, specializes in animal welfare. As a fundamental in the Concept of the 3R, animal welfare in the field of Refinement is not only about the improvement of husbandry, but also in implementing a Culture of Care and 3R-Education.

As the ICAR3R considers interdisciplinarity to be crucial for the implementation of the 3R, not only veterinary medicine and human medicine are represented, but also an exchange is sought with research disciplines that are not initially associated with the topic of animal testing. Being an active contributor to the discourse on the subject of animal experiments the ICAR3R for example cooperates with the humanities at the Justus Liebig University to approach the discussion from an ethical and moral perspective. The highly interconnected structures of the Research Campus of Central Hesse form suitable conditions, so that ICAR3R covers a sustainable contribution to the implementation of the 3R concept of Russell & Burch.

ID 126 - Refinement Perspectives on Aortic Aneurysm Rupture: Insights from a Mouse Model

Viktoriia-Anna Boiko, Prof. Dr. Sebastian Zimmer, Dr. Marta Stei

University Hospital Bonn, Germany

Human abdominal aortic aneurysms (AAAs) often remain asymptomatic until rupture. Although no animal model can fully replicate the human condition, preclinical models remain indispensable for studying disease mechanisms and identifying factors contributing to rupture. This study aims to characterize an AAA mouse model in order to elucidate the features associated with rupture, while addressing considerations related to animal welfare.

Male C57BL/6J mice (n=29) were administered Angiotensin II via a subcutaneous osmotic mini-pump and received β-Aminopropionitrile (BAPN) in drinking water over 28 days. Mice were subjected to weekly echocardiography and blood pressure measurements.

All mice developed an AAA. Aortic rupture occurred in 44.8% [25%; 66,7%] and the mean time to death was 12 days. AAA progression, characterized by an increase in aortic diameter, was found to correlate with an increase in maximum blood flow velocity (d7: r2=0,58) and a decrease of heart rate (d28: r2=0,76). The incidence of rupture correlated with body weight loss on day 7 (p=0,039, ∅ 4,73±1,29% vs. ∅ 0,33±1,31%). In histomorphometric analyses, a notable increase in elastic fiber breaks was observed in ruptured aortas.

This mouse model reliably reflects key components of human AAA during progression and in histologic analysis. There is no significant difference in the measured parameters between ruptured and non-ruptured aneurysm mice other than body weight loss at day 7, making weight monitoring critical for animal care and relevant for establishing it as a prognostic factor. Although statistically significant correlations were identified, there is considerable variability across all measured parameters.

ID 127 - Human and murine metabolism – A comparison

Thorsten Cramer, Antje Egners

RWTH Aachen University Hospital, Germany

Rodents especially mice have been widely used model organisms to study human biology and disease for more than a hundred years. This stems from physiological similarities with humans and the fact that many manifestations of human diseases, for example the metabolic syndrome, can also develop in mice. Several interspecies differences, however, not least regarding metabolic parameters and mechanisms highlight the need for cautious interpretation of results when it comes to extrapolation from mice to the human context. Based on systematic literature review, we compiled an overview of metabolic disparities between both species. Not only anatomical differences of the gastrointestinal tracts and adipose tissue are apparent but - amongst others - aspects of carbohydrate, lipid, bile acid, drug and bone metabolism as well. In resesarch projects based on murine models, considering these species differences and integrating their consequences in experimental planning and results interpretation should be mandatory and are highly meaningful in view of the 3R strategy.