5th CCP Phenogenomics Conference 2023

14–15 September 2023

Hybrid Conference

ABSTRACT BOOK

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Czech Centre for Phenogenomics

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Czech Academy of Sciences

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Content

POSTER SESSION 1

POSTER SESSION 2

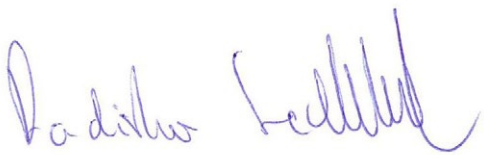
Dear Colleagues,

It is my great pleasure to welcome you to the fifth CCP Phenogenomics Conference.

The scientific committee has selected the topic of rare diseases: experimental models, delivery of therapies and non-coding elements as the main thematic focus of this year Conference.

We believe that the Conference will provide again an excellent opportunity to support networking and interactions among the researchers, CCP staff, users and experts from the commercial sector.

Yours sincerely,



On behalf of the CCP Organizing Committee,
Radislav Sedláček
Director of the Czech Centre for Phenogenomics

Photo © Jana Plavec / Czech Academy of Sciences

ORGANIZER – CZECH CENTRE FOR PHENOGENOMICS

The Czech Centre for Phenogenomics (CCP) is a large research infrastructure unique in combining genetic engineering capabilities, advanced phenotyping and imaging modalities, SPF animal housing and husbandry, as well as cryopreservation and archiving, all in one central location – at BIOCEV campus.

CCP is the only specialized place in the Czech Republic that, at the level of the world's best centres, creates genetically modified mouse and rat models for indispensable biomedical research and at the same time uses standardized but the most advanced phenotyping to characterize the expression of gene functions. CCP outputs are utilized solving the role of genes in the development and treatment of human diseases. CCP provides unique comprehensive preclinical research services in the Czech Republic. With the quality of service and publication results, CCP has gained a worldwide reputation, it has a strong position in international consortia such as the global IMPC (to determine the role of all genes), the European Infrafrontier, and EuroPDX. CCP is involved in a number of international scientific projects.

www.phenogenomics.cz

ACKNOWLEDGEMENTS:

MINISTRY OF EDUCATION, YOUTH AND SPORTS

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Czech Academy of Sciences

STRATEGY AV21
Top research in the public interest

"Towards precision medicine and gene therapy" research programme of the Strategy AV21 is coordinated by the Institute of Molecular Genetics of the Czech Academy of Sciences.

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Tento projekt je spolufinancován se státní podporou Technologické agentury ČR v rámci Programu Národní Centra kompetence
www.tacr.cz
Výzkum užitečný pro společnost.

The project TN02000132/National Centre for New Methods of Diagnosis, Monitoring, Treatment and Prevention of Genetic Diseases is co-financed with state support from the Technology Agency of the Czech Republic under the National Centres of Competence Programme.

The Centre aims to improve diagnostic quality beyond the identification of mutations in the genome and to create a base for monitoring of disease progression and therapy effectiveness as well as for testing and development of cell and gene therapies.

Thursday 14 September 2023	
9:00 – 10:00	Registration + refreshment
SESSION 1 — OPENING + RARE DISEASES & MODELS I (CHAIR: RADISLAV SEDLÁČEK)	
10:00 – 10:20	Radislav Sedláček, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "Czech Centre for Phenogenomics: Leveraging the mouse models resources & challenges" Anna Arellanesová, Czech Association for Rare Diseases, Czech Republic – Opening greeting
10:20 – 11:00	**James Noonan, Yale School of Medicine, United States – Keynote lecture – "Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species"**
SESSION 2 — SESSION 2 – RARE DISEASES & MODELS I (CHAIR: JAN PROCHÁZKA)	
11:00 – 11:20	Illana Gozes, Tel Aviv University, Israel – "The autistic ADNP syndrome and davunetide: From gene to drug development"
11:20 – 11:40	Hana Hanzlíková, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "DNA repair defects and human neurological disease"
11:40 – 12:00	Fatima Bosch, Autonomous University of Barcelona, Spain – "Gene Therapy for Lysosomal Storage Diseases: Treatment of Severe Mucopolysaccharidoses"
12:00 – 12:20	Discussion with speakers
12:20 – 13:30	**Lunch break**
SESSION 3 — DISEASE MODELS & THERAPY DELIVERY: SHORT AND TECHNOLOGY TALKS (CHAIRS: KAREL CHALUPSKÝ & PETR NICKL)	
13:30 – 13:50	Petr Nickl, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "EV-AAV Vector: A Novel Tool for Efficient and Non-Invasive Genetic Delivery into Zygotes"
13:50 – 14:05	Pavel Krist, ZEISS Research Microscopy Solutions, Czech Republic – "ZEISS solutions for the most advanced imaging of animal models"
14:05 – 14:20	Lutz Büchner, Vizgen Inc., USA (Accela sponsored talk) – "Explore new dimensions through spatial context using Vizgen MERSCOPE"
14:20 – 14:35	Katarzyna Kowalczyk, BIO-RAD spol. s r.o., Czech Republic – "BIO-RAD – Your partner in genomics experiments: Application possibilities of ddPCR"
14:35 – 14:50	Florine Samain, Flash Therapeutics, France – "A game-changing RNA delivery platform for next generation therapy to transfer multiple RNA in vivo: LentiFlash® for the treatment of lymphedema"
14:50 – 15:10	Discussion with speakers
15:10 – 16:00	**Poster session 1 – on-site including coffee break**
SESSION 4 — GENETIC DISEASES & THERAPY (CHAIR: RADISLAV SEDLÁČEK)	
16:00 – 16:20	Axel Schambach, Hannover Medical School, Germany – "Gene therapy for the treatment of inherited and acquired diseases"
16:20 – 16:40	Ras Trokovic, University of Helsinki, Finland – "CRISPRa: Unlocking the Potential of Epigenetic Reprogramming to Pluripotency"
16:40 – 17:00	Marcello Maresca, AstraZeneca, Sweden – "Targeting the human genome with SpOT-ON Cas9"
17:00 – 17:20	Discussion with speakers
18:00 – 21:00	**Informal dinner**

Friday 15 September 2023	
SESSION 5 — RARE DISEASES & MODELS II (CHAIR: RADISLAV SEDLÁČEK)	
9:30 – 9:50	Marián Hajdúch, Institute of Molecular and Translational Medicine, Czech Republic – "Drug repurposing strategies for treatment of rare diseases"
9:50 – 10:10	Norbert Weis, Third Faculty of Medicine, Charles University, Czech Republic – "T-type calcium channelopathies"
10:10 – 10:30	Jan Tuckermann, University of Ulm, Germany – "Mouse models uncovering regulation of Immune Metabolism by glucocorticoids"
10:30 – 10:50	Luca Varani, Institute for Research in Biomedicine, Universita' della Svizzera italiana, Italy – "A conformational switch controlling the toxicity of the prion protein, rational design of neuroprotective antibodies"
10:50 – 11:10	Discussion with speakers
11:10 – 11:30	**Coffee break**
SESSION 6 — SHORT & TECHNOLOGY TALKS SELECTED FROM POSTER PRESENTATIONS (CHAIR: JAN PROCHÁZKA)	
11:30 – 11:45	Miles Joseph Raishbrook, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "The impact of Fam84b in retinal homeostasis"
11:45 – 12:00	Poulami Banik, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "Correcting splicing of Prpf31 in retinitis pigmentosa – A step towards finding a cure"
12:00 – 12:15	Marketa Kolarikova, University of Palacky, Olomouc, Czech Republic – "The Enhancement of Anticancer Therapy with Liposome-Enclosed Porphyrin"
12:15 – 12:30	Zuzana, Nichtova, Thomas Jefferson University, Philadelphia, United States – "Chronic Enhancement of Mitochondria-SR Tethering in the Heart through Genetic Engineering Triggers Adaptive Cardiac Muscle Remodeling"
12:30 – 12:45	Discussion with speakers
12:45 – 14:00	**Lunch break & poster session 2**
SESSION 7 — GENETICS OF DISEASES & NON-CODING GENOME (CHAIR: DANNY HUYLEBROECK)	
14:00 – 14:20	Annique Claringbould, European Molecular Biology Laboratory, Germany – "Pooled multiomic single-cell CRISPRi screen of schizophrenia risk genes"
14:20 – 14:40	Hans Tómas Björnsson, University of Iceland, Iceland – "The histone methylation machinery: roles in rare disease and environmental responses"
14:40 – 15:00	David U. Gorkin, Emory University, United States – "Single-cell epigenomic phenotyping of mice lacking chromatin regulators"
15:00 – 15:40	**Matthew T. Maurano, Institute for Systems Genetics & Dept. of Pathology, NYU Medical Center, United States – Keynote lecture – "Synthetic genomic dissection of enhancer context sensitivity and synergy"**
15:40 – 16:00	Discussion with speakers
SESSION 8 — CLOSING	
16:00 – 16:10	Radislav Sedláček, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic – "Closing remarks"

James Noonan, Yale School of Medicine, United States

"Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species"

Jim received his undergraduate degree in Biology and English Literature (Honors) from Binghamton University in upstate New York. He carried out his graduate work with Dr. Richard Myers in the Department of Genetics at Stanford University, where he contributed to the Human Genome Project and characterized the evolutionary history of protocadherin cluster genes in vertebrates.

He received his Ph.D. in 2004. He did his postdoctoral work in Dr. Edward Rubin's lab at the Lawrence Berkeley National Laboratory and the U.S. Department of Energy Joint Genome Institute.
At Berkeley, Jim developed methods to sequence and analyze ancient genomic DNA, providing the first insight into the Neanderthal genome. He also pioneered the discovery and analysis of uniquely human genetic changes that altered developmental gene regulation during human evolution. Jim joined the Yale Genetics faculty in 2007. He is currently the Albert E. Kent Professor of Genetics, with secondary appointments in Ecology and Evolutionary Biology and Neuroscience.

He is also a member of the Wu Tsai Institute, and Executive Director for Genome Sciences at the Yale Center for Genome Analysis. Jim was also named a NOMIS Foundation researcher in 2020.

Matthew T. Maurano, NYU Medical Center, United States

„Synthetic genomic dissection of enhancer context sensitivity and synergy"

Dr. Maurano is an Assistant Professor at the NYU Institute for Systems Genetics and the Department of Pathology. He received B.A.s in Molecular and Cell Biology and Computer Science with Honors from the University of California, Berkeley in 2005. He then moved to Rosetta Inpharmatics (Merck). He returned to academia to train with Dr. John Stamatoyannopoulos at the University of Washington and received his Ph.D. in Genome Sciences in 2013. He established his laboratory in 2015, which focuses on the systems level study of regulatory variation. His laboratory employs both experimental and computational approaches to understanding transcription factor interaction with the nuclear genome, including genome-wide regulatory profiling, novel technologies for genome rewriting, and computational modeling of the sequence determinants of transcription factor binding.

Keynote lectures

- **Thursday 14 September (10:20 – 11:00)**

James Noonan, Yale School of Medicine, United States

"Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species"

- **Friday, 15 September (15:00 – 15:40)**

Matthew T. Maurano, Institute for Systems Genetics & Dept. of Pathology, NYU Medical Center, United States

"Synthetic genomic dissection of enhancer context sensitivity and synergy"

Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species

James Noonan [1]

1. Yale School of Medicine, United States

Email of the presenting author: james.noonan@yale.edu

The development of rapid and efficient genome editing technologies has greatly accelerated the generation and use of genetically modified mouse models, even models with complex designs. Here I will discuss our recent work using these approaches to study the role of gene regulatory variation in human evolution using humanized mice. Our work focuses on a particular class of elements, Human Accelerated Regions (HARs), which encode transcriptional enhancers with uniquely human functions during development. We are generating mouse models for HARs with novel functions in the developing limb, pharyngeal arches and brain, and using a variety of approaches to identify cell-type specific changes in gene expression and regulation that may contribute to developmental phenotypes. I will also discuss ongoing challenges, notably the need for combinatorial technologies to generate models with multiple genetic modifications, which is necessary if we wish to understand how pathways were rewired during human evolution.

Synthetic genomic dissection of enhancer context sensitivity and synergy

Matthew T Maurano [1], Raquel Ordoñez [1], Gwen Ellis [1], André M. Ribeiro-dos-Santos [1], Hannah J Ashe [1]

1. Institute for Systems Genetics, NYU School of Medicine, New York, NY 10016, USA

E-mail of the presenting author: maurano@nyu.edu

Noncoding disease and trait-associated genetic variation is frequently interpreted in the context of genomic regulatory elements such as DNase I hypersensitive sites (DHSs). But while most DHSs lie within a few kilobases of another DHS, these regulatory elements are typically analyzed individually without accounting for their surrounding context. We have recently developed and applied the Big-IN technology for rewriting large segments of intact genomic loci (upwards of 160 kb). Using this synthetic regulatory genomics approach, we characterized distance-dependent synergy among the DHSs at the Sox2 Locus Control Region (LCR) in mouse embryonic stem cells. Here, we deliver and characterize multiple heterotypic combinations DHSs from different critical mESC loci through delivery in place of the Sox2 LCR. We identify widespread genomic examples of context-dependent enhancers which have no activity on their own but impact the activity of a nearby DHS. We show that this synergy between nearby DHSs decays as a characteristic function of distance with its influence extending up to several kilobases. We fine map this context dependency to the contribution of individual TF recognition sequences. Our approach implicates specific sequence and architectural features underpinning the influence of genomic context, and suggests that interpretation of noncoding variation must be done at a haplotype level rather than implicitly assuming the surrounding sequence matches the genomic reference.

Session 1 – Opening + Rare diseases & models I

Thursday 14 September 2023 (10:00 – 11:00)

10:00 – 10:20
Radislav Sedláček, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"Czech Centre for Phenogenomics: Leveraging the mouse models resources & challenges"

Anna Arellanesová, Czech Association for Rare Diseases, Czech Republic
Opening greeting

10:20 – 11:00
James Noonan, Yale School of Medicine, United States
"Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species"

Czech Association for Rare Diseases

Anna Arellanesová [1]

1. Czech Association for Rare Diseases, Czech Republic

E-mail of the presenting author: cavo@vzacna-onemocneni.cz

The Czech Association for Rare Diseases (abbreviation CAVO) was founded in March 2012. The mission of CAVO is to bring together rare disease patient organisations and individual patients, to represent their interests and to strengthen awareness of the specific issue of rare diseases among health professionals, representatives of national and international institutions and the public.

CAVO website: https://www.vzacna-onemocneni.cz/ (in Czech)

Mouse models as an entry point for understanding gene regulatory variation and its effects on traits within and across species

James Noonan [1]

1. Yale School of Medicine, United States

Email of the presenting author: james.noonan@yale.edu

The development of rapid and efficient genome editing technologies has greatly accelerated the generation and use of genetically modified mouse models, even models with complex designs. Here I will discuss our recent work using these approaches to study the role of gene regulatory variation in human evolution using humanized mice. Our work focuses on a particular class of elements, Human Accelerated Regions (HARs), which encode transcriptional enhancers with uniquely human functions during development. We are generating mouse models for HARs with novel functions in the developing limb, pharyngeal arches and brain, and using a variety of approaches to identify cell-type specific changes in gene expression and regulation that may contribute to developmental phenotypes. I will also discuss ongoing challenges, notably the need for combinatorial technologies to generate models with multiple genetic modifications, which is necessary if we wish to understand how pathways were rewired during human evolution.

Session 2 – Rare diseases & models I

Thursday 14 September 2023 (11:00 – 12:20)

11:00 – 11:20
Illana Gozes, Tel Aviv University, Israel
"The autistic ADNP syndrome and davunetide: From gene to drug development"

11:20 – 11:40
Hana Hanzlíková, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"DNA repair defects and human neurological disease"

11:40 – 12:00
Fatima Bosch, Autonomous University of Barcelona, Spain
"Gene Therapy for Lysosomal Storage Diseases: Treatment of Severe Mucopolysaccharidoses"

The autistic ADNP syndrome and davunetide: From gene to drug development

Illana Gozes [1]

1. Elton Laboratory for Molecular Neuroendocrinology, Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine, Adams Super Center for Brain Studies and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 69978, Israel

E-mail of the presenting author: igozes@tauex.tau.ac.il

We revealed activity-dependent neuroprotective protein (ADNP), a critical participant in brain formation. When mutated in humans (de novo or somatically), ADNP drives tauopathy/intellectual disability, cognitive impairments, and social deficits. Interestingly, we discovered a short neuroprotective motif in ADNP, NAP (NAPVSIPQ, davunetide, AL-108, CP201), providing neuroprotection through cytoskeletal fortification. We have developed two unique mouse models, one encompassing ADNP haploinsufficiency and one (together with Biocev's Czech Centre for Phenogenomics) including heterozygous gain of toxic function mutation leading to early-onset Alzheimer's disease-like tauopathy, accentuated in male compared to female mice and corrected by NAP treatment. Previously looking at ADNP hypothalamic expression, we revealed sex-dependency, synchronized by the estrous cycle. In turn, ADNP regulates sex-steroid biosynthesis genes, while controlling hundreds of genes, associated with chromatin remodeling. In this respect, ADNP/NAP regulate the chromosome Y-linked lysine demethylase 5d. Furthermore, axonal transport is sex-dependently controlled in the haploinsufficient ADNP mice, while differential regulation of dendritic spine formation was most obvious in the genome-edited ADNP heterozygous mutated mice (males showing hippocampal deficits and females exhibiting motor cortical deficits, translated to a more severe female motor deficit, corrected by NAP). Also, ADNP deficient neurons had greater and more frequent spontaneous calcium influx in female mice, translated into sex-dependent ADNP-controlled environmental sensation. Clinically, our recent findings reveal surprising sex differences indicating NAP (davunetide) mediated brain protection and clinical efficacy in women suffering from tauopathy (progressive supranuclear palsy).

References:
Biol Psychiatry. 2022 Jul 1;92(1):81-95.
Research Square (Preprint, 2023). doi: https://doi.org/10.21203/rs.3.rs-2457393/v1

DNA repair defects and human neurological disease

Hana Hanzlikova [1,2]

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2. Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, 3012 Bern, Switzerland

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ADP-ribosylation, which is a widespread and transient post-translational protein modification, plays a crucial role in numerous essential cellular and biological functions. These include DNA damage repair, cellular proliferation and differentiation, metabolic processes, stress responses, and immune reactions. Our research is focused on unraveling the complexities of ADP-ribosyl transferases, a subclass of DNA repair enzymes responsible for detecting DNA single-strand breaks (SSBs). These enzymes signal the presence of such breaks by catalyzing the rapid generation of mono(ADP-ribose) and poly(ADP-ribose). Furthermore, our investigations extend to hydrolases, enzymes tasked with eliminating specific ADP-ribosyl modifications from proteins.

SSBs constitute some of the most prevalent DNA lesions that emerge within cells. These lesions have the potential to disrupt replication, RNA processing and transcription, posing threats to both genetic stability and cellular viability. Significantly, deficiencies in DNA single-strand break repair and ADP-ribose metabolism are intimately linked to hereditary neurodevelopmental and neurodegenerative disorders in humans. This underscores the critical importance of these processes, particularly in neurons characterized by extended lifespans and post-mitotic properties.

We investigate the molecular mechanisms by which SSBs are detected and aim to identify and characterize the protein factors and pathways that establish connections between aberrant ADP-ribose metabolism and neurodegenerative diseases including a use of CRISPR/Cas9 gene-edited mouse models. Our ultimate goal is to find whether the perturbed ADP-ribose metabolism at SSBs extends beyond rare DNA repair-deficient conditions to more common dementia.

Session 3 – Disease models & therapy delivery: Short and technology talks

Thursday 14 September 2023 (13:30 – 15:10)

13:30 – 13:50
Petr Nickl, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"EV-AAV Vector: A Novel Tool for Efficient and Non-Invasive Genetic Delivery into Zygotes"

13:50 – 14:05
Pavel Krist, ZEISS Research Microscopy Solutions, Czech Republic
"ZEISS solutions for the most advanced imaging of animal models"

14:05 – 14:20
Lutz Büchner, Vizgen Inc., USA (Accela sponsored talk)
"Explore new dimensions through spatial context using Vizgen MERSCOPE"

14:20 – 14:35
Katarzyna Kowalczyk, BIO-RAD spol. s r.o., Czech Republic
"BIO-RAD – Your partner in genomics experiments: Application possibilities of ddPCR"

14:30 – 14:50
Florine Samain, Flash Therapeutics, France
"A game-changing RNA delivery platform for next generation therapy to transfer multiple RNA in vivo: LentiFlash® for the treatment of lymphedema"

EV-AAV Vector: A Novel Tool for Efficient and Non-Invasive Genetic Delivery into Zygotes

Petr Nickl [1], Maria Barbiera [2], Jacopo Zini [2], Tereza Nickl [1] Irena Jenickova [1], Jana Kopkanova [1], Marjo Yliperttula [2], Aki Ushiki [3,4], Nadav Ahituv [3,4] and Radislav Sedlacek [1]

1. Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Centre for Phenogenomics, Transgenic and Archiving Module, Prague, Czech Republic
2. University of Helsinki, Division of Pharmaceutical Biosciences, Helsinki, Finland
3. Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA
4. Institute for Human Genetics, University of California San Francisco, San Francisco, California, USA

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Recombinant Adeno-associated viruses (AAVs) are characterized by their relative safety, long-term expression, and persistence in the nucleus in the form of episomes. Due to long-term expression with low integration frequency, rAAV is widely used for gene therapies. In the context of transgenic or disease modeling, AAVs can be used in vivo for pre-clinical studies, conditional model validation, humanization, or generation of CDX models. In addition, specific AAV serotypes can serve as ssDNA HDR donor vectors for zygote transgenesis in a method known as CRISPR-READI.

AAV serotypes 1 and 6 are able to pass through zona pellucida, cellular membrane, and reach the pronucleus without any significant negative impact on the viability of the zygote/embryo. Chen et al. 2012 has demonstrated a beneficial combination of CRISPR/Cas9 system and AAV vectors as homology template, known as CRISPR-READI. We sought to elaborate on this method and introduced a new method of acquiring rAAV vectors via co-isolation with extracellular vesicles (EV) and obtaining compound particle EV-AAV. We have proven that EV-AAV can be used as a full-fledged substitution for standard rAAV vectors in the CRISPR-READI method, with the advantage of straightforward production regardless of AAV serotype. Moreover, it allows pseudotyping of EV-AAV particles, resulting in simple characterization, higher EV yield, different tropism or labelling.

In this study, we introduce a novel application of the EV-AAV vector, a compound vector that combines the strengths of extracellular vesicles (EVs) and adeno-associated virus (AAV). Our research focuses on utilizing this innovative vector system as a non-invasive and efficient method for delivering genetic material into zygotes. We successfully evaluated the effectiveness of EV-AAV vectors as carriers for homology templates using the CRISPR-READI method, and demonstrated that the EV-AAV compound vector can deliver AAV genome sequences exceeding the 5kb capacity limit. Additionally, we conducted further investigations into the potential of the EV-AAV vector in delivering the PiggyBac transposon system, enabling us to explore delivery efficiency and potential toxicity. Our results show that the EV-AAV vector outperforms the standard AAV vector, exhibiting superior delivery efficiency and reduced toxicity profiles. In this poster, we also provide a brief description of the production and characterization of EV-AAV particles and their application in transgenic practices.

ZEISS solutions for the most advanced imaging of animal models

Pavel Krist [1]

1. ZEISS Research Microscopy Solutions

E-mail of the presenting author: pavel.krist@zeiss.com

Light microscopes have been available for centuries and they are used in different research and industry applications. Since microscopy is one of the basic methods, there is great interest in the development of new technologies that would allow users to discover and observe new details of the unknown. ZEISS company as a leading manufacturer of microscopes (since 1846) offers inspiring solutions and services for your life sciences and materials research, teaching and clinical routine. You can focus on you research while we focus on the development of new microscopes.

In the short talk we would like to introduce to you the recent technologies which will allow you to observe you specimen with more details and much longer without the risk of bleaching your samples - Lattice Lightsheet 7, true spectral – LSM 980 with 390 – 900 nm detection range, much deeper – Lightsheet 7, X-ray non-destructive nanotomography for 3D imaging - Xradia, scanning electron microscopes with focused ion beam - ZEISS FIB-SEM, software for correlative microscopy - ZEN Connect, all with the possibility to evaluate the results with Deep Machine Learning and sophisticated Artificial Intelligence algorithms - ZEN Intellesis and ZEISS arivis.
While you focus on your research and advance science, ZEISS Microscopy continues to support you.
If you need more information, please, visit our web pages: www.zeiss.com/microscopy

www.zeiss.com/microscopy

ZEISS

Seeing beyond

Explore new dimensions through spatial context using Vizgen MERSCOPE

Lutz Büchner [1]

1. Vizgen Inc., United States of America

Email of the presenting author: lutz.buechner@vizgen.com

Biological systems are comprised of numerous cell types, intricately organized to form functional tissues and organs. Cell atlas initiatives with single-cell RNA sequencing have begun to characterize cell types based on their RNA expression profiles. However, the tissue organization is lost when cells are dissociated for single-cell sequencing, making it difficult to study how the cellular heterogeneity is contributing to the function of the tissue. In this presentation, we will introduce you to Vizgen's all-in-one in situ single-cell genomics platform MERSCOPE which enables the direct profiling of the spatial organization of intact tissue with subcellular resolution.

BIO-RAD - Your partner in genomics experiments; application possibilities of ddPCR

Katarzyna Kowalczyk [1]

1. Field Application Specialist Genomics, CEE, BIO-RAD spol. s r.o.

E-mail of the presenting author: katarzyna_kowalczyk@bio-rad.com

Droplet Digital PCR (ddPCR) provides ultrasensitive nucleic acid detection and direct absolute quantification without the need for standard curves. Not only does this simplify experiment set-up but it also increases reproducibility. Bio-Rad provide a range of solutions to provide unparalleled precision with high flexibility. With the capacity to run 8 to 96 samples per run (in a 96-well format) scalability is easy. New solutions and advanced techniques (with supportive bulletins) can enable the highest multiplexing solution on the market, with up to 5-6-plex on our QX200 flagship system, to 8-plex on our fully integrated QXONE platform and 12-target multiplexing in a single well using the latest QX600 reader.

Some but not all applications include environmental monitoring of pathogens, species determination, residual host cell contamination assessment, viral load analysis, mutation detection, copy number variation (CNV), minimal residual disease (MRD), microbial quantification, NGS library quantification, genome editing assessment (HDR and NHEJ), small-fold change gene expression analysis, miRNA quantification and methylation sensitive restriction enzyme (MSRE) ddPCR without the need for bisulfite conversion. ddPCR could be used in production of viral vectors, gene editing in human cells, validation of IPS cells and transgenic models. ddPCR is more sensitive than qPCR and can detect rare targets, providing accurate data. Critical in cell therapy products is to check tumorigenicity of human induced pluripotent stem cells (IPS) so ddPCR could be best for checking safety and quality control of those products.

Copy number variation, including genomic deletions or duplications, is a prominent source of variability, and copy number variation of specific loci has been associated with cancers, neurological disease, and adverse drug response. The ability to reliably discriminate between copy number states across samples and to detect copy number aberrations in heterogeneous samples is crucial for tumor profiling, detecting mosaicism, and population studies.
ddPCR enables high – resolution CNV analysis through ultraprecise, absolute quantification of specific nucleic acid sequences. Using a reference with known copy number per cell, it is easy to determine the copy number of 1 to 11 targets of interest per well (depending on platform see above). First droplets are generated for 8-96 wells (for low to medium throughput suggest manual droplet generation and for higher throughput suggest automated droplet generation. Samples are then amplified to end point and the fluorescence is measured after PCR is completed (not in real time like qPCR) that is why the measurement is not relying on kinetics of the reaction – accuracy relies on dividing positive and negative.

Precise and accurate viral quantification is critical to assess disease progression and virologic response to treatment, and for the determination of viral load, infectivity, and potency.

Droplet Digital PCR enables exquisitely precise, sensitive, and absolute nucleic acid quantification without a standard curve. Bio-Rad's ddPCR systems enable measurement of low-abundance targets, targets in complex backgrounds, and subtle changes in target levels undetectable by real-time PCR, giving ddPCR assays the ability to accurately quantitate even very low copy numbers.
A novel application of ddPCR is linkage assessment. Which can be used to identify intact proviral DNA, the integrity of viral vectors, inversion assessment, plasmid impurities, cis/trans mutations, and whole cell DNA analysis. Linkage works by using two or more assays (FAM and HEX) at either end of your target that are expected to be co-amplified (e.g. vectors, viral pathogen, suspected cis-mutations,

whole cells isolated in droplets). The software can then determine the amount of linkage above that of random distribution to determine the concentration of linked loci and the %linkage, this technique is solely unique to digital PCR.

Across the gene therapy development process, from early R&D to full-scale manufacturing, viral vector characterization is critical for process optimization, ensuring safety, and maximizing efficacy. One of the more complex viral vector characterization assays is to understand how much of the viral genome has been packaged into the capsid. Without an accurate measurement of the percentage of capsids that have fully intact vector versus completely empty or partially filled capsids, you may be overestimating the potency of your gene therapy. Especially with partially-filled capsids, you may or may not have enough vector to elicit a therapeutic response, leading to a great deal of uncertainty around potency.

Fortunately, with ddPCR technology, you can develop a robust assay that detects multiple parts of the viral genome to evaluate viral vector intactness.
For validating product quality Bio – Rad has also in his offer kits allowing to find contaminants in biopharmaceutical development processes that are typically not detectable by standard microscopic methods due their small size. The European, U.S., and Japanese pharmacopoeias define the levels of Mycoplasma that should be present in any given product. Bio-Rad's Vericheck ddPCR Mycoplasma Detection Kit is the first droplet digital PCR based Mycoplasma testing solution. Contaminants such as host cell proteins (HCPs) and host cell DNA (hcDNA) can be reduced to safe levels by employing a robust purification strategy. To meet regulatory requirements, drug manufacturers employ sensitive and reliable detection methods. Quantifying residual hcDNA by Droplet Digital PCR (ddPCR) provides the most sensitive absolute quantification. Traditionally, qPCR has been used for hcDNA quantification, but this technique can be susceptible to PCR inhibitors found in complex matrices, such as biological material, and thus often requires a DNA extraction step. Bio-Rad's ddPCR technology eliminates the need for DNA extraction and allows direct quantification of residual DNA from multiple species without compromising accuracy, sensitivity, or precision.

ddPCR provides reliable and accurate nucleic acid quantification and can be used for a wide variety of applications, some only application to ddPCR. Bio-Rad has a wide range of online tools, bulletins, kits, assays, online assay design tools, experienced specialist, specialized supermixes and optimized software (drop-off analysis function for mutation quantification and genome editing, 2D plate view for experimental optimization, amplitude multiplex, probe mix triplex, advanced classification mode, tilt correction, heat-map for thresholding, positive sample thresholding, regulatory editions and more). This enables Bio-Rad to provide the best possible support during experimental design, trouble shooting and data analysis that we need and want to use.

A game-changing RNA delivery platform for next generation therapy to transfer multiple RNA in vivo: LentiFlash® for the treatment of lymphedema

Florine Samain [1], Alexandra Iché [1], Nicolas Martin [1], Christine Duthoit [1], Régis Gayon [1], Emeline Benuzzi [2], Barbara Garmy-Susini [2], Pascale Bouillé [1]

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Gene & cell therapy approaches show that there is no universal delivery tool for all therapeutic strategies. Compared to DNA delivery therapies mediated by integrative lentiviral vectors and AAVs, RNA therapies are more versatile, cover a broad range of applications with minimal regulatory concerns and thus address a large variety of diseases. RNA delivery targets applications in which a transient expression is expected.

As a game-changing biological RNA carrier, LentiFlash, a bacteriophage-lentivirus chimera, can efficiently and safely deliver multiple RNA species that are transiently expressed directly in the cell cytoplasm. RNA delivery mediated by a lentiviral particle is an attractive approach as it combines most of the lentiviral particles properties (cell entry and tropism) without the potential adverse effects from long-lasting expression nor genomic integration. Indeed, as there are no HIV sequences on the RNA packaged into lentiviral particles anymore, this enables the encapsidation of multiple and heterologous RNA sequences into the same LentiFlash particle without any risk of integration.

We are developing a regenerative therapy with LentiFlash® expressing two different mRNAs to restore the lymphatic function in the arm of patients who developed a lymphedema after breast cancer surgery and lymph node removal. Lymphedema is developed by 10-15% of women after breast cancer and there is no curative treatment. Results show that dual mRNAs delivery abolished lymphedema and restored the lymphatic flow in the limb showing that this strategy allows enough synthesis of the two proteins to observe a beneficial effect. LentiFlash® expressing 2 mRNAs will be used for a Phase I/II clinical trial called Theralymph that is in the process of being launched in Toulouse University Hospital.
These properties and the ability to produce LentiFlash® using a cGMPs platform, makes this innovating technology a very efficient and safe therapy compared to other approaches.

Session 4 – Genetic diseases & therapy

Thursday 14 September 2023 (16:00 – 17:20)

16:00 – 16:20
Axel Schambach, Hannover Medical School, Germany
"Gene therapy for the treatment of inherited and acquired diseases"

16:20 – 16:40
Ras Trokovic, University of Helsinki, Finland
"CRISPRa: Unlocking the Potential of Epigenetic Reprogramming to Pluripotency"

16:40 – 17:00
Marcello Maresca, AstraZeneca, Sweden
"Targeting the human genome with SpOT-ON Cas9"

Gene therapy for the treatment of inherited and acquired diseases

Axel Schambach [1,2]

1. Institute of Experimental Hematology, Hannover Medical School, Germany
2. Division of Hematology / Oncology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

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Gene therapy, as an innovative new strategy, has revolutionized the face of modern medicine by addressing the roots of various genetic and acquired disorders at the molecular level, and, thus, by altering the genetic material of an individual to treat or prevent diseases. In this talk, we will give an overview of recent developments in the fields of inherited and acquired diseases. First, as an example of a rare monogenetic disease, we will talk about the evolution of gene therapy for severe combined immunodeficiency (SCID). Here, by introducing functional copies of the faulty gene, we aim to restore normal cellular functions and immunity to alleviate the devastating symptoms of this disorder. Second, as an example for a severe acquired disease, we will explain novel approaches to fight cancer cells by genetic modification of T and NK cells with chimeric antigen receptors (CARs) and TRUCKs, and how this can be used to target solid cancers.

CRISPRa: Unlocking the Potential of Epigenetic Reprogramming to Pluripotency

Ras Trokovic [1,2]

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Traditional reprogramming techniques involve inducing pluripotent stem cells (iPSCs) from somatic cells through the forced expression of transcription factors. However, this approach can trigger unintended gene activation and result in abnormal iPSCs. The application of CRISPR activation (CRISPRa) to stimulate endogenous pluripotency factors can result in improved iPSCs quality. This study outlines a highly efficient approach for reprogramming human somatic cells into iPSCs using finely-tuned CRISPRa. Achieving efficient reprogramming hinged on targeting the Alu-motif, enriched in embryo genome activation, alongside the miR-302/367 locus. Single-cell transcriptome analysis unveiled that optimized CRISPRa directed cells more precisely and specifically towards the pluripotent state compared to conventional methods. These findings underscore the potential of CRISPRa for superior-quality pluripotent reprogramming of human cells.

SPOT-ON is a Cas9 nuclease with no detectable off-targets and reduced chromosomal translocations in vivo

Marcello Maresca [1]

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The CRISPR-Cas9 systems have revolutionized the field of genome medicines. However, only a few Cas9 enzymes work efficiently in the in vivo setting and even fewer made it to clinical trials. While the first enzyme, SpCas9 has been successful in early trials, its aberrant activity raises safety concerns due to off-target editing.
Here, we describe the discovery of a new Cas9 member of the Type II-B family from the human gut microbiome metagenomic database, which we named SpOT-ON. We characterized SpOT-ON biochemically and showed that its gene editing activity in vitro and in vivo is comparable to SpCas9. Remarkably, SpOT-ON displayed intrinsic high-fidelity and undetectable levels of off-target editing evaluated by conventional methods and high-sensitive DUPLEX-sequencing. Genome editing with SpOT-ON results in reduced levels of genomic translocation compared to SpCas9. Overall, SpOT-ON could serve as an alternative to SpCas9 for clinical use with a significantly improved safety profile.
At the conference, we will present the discovery and engineering of SpOT-ON and our latest efforts to further improve its editing properties. Furthermore, we will share the results of in vivo studies using SpOT-ON.

Session 5 – Rare diseases & models II

Friday 15 September 2023 (9:30 – 11:10)

9:30 – 9:50
Marián Hajdúch, Institute of Molecular and Translational Medicine, Czech Republic
"Drug repurposing strategies for treatment of rare diseases"

9:50 – 10:10
Norbert Weis, Third Faculty of Medicine, Charles University, Czech Republic
"T-type calcium channelopathies"

10:10 – 10:30
Jan Tuckermann, University of Ulm, Germany
"Mouse models uncovering regulation of Immune Metabolism by glucocorticoids"

10:30 – 10:50
Luca Varani, Institute for Research in Biomedicine, Universita' della Svizzera italiana, Italy
"A conformational switch controlling the toxicity of the prion protein, rational design of neuroprotective antibodies"

T-type Calcium channelopathies

Norbert Weiss [1]

1. Third Faculty of Medicine, Charles University, Czech Republic

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T-type calcium channelopathies encompass a group of human disorders either caused or exacerbated by mutations in the genes encoding different T-type calcium channels. Through several examples of rare disease-causing variants, I will illustrate how these mutations can affect various aspects of the channels, ranging from alteration of their molecular structure, trafficking to the cell surface, gating properties, and regulation. I will also discuss, based on this knowledge, why a specific T-type channel variant often associates with one specific disorder without overleaping with other T-type channelopathies.

Mouse models uncovering regulation of Immune Metabolism by glucocorticoids

Jan Tuckermann [1]

1. Institute of Comparative Molecular Endocrinology, University of Ulm, Germany

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Glucocorticoids are stress hormones that regulate energy metabolism and are in widespread clinical use to limit inflammation, despite their metabolic side effects, including diabetes and osteoporosis. Glucocorticoids act via nuclear receptors, in particular the glucocorticoid receptor GR, a ligand-induced transcription factor. For a long time, the reduction of cytokine expression and induction of gluconeogenetic enzymes were considered the main molecular mechanisms of GR function in immune modulation and regulating energy metabolism, respectively. Now evidence is emerging that GR is at the crossroads of extrinsic and intrinsic immunometabolism. Using conditional mutations of the GR exploited in preclinical models we present examples of how GR in immune cells regulates fundamental energy metabolism during fasting and obesity and how GR impacts on cellular metabolism and metabolites to induce anti-inflammatory activity in cells to resolve inflammation.

A conformational switch controlling the toxicity of the prion protein, rational design of neuroprotective antibodies

Luca Varani [1]

1. Institute for Research in Biomedicine

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Prions became famous in the 90s due to Mad Cow disease. Unbeknown to most, the human forms kill ~700 people in Europe each year. Prion diseases are invariably fatal and no cure is available. The disease is characterized by misfolded protein aggregates in the brain, much like other neurodegerative diseases, but the molecular mechanism remains largely unknown.
We pinpointed the molecular determinant triggering neurodegeneration, identifying a toxicity triggering site responsible for converting prion protein into a toxic conformer. Rational molecular engineering allowed triggering or inhibiting the toxic conversion, and occupying the toxicity triggering site with aptly discovered antibodies resulted in protection from neurodegeneration, even if administered when signs of neurodegeneration where already evident ex vivo.

Session 6 – Short & technology talks selected from poster presentations

Friday 15 September 2023 (11:30 – 12:45)

11:30 – 11:45
Miles Joseph Raishbrook, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"The impact of Fam84b in retinal homeostasis"

11:45 – 12:00
Poulami Banik, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic
"Correcting splicing of Prpf31 in retinitis pigmentosa – A step towards finding a cure"

12:00 – 12:15
Marketa Kolarikova, University of Palacky, Olomouc, Czech Republic
"The Enhancement of Anticancer Therapy with Liposome-Enclosed Porphyrin"

12:15 – 12:30
Zuzana, Nichtova, Thomas Jefferson University, Philadelphia, United States
"Chronic Enhancement of Mitochondria-SR Tethering in the Heart through Genetic Engineering Triggers Adaptive Cardiac Muscle Remodeling"

The impact of Fam84b in retinal homeostasis

Miles Raishbrook [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vestec, Czech Republic

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Fam84b is an incompletely characterised protein. Despite reports associating its overexpression with breast, oesophageal and prostate cancer, its function is still unclear. The first FAM84B knockout mouse line was developed at CCP using CRISPR/Cas9 to create an indel in exon 2. FAM84B -/- mice display a degenerative retinal phenotype that becomes more severe with age. This phenotype, which bears similarities to the human retinal disease age-related macular degeneration, has been extensively characterised in our lab. FAM84B -/- mice demonstrate thinner and more disorganised retinal morphology from 12 weeks of age with photoreceptor loss, reduced responses to light stimulation, as well as signs of choroidal neovascularisation at older ages. Localised expansion of the retinal pigmented epithelial layer is also prominent in knockout eyes. The aim of this ongoing project will be to explain how the lack of Fam84b unsettles retinal homeostasis and induces the presented phenotype.

Correcting splicing of Prpf31 in retinitis pigmentosa - A step towards finding a cure

Poulami Banik [1], David Staněk [1]

1. Laboratory of RNA biology, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

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Retinitis pigmentosa(RP) is a genetic disorder that causes the loss of photoreceptors and consequent loss of central and peripheral vision. Pre-mRNA processing factor 31 (Prpf31) is a splicing factor associated with the assembly and stability of the U4/U6•U5 tri small nuclear ribonucleoprotein particle (snRNP), which is an essential component of the spliceosome. Various mutations in Prpf31 are associated with ~10% of cases of the autosomal dominant form of RP. In this report, we describe a novel point mutation in intron 10 of the PRPF31 gene. This mutation leads to RP in one of the affected siblings, despite the absence of phenotypic abnormalities in the parents. We tested the splicing efficiency of intron 10 using human cell lines and blood samples and found that intron 10 is not efficiently removed even in wild type samples. Next, we established a splicing reporter based on PRPF31 intron 10 and inserted the intronic mutation observed in the patient. The RP mutation completely abolished splicing of intron 10. We then analyzed available data sets to identify potential binding sites of splicing regulators in intron 10 and surrounding exons. Next, we designed antisense oligonucleotides (ASOs) targeting the potential binding sites in two exons (10 and 11) and intron 10. We introduced ASOs individually or in combination into cells and evaluated their effect on splicing of wild type and mutated Prpf31. We identified ASO(s) that enhanced splicing of both wild type and mutated Prpf31. ASOs are becoming a powerful tool to manipulate RNA processing and hold a therapeutic potential to treat various genetic disorders, Here, we identified ASO to improve splicing of PRPF31, one of the most mutated genes causing RP, which may open a way to treat this rare disease.

The Enhancement of Anticancer Therapy with Liposome-Enclosed Porphyrin

Marketa Kolarikova [1], Barbora Hosikova [1], Lukas Malina [1], Jakub Hosik [1], Martin Snehota [1], Klara Balazova [1], Robert Bajgar [1], Hana Kolarova [1]

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Photodynamic therapy is an alternative treatment for cancerous but also noncancerous diseases. The anticancer Photodynamic therapy (PDT) is based on the application of a photodynamically active substance, so-called photosensitizer, its selective accumulation in malignant tissue and subsequent irradiation with a specific wavelength leading to the activation of the photosensitizer further causing the cell death. Although the concept of PDT is dated to 1900, drug delivery modifications to enhance the therapeutic effect of this therapy present an unremitting challenge in clinical field. As nanoparticle carrier systems tend to accumulate passively in tumor tissues through the leaky tumor vasculature, there is a demand for the delivery systems of a nanoparticle size in this therapy. Thus, liposomes present a type of clinically well-established nanoparticles, consisting of inner aqueous core where hydrophilic molecules can be captured, and lipid bilayers where hydrophobic molecules accumulate. Here, the extrusion method for the formation of 200 nm DPPC:Chol liposomes was used to encapsulate a porphyrin photosensitizer (TMPyP). Therapy was induced with blue LED light 4 and 24 hours after the TMPyP administration to HeLa and NIH3T3 cells. To assess the treatment efficiency, fluorescence lifetime imaging microscopy (FLIM) was used to detect the lifetime of free/bound states of FAD. The liposomal size was detected using DLS (Dynamic Light Scattering) measurement. The intracellular localization of free TMPyP and TMPyP enclosed in liposomes was assesed on HeLa cell line using confocal spinning disk imaging. The viability of HeLa cell line after the therapy application was determined via MTT assay.
This work was supported by Ministry of Health of the Czech Republic, grant nr.NU21J-03-00062.

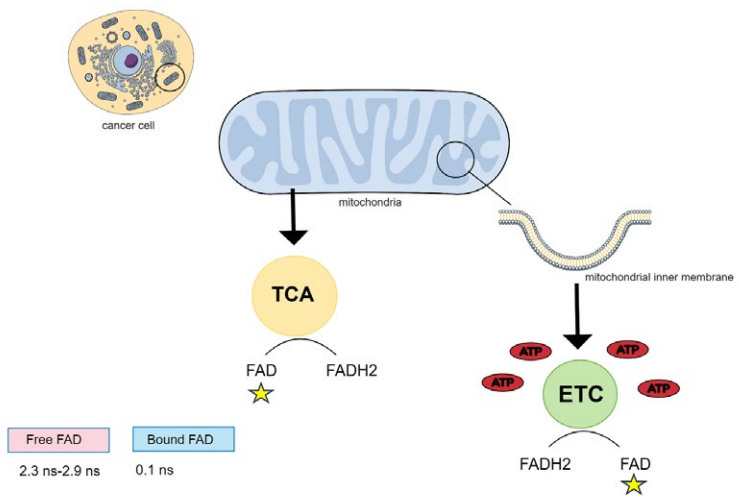

Fig. 1: Autofluorescence as a tool to assess a treatment efficiency based on ratio differences of free/bound states of FAD using FLIM system.

Chronic Enhancement of Mitochondria-SR Tethering in the Heart through Genetic Engineering Triggers Adaptive Cardiac Muscle Remodeling

Zuzana Nichtová [1], Celia Fernandez-Sanz [1], Sergio De La Fuente [1], Yuexing Yuan [1], Stephen Hurst [1], Sebastian Lanvermann [1], Hui-Ying Tsai [1], David Weaver [1], Ariele Baggett [1], Christopher Thompson [2], Cedric Bouchet-Marquis [2], Péter Várnai [3], Erin L Seifert [1], Gerald W Dorn [2, 4], Shey-Shing Sheu [1], György Csordás [1]

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2. Thermo Fisher Scientific, Hillsboro, OR (C.T., C.B.-M.)
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4. Center for Pharmacogenomics, John T. Milliken Department of Medicine, Washington University School of Medicine, St Louis, MO (G.W.D.)

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Cardiac contractile function relies heavily on the energy supplied by mitochondria and the regulation of calcium ions (Ca2+) from the sarcoplasmic reticulum (SR). This delicate balance is crucial for excitation-bioenergetics coupling, ensuring that mitochondrial ATP production matches the surges in demand during excitation. However, under pathological conditions, mitochondrial Ca2+ overload can lead to excessive reactive oxygen species production and permeability transition, jeopardizing cellular homeostasis and myocyte viability.

The role of mitochondria-SR tethering in cardiac physiology and pathology has been a subject of debate. Endogenous tether proteins serve multiple functions, making it challenging to dissect their specific roles in interorganelle linkage. In this study, we investigate the physiological and pathological implications of selectively enhancing cardiac mitochondria-SR tethering via introduction of a cardiac muscle-specific engineered tether (linker) transgene with a fluorescent protein core into mice.

Our findings revealed that the linker expanded and reinforced individual mitochondria-junctional SR contacts. Surprisingly, it also induced significant remodeling, resulting in large, dense mitochondrial clusters that excluded dyads. Nevertheless, excitation-bioenergetics coupling remained robust, possibly due to increased longitudinal mitochondria-dyad contacts and nanotunneling between mitochondria in proximity to junctional SR and those positioned away from it.

Remarkably, the linker reduced female susceptibility to acute massive β-adrenergic stress and mitigated myocyte death and myocardial impairment associated with mitochondrial calcium overload during ex vivo ischemia/reperfusion injury.

We propose that mitochondria-SR/endoplasmic reticulum contacts operate optimally at a specific structural configuration. While acute changes in tethering may disrupt function, chronic enhancement of contacts from an early age triggers adaptive remodeling of organelles, shifting the system to a new, stable structural equilibrium. This remodeling enhances the interconnected mitochondrial pool and, presumably, the capacity for handling Ca2+ and reactive oxygen species, thus improving resilience to stresses associated with dysregulated hyperactive Ca2+ signaling. These findings shed light on the intricate interplay between organelle dynamics and cardiac function in health and disease.

Session 7 – Genetics of diseases & non-coding genome

Friday 15 September 2023 (14:00 – 16:00)

14:00 – 14:20
Annique Claringbould, European Molecular Biology Laboratory, Germany
"Pooled multiomic single-cell CRISPRi screen of schizophrenia risk genes"

14:20 – 14:40
Hans Tómas Björnsson, University of Iceland, Iceland
"The histone methylation machinery: roles in rare disease and environmental responses"

14:40 – 15:00
David U. Gorkin, Emory University, United States
"Single-cell epigenomic phenotyping of mice lacking chromatin regulators"

15:00 – 15:40
Matthew T. Maurano, Institute for Systems Genetics & Dept. of Pathology, NYU Medical Center, United States
"Synthetic genomic dissection of enhancer context sensitivity and synergy"

Pooled multiomic single-cell CRISPRi screen of schizophrenia risk genes

Annique Claringbould [1], Umut Yildi [1]z, Mikael Marttinen [1], Judith Zaugg [1], Kyung Min Noh [1]

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Schizophrenia is a highly heritable disease that primarily manifests in neurons, yet the molecular function of risk genes is still unclear. Changes in the epigenetic and transcriptional landscape throughout neurodevelopment implicate epigenetic modulators and transcription factors (TFs) as key drivers in the disease[1]. In this study, we investigate the role of such regulators in neuronal gene regulation and differentiation by repressing their transcription with CRISPR interference (CRISPRi) and single cell profiling the subsequent changes in chromatin accessibility (scATAC-seq) and transcription (scRNA-seq).
We identified 65 schizophrenia-associated neurodevelopmental regulators and designed four guide RNAs (gRNAs) to target each gene. We generated an induced pluripotent stem cell (iPSC) line expression a CRISPR repressor (dCas9-KM2), transduced the cells with the gRNA library, and differentiated them into neural progenitor cells (NPCs) or neurogenin-2-expressing neurons. To increase the throughput of the screen, we developed and employed single-cell combinatorial fluidic indexing for scRNA-seq[2] and scATAC-seq. We also sequenced the gRNA library to match perturbations to cells in both modalities.
gRNAs targeting known essential genes, including THOC7, EXOSC5 and MED8, were found in a low number of cells, indicating that they were unable to survive and thereby showing that the cells were perturbed successfully. We detected a significant effect of perturbation on 43% of the 65 gnes in a targeted scRNA-seq panel. Differential TF activity and gene expression analyses show converging effects on reactive oxygen species metabolism. In spite of the homogeneous differentiation protocols to generate neurons and NPCs, we observe subpopulations of cells that seem to have undergone changes in differentiation. For examples, one group of neurons is much more similar to NPCs. Interestingly, this cluster is enriched for perturbations of PPM1G, DISC1 and JDP2, among others. We hypothesize that these perturbations stall neuronal differentiation and leave the cells stuck as progenitors. Conversely, a cluster of NPCs enriched for the gRNA targeting MCRS1 shows a neuronal phenotype, indicating accelerated differentiation. Cells in this neuron-like cluster are enriched for neuronal genes differentially expressed in schizophrenia patients versus controls[3,4], hinting at disrupted neuronal differentiation as a disease mechanism. This is also supported by a cell viability screen, where MCRS1 perturbation leads to fewer NPCs, but not to increased apoptosis.
In summary, the downregulation of schizophrenia-related regulators affects gene regulation at the level of expression and chromatin accessibility. The integration of both modalities identifies cell clusters driven to distinct cell states by specific perturbations. As such, this high throughput functional screen elucidates the molecular mechanisms at the core of neurodevelopmental disorders.

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The histone methylation machinery: roles in rare disease and environmental responses

Hans Bjornsson [1]

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Disease causing variants in the epigenetic machinery are an emerging cause of intellectual disability with at least 90 such factors known. Our group has assembled a list of all robustly defined epigenetic machinery factors (http://www.epigeneticmachinery.org/) and shown that a subset of these factors (74) are highly co-expressed with one another, and interestingly those that are highly co-expressed show the strongest haploinsufficiency and links to neurological disease. Working on one of these Mendelian disorders of the Epigenetic Machinery (Kabuki syndrome), which is most commonly caused by heterozygous mutations in the histone methyltransferase KMT2D, we´ve demonstrated postnatal rescue of neurological phenotypes (hippocampal memory defects, disruption of adult neurogenesis) using several strategies that counter the epigenetic defect. This serves as proof-of-principle that this disorder may be a treatable cause of intellectual disability and plans are to initiate a clinical trial in 2023 to test this strategy in individuals with Kabuki syndrome. Finally since epigenetic modification often mediates environmental stimuli in various organisms, we´ve done unbiased studies combining a novel mild hypothermia indicator with a CRISPR-Cas9 forward screen to chart regulators of the mild hypothermia response. These studies have yielded SMYD5, a histone methyltransferase, which appears to be a key regulator of the mild hypothermia response. Together, these studies illustrate the diverse roles of histone methylation in health and disease.

Single-cell epigenomic phenotyping of mice lacking chromatin regulators

David U. Gorkin [1]

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Many chromatin regulators are essential for mammalian development, and mutations in chromatin regulators cause a variety of human developmental disorders. However, the developmental cell types and gene regulatory networks networks that rely on each chromatin regulator for their proper function are not well characterized. Together with collaborators at the Jackson Laboratory (Co-I Kevin Peterson, PhD) and UCSD (Co-I Allen Wang, PhD), we are leveraging mouse knockout lines lacking key epigenetic regulators in order to interrogate the effects of chromatin regulator loss during developmental at the molecular level, and with cell-type resolution. More specifically, we are phenotyping mutant lines at prenatal time points with single-cell multiomic approaches that capture mRNA expression and chromatin accessibility from the same single nuclei. By integrating our data with existing resources and genomic datasets, we are developing a tissue- and cell-type-resolved map of the gene regulatory defects caused by loss of epigenetic regulators relevant to human health and disease. In my talk I will give an update on the progress of this effort, and look ahead to future opportunities and challenges.

Synthetic genomic dissection of enhancer context sensitivity and synergy

Matthew T Maurano [1], Raquel Ordoñez [1], Gwen Ellis [1], André M. Ribeiro-dos-Santos [1], Hannah J Ashe [1]

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Noncoding disease and trait-associated genetic variation is frequently interpreted in the context of genomic regulatory elements such as DNase I hypersensitive sites (DHSs). But while most DHSs lie within a few kilobases of another DHS, these regulatory elements are typically analyzed individually without accounting for their surrounding context. We have recently developed and applied the Big-IN technology for rewriting large segments of intact genomic loci (upwards of 160 kb). Using this synthetic regulatory genomics approach, we characterized distance-dependent synergy among the DHSs at the Sox2 Locus Control Region (LCR) in mouse embryonic stem cells. Here, we deliver and characterize multiple heterotypic combinations DHSs from different critical mESC loci through delivery in place of the Sox2 LCR. We identify widespread genomic examples of context-dependent enhancers which have no activity on their own but impact the activity of a nearby DHS. We show that this synergy between nearby DHSs decays as a characteristic function of distance with its influence extending up to several kilobases. We fine map this context dependency to the contribution of individual TF recognition sequences. Our approach implicates specific sequence and architectural features underpinning the influence of genomic context, and suggests that interpretation of noncoding variation must be done at a haplotype level rather than implicitly assuming the surrounding sequence matches the genomic reference.

Poster session 1 (on-site) - Thursday 14 September 2023 (15:10 – 16:00)

Poster session 2 (on-site) – Friday 15 September 2023 (12:45 – 14:00)

A) Research poster presentations

(PO-01) Gunay Akbarova-Ben-Tzvi: Correction with an extract of Actinidia Chinensis of an inducted mutagenesis on mammals

(PO-02) Goretti Aranaz Novaliches: Cytoplasmic polyadenylation by Tent5a is essential for teeth formation

(PO-03) Federica Gambini: Novel mouse models to study the pathogenecity of SARS-CoV-2

(PO-04) Barbora Hosikova: Sonodynamic sensitive liposomes for in vitro anti-tumor therapy

(PO-05) Marketa Kolarikova: The Enhancement of Anticancer Therapy with Liposome-Enclosed Porphyrin

(PO-06) Betul Ogan: Role of FAM83H in immune system homeostasis

(PO-07) Michaela Procházková: Proper function of CRL4/Cop1 ubiquitin ligase complex is substantial for tooth morphogenesis

(PO-08) Jolana Turečková: Ablation of Atf2 results in highly invasive tumors in an AOM/DSS model of colorectal cancer

(PO-09) Matilde Vale: Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

(PO-10) Michaela Šímová: Uncovering the origins of yolk-sac-derived hematopoiesis

(PO-11) Jana Balounová: Twenty-two-color full spectrum flow cytometry panel for IMPC immuno-phenotyping of major cell subsets in the mouse spleen

(PO-12) Petr Nickl: Adeno-Associated Virus Vectors Enable Efficient Allele Conversion in Mouse Embryos

(PO-13) David Pajuelo Reguera: Effect of the number of mice per cage on the amount of body weight lost during overnight fasting, its calculation using an equation and possible influences on IPGTT

(PO-14) Igor Varga: Statistical shape analysis of mouse skull

(PO–01) Correction with an extract of Actinidia Chinensis of an inducted mutagenesis on mammals

Gunay Akbarova-Ben-Tzvi [1]

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According to the conception of the compositional preservation of the genome, the base for an effective regulation of mutagenesis is the synergy of the effect of each working component. In this regard, research focused on discovery of gene-protective features of new natural and complex drugs and the determination of the mechanism increasing the endurance of the organism to the effect of the mutagens, which is necessary for production of highly effective gene-protective medicaments.

The given work is a complex genetic and bio-chemical research of the gene-protective features of Actinidia Chinensis (Kiwi) on white, 28 weeks, semi-mature mice of both genders, with mean weight 160±10 g.

An extract in a dosage of 0.2-0.5 mg per 100 g of animal mass was tested on the background of the effect of gamma-radiation (60Co in a dosage of 3.008Gy); antibiotics of cytostatic effect: direct mutagen N-methyl-N'-nitro-N-nitrosoguanidine (3.0 mg/100g) and promutagen cyclophosphamide (2.0 mg/100g).

Under the effect of the mutagen, the frequency of chromosome aberrations is increased 6 times; and the quantity of the malondialdehyde in the mitochondrial fraction of the animal's liver, as an indication of free-radical processes. At the same time, the general and the free activity of the enzyme, Glucose-6-phosphotasa, is decreased, as the most responsive indication of the Lipid peroxidation. Our research establishes the quantitative and qualitative changes in the composition of lipid fraction, which points to the intensity of the free-radical oxidation and structural and functional condition of bio-membranes.

When the extract of kiwi seeds in the most effective dosage, 0.4 mg/100g are infused in bone marrow in the bones of the hip before and after mutagen manipulation, the frequency of the induced chromosome aberrations is reduced, which is followed by stabilization of cell metabolism. Although it has been shown that the extract has a correcting effect, at least in two early key stages in the process of creation and formation of pre-mutation, the experimental findings convincingly demonstrate the high effectiveness of the extract in the protection of the mammal genome in its realization under the condition of the primary potential damage of the DNA.

(PO–02) Cytoplasmic polyadenylation by Tent5a is essential for teeth formation

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Tooth enamel formation, known as amelogenesis, is a complex process orchestrated by specialized cells called ameloblasts. These cells secrete enamel matrix proteins (EMPs), including Amelogenin (Amelx) and Ameloblastin (Ambn), which play a crucial role in depositing hydroxyapatite crystals for mineralization. In this study, we utilized a mouse model with a Tent5a knock-out (KO) show that Tent5a non-canonical poly-A polymerase is crucial for EMPs synthesis, secretion and enamel mineralization. Tent5a belongs to the Terminal nucleotidyl transferases (TENTs) superfamily and is responsible for protecting mRNA from degradation, ensuring stability, and promoting mRNA translation.

Our findings using micro-computed tomography revealed that mice lacking Tent5a exhibited teeth hypomineralization, thinner enamel layers, and disrupted enamel patterning. Through nanopore direct mRNA sequencing, we identified that Tent5a polyadenylates Amelx and other mRNAs encoding secreted proteins, thereby increasing their expression during amelogenesis. Tent5a is predominantly localized in the cytoplasm and the endoplasmic reticulum, where it regulates the synthesis of Amelx. Moreover, our results demonstrated that the self-assembly of Amelx in the extracellular organic matrix was impaired in Tent5a KO mice, which is essential for directing hydroxyapatite deposition during enamel formation.

In conclusion, this study establishes the critical role of Tent5a-mediated cytoplasmic polyadenylation in the biomineralization of teeth. Our findings enhance our understanding of the new posttranscriptional regulation mechanisms underlying tooth formation.

(PO–03) Novel mouse models to study the pathogenecity of SARS-CoV–2

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SARS-CoV-2 is the etiologic agent of Covid-19, a highly contagious respiratory illness that emerged in 2019 and rapidly developed into a global pandemic. The lack of suitable animal models to study Covid-19 has posed significant challenges in fully understanding the development of the disease. For instance, Long Covid or sex-specific outcomes of the infection remain poorly understood. Appropriate mouse models are therefore required to investigate the causes, risk factors, and optimal therapeutic approaches. Long Covid is broadly defined as a set of symptoms and conditions that continue or develop after initial infection. Some studies suggested that around 10-30% of Covid-19 patients experience persistent symptoms beyond the acute phase. One of the aims of our project is to study the acute phase and the long-term effects of the infection, including the sex differences that contribute to the outcome of the infection. We have developed a conditional mouse model where human-ACE2 (hACE2) expression could be driven from tissue-specific promoters using an inducible Cre-lox system. Mice with ubiquitous expression of hACE2 were susceptible to the Wuhan variant of SARS-CoV-2. We observed that the infection led to a more severe outcome in males than in females. Moreover, females were more likely to develop milder symptoms and entered the recovery phase. Additionally, we will use different tissue-specific drivers to address the possible consequences of SARS-CoV-2 infection beyond the respiratory system. Moreover, we are developing a model, where hACE2 expression is driven by mouse-ACE2 endogenous regulatory sequences. In conclusion, these models will allow us to deepen our knowledge about crucial aspects of Covid-19 such as Long Covid and sex-specific outcomes of the infection. With these tools, we aim to unravel the mechanisms underlying SARS-Cov2 pathogenesis and improve the overall health outcomes.

(PO–04) Sonodynamic sensitive liposomes for in vitro anti-tumor therapy

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The study is focused on the liposomes containing a sonodynamically active substance, and in vitro research of the effect of high-frequency ultrasound, causing the release of these substances in target cells in order to increase the effectiveness of antitumor therapy. DPPC lipids (Avanti Polar Lipids, USA) were used for the formation of liposomes. Liposomal carriers (200 nm) were created using extrusion followed by purification of the non-encapsulated sonosensitizer by centrifugation. The size of the liposomes and zeta potential were evaluated by DLS (dynamic light scattering; Zetasizer Nano ZS; Malvern). The fluorescence and atomic force microscopy (AFM) were used to visualize the liposomes. The MTT assay assessed cell viability. DNA damage in terms of double strand breaks formation was evaluated by the confocal microscopy and FiJi software.

A difference in size distribution and zeta potential related to the number of passages through the extruder membrane was found. The incorporation of the fluorescent substance into the liposomes and their presence in the target cells was confirmed. The use of liposomes led to a reduction in the required drug incorporation time. When TMPyP was incorporated into liposomes, there was a change in cell localization, compared to the sensitizer itself. In the case of TMPyP alone, localization was mainly in the nucleus, while for lipo-TMPyP in lysosomes.
Treatment of HeLa cells with DPPC:TMPyP and DPPC caused higher number of foci with a smaller foci area in case of DPPC:TMPyP. Intracellular localization of free TMPyP in cell nuclei points to a possible mutagenic effect, representing a limitation in therapy. Thus, the use of lipo-TMPyP with a majority localization in lysosomes could lead to a reduction of the negative side effects of antitumor treatment.
Supported by Ministry of Health of the Czech Republic, grant nr.NU21J-03-00062.

(PO–05) The Enhancement of Anticancer Therapy with Liposome-Enclosed Porphyrin

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Photodynamic therapy is an alternative treatment for cancerous but also noncancerous diseases. The anticancer Photodynamic therapy (PDT) is based on the application of a photodynamically active substance, so-called photosensitizer, its selective accumulation in malignant tissue and subsequent irradiation with a specific wavelength leading to the activation of the photosensitizer further causing the cell death. Although the concept of PDT is dated to 1900, drug delivery modifications to enhance the therapeutic effect of this therapy present an unremitting challenge in clinical field. As nanoparticle carrier systems tend to accumulate passively in tumor tissues through the leaky tumor vasculature, there is a demand for the delivery systems of a nanoparticle size in this therapy. Thus, liposomes present a type of clinically well-established nanoparticles, consisting of inner aqueous core where hydrophilic molecules can be captured, and lipid bilayers where hydrophobic molecules accumulate. Here, the extrusion method for the formation of 200 nm DPPC:Chol liposomes was used to encapsulate a porphyrin photosensitizer (TMPyP). Therapy was induced with blue LED light 4 and 24 hours after the TMPyP administration to HeLa and NIH3T3 cells. To assess the treatment efficiency, fluorescence lifetime imaging microscopy (FLIM) was used to detect the lifetime of free/bound states of FAD. The liposomal size was detected using DLS (Dynamic Light Scattering) measurement. The intracellular localization of free TMPyP and TMPyP enclosed in liposomes was assesed on HeLa cell line using confocal spinning disk imaging. The viability of HeLa cell line after the therapy application was determined via MTT assay.

This work was supported by Ministry of Health of the Czech Republic, grant nr.NU21J-03-00062.

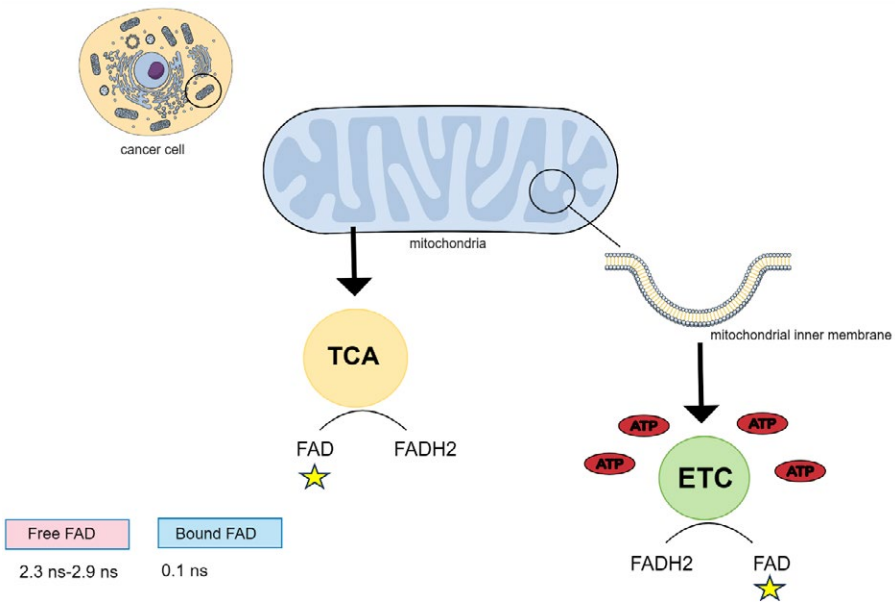

Fig. 1: Autofluorescence as a tool to assess a treatment efficiency based on ratio differences of free/bound states of FAD using FLIM system.

(PO–06) Role of FAM83H in immune system homeostasis

Betul Ogan [1], Laura Jane Dowling [2], Michaela Šímová [1,2], František Špoutil [2], Jan Procházka [1,2], Radislav Sedláček [1,2], Jana Balounová [1,2]

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FAM83H is expressed mainly in epithelial cells and has been suggested to be responsible for intracellular transport, regulation of cytoskeletal networks, and enamel formation. A deficiency of FAM83H was reported to be the cause of amelogenesis imperfecta (AI), a soft enamel disease. Interestingly, two AI patients from one family in Czechia with confirmed FAM83H mutation developed juvenile rheumatoid arthritis. To understand the function of FAM83H in immune system homeostasis, we generated Fam83h knock-out (KO) and mutant mouse (Fam83htg/tg). Both mutant and KO animals exhibit decreased body size, sparse and scruffy coats, scaly skin, weakness, and hypoactivity. While we have not observed dentin-related phenotype, mutant, and KO pups show severe swelling of their forepaws accompanied by bone deformation at as early as 3 weeks. However, the soft tissue lesions are being resolved and peripheral blood leukocytes return back to normal levels at 7 weeks of age. Moreover, mutant juvenile animals have increased neutrophils as well as G-CSF and inflammatory cytokine levels in their peripheral blood. Additionally, the development of leukocytes including T, B, and NK cells is severely impaired in Fam83h mutants. Specifically, T cell development is arrested at the double-negative stage, B cells at the pro- and pre-B cell stages, and NK cells at the immature NK cell stage. Altogether, our findings advocating for the importance of FAM83H's role in the hematopoietic niches and leukocyte development will contribute to the unraveling of the role of Fam83h in the onset of arthritic lesions and, in general regulation of the immune system.

(PO–07) Proper function of CRL4/Cop1 ubiquitin ligase complex is substantial for tooth morphogenesis

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The main role of the Cullin-RING ubiquitin ligase (CRL) complexes is the ubiquitination of substrate proteins. Altered expression of genes from CRL family has been associated with many different types of invasive cancer diseases. Even though it has been shown that many cancerogenic processes are based on misused developmental molecular programs, the role of CRLs in these processes is largely unknown with exception of the connection between CRL4 and the teratogenic potential of thalidomide during prenatal limb outgrowth via regulation of Fgf8 (Ito et al., 2010). Notably, Fgf8 expressing population is essential also for development of tooth primordia (Prochazka et al., 2015).

We have mapped the expression pattern of distinct CRL genes and revealed that CRL4 genes are specifically expressed within developing mouse tooth primordia. Using explant tissue culture approach we uncovered that inhibition of CRL complexes severely disrupts embryonic tooth development – the migratory epithelial cells fail to form the molar primordia.

We have further identified Cop1 as putative substrate binding protein within CRL4 complex in the developing mandible via coIP followed by mass-spectrometry analysis. In order to evaluate the role of Cop1 and CRL4 as such in odontogenic tissue in vivo we crossed Cop1 flox and Ddb1 flox with Fgf8CreER mouse line. Phenotypization of these models revealed that conditional deletion of CRL4 genes severally affects size and morphology of molar primordia and future teeth. We also examined the ETS transcription factors, downstream effectors of Fgf pathway, as plausible targets of CRL4/Cop1 complex during odontogenesis.

Our results uncovered that CRL4 ubiquitin ligase complex is essential for proper behaviour of orofacial epithelium during tooth formation and morphogenesis.

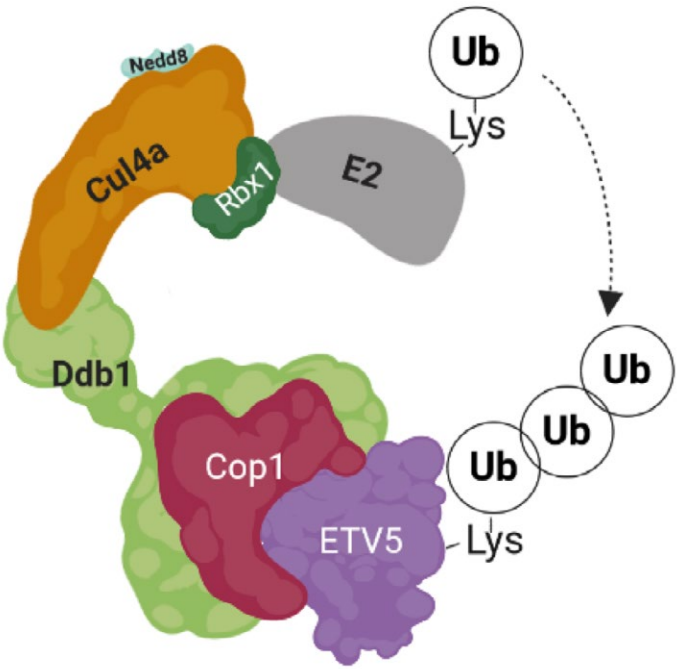

Fig. 1: CRL4/Cop1 ubiquitin ligase complex

(PO–08) Ablation of Atf2 results in highly invasive tumors in an AOM/DSS model of colorectal cancer

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Aim: Activating transcription factor 2 (Atf2) is involved in many physiological and developmental processes including colorectal cancer (CRC). Yet, the role of Atf2 in CRC is poorly understood. Therefore, we aimed to unravel a role of Atf2 in tumor growth using a novel conditional Atf2KO mouse model.

Method: We established conditional Atf2fl/fl mouse strain bred with tamoxifen-inducible villin-CreERT2 mice to delete Atf2 transcription in intestinal mucosa. The latter mice were first injected with two doses of tamoxifen. The animals were subsequently injected with one dose of AOM and then underwent chronic colitis induced by two runs of treatment with 2% DSS. After additional 4-week period, we compared tumor development in vil-CreERT2- and vil-CreERT2+ animals. Colons were pre-stained with Alcian blue to estimate tumor numbers. Then, tissues were fixed and paraffin-embedded for immunohistochemical analysis.

Results: We performed a detailed histological analysis of colorectal tumors formed in our Atf2KO model. Both early and late tamoxifen-induced ablation of Atf2 led to a significantly early onset of invasive tumor growth. Importantly, while most of vil-CreERT2- mice developed sessile serrated lesions of low- and high-grade dysplasia, vil-CreERT2+ mice displayed invasive serrated adenocarcinoma with infiltrations, desmoplastic stroma and mucinous areas. We also found differences in number and size of the lesions in both genotypes. According to in silico analysis of Atf2KO gene signature it resembles that of the CMS3 CRC subtype. We evaluated some of genes of the signature by IHC in the AOM/DSS-derived tumors.

Conclusion: Our results show that ablation of Atf2 accelerates colorectal cancer. This Atf2KO mouse might present a suitable model for therapeutic strategies.

(PO–09) Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

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Diamond-Blackfan Anemia (DBA) is an inherited bone marrow failure syndrome characterized by rare congenital bone marrow erythroid hypoplasia. In addition to anemia, approximately 50% of DBA patients also develop congenital malformations, primarily located in the cephalic region and the thumbs. This condition is further associated with short stature, cardiac and urogenital tract abnormalities. DBA is classified as a ribosomopathy due to its origin in mutations within ribosomal protein (RP) genes, which result in defective maturation of ribosomal RNA (rRNA).

The primary treatment for DBA involves the use of corticosteroids. However, owing to their long-term side effects, patients typically require chronic blood transfusions alongside concurrent iron chelation therapy. In some cases, patients may undergo hematopoietic stem cell transplantation (HSCT), the sole curative approach for the hematological aspect of DBA. Nevertheless, HSCT carries risks of rejection and infection. Consequently, gene therapy presents an appealing potential solution when an underlying genetic mutation is identifiable.

This project aims to develop gene therapies utilizing Extracellular Vesicle (EV) and lentivirus (LV) methods to rescue DBA mouse models. We isolate exosomes from mice plasma using ultracentrifugation, followed by sample characterization in terms of purity, particle heterogeneity, and size. Various techniques, including MADLS, TEM, lipidomics, proteomics, and clinical chemistry analysis, are employed for this purpose.

Concurrently, we are striving to establish lentiviral vectors for gene therapy. Our initial approach involved generating an integrating lentivirus that expresses luciferase, which was then delivered to mouse bone marrow via intrafemoral injection (IF). Additionally, the same procedure was adapted for a non-integrating lentivirus expressing luciferase, developed by Flash Therapeutics. This non-integrating strategy offers a significant advantage to our project, as it permits transient utilization of the CRISPR-Cas9 system.

(PO–10) Uncovering the origins of yolk-sac-derived hematopoiesis

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The emergence of blood cells during mammalian embryonic development occurs in three independent waves. The first blood cells emerge in the extra embryonic Yolk sac (YS) and they are essential for successful embryonic development. YS provides the embryo with primitive erythrocytes (wave 1) and later with erythro-myeloid progenitors (EMPs; wave 2). Hematopoietic stem cells (HSC, wave 3) emerge later in the intraembryonic aorta-gonad-mesonephros region. The 2nd and 3rd wave progenitors then colonize the fetal liver, where they expand and differentiate. Before birth, these progenitors seed the bone marrow, where the HSCs gradually take over for the hematopoiesis.

Due to the high similarity of all hematopoietic progenitors, there is a lack of genetic models that would enable the specific tracing of individual hematopoietic waves. Consequently, the molecular details behind the niche-dependent specification of different progenitors are still poorly understood.

The goal of our project is to map the origins and development of Yolk sac (YS)-derived hematopoietic lineages. We are utilizing scRNA-Seq in combination with flow cytometry to identify new markers of distinct hematopoietic progenitors. To uncover the molecular mechanisms behind the emergence and differentiation of erythro-myeloid progenitors (EMPs), we are analyzing knockout models of EMP-relevant genes. Additionally, we are in the process of developing more precise lineage tracing models that will enable us to distinguish the hematopoietic waves in their respective niches.

By focusing on EMP hematopoiesis using the aforementioned tools, our objective is to map the cell differentiation and the modulation of EMPs by diverse niches, as well as to reveal the origin of primitive erythrocytes and assess the existence of primitive macrophages.

(PO–11) Twenty-two-color full spectrum flow cytometry panel for IMPC immuno-phenotyping of major cell subsets in the mouse spleen

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Besides other assays, IMPC immuno-phenotyping includes characterization of mouse splenic immune cell populations in terms of their cellularity and phenotype using multicolor flow cytometry (FCM). According to the International Mouse Phenotyping Resource of Standardised Screens (IMPReSS) guidelines (https://www.mousephenotype.org/impress/ProcedureInfo?action=list&procID=1225), two panels (IMPC Panel A & Panel B) are utilized to discriminate subsets of lymphoid and myeloid cells in the mouse spleen at CCP.
To make this effort more efficient, we have optimized a 22- color panel that combines all mandatory IMPC immuno-phenotyping parameters that should be measured by all IMPCs plus one optional marker that enables to determine the antigen inexperienced (AIM) memory T cells. We have primarily designed this panel for a spectral flow cytometer, however to enable its broad use, we chose the fluorochromes that could be easily separated using conventional flow cytometers equipped with 5 lasers (UV/V/B/YG/R).

The final panel covers all main leukocyte subtypes, including T cells, B cells, NK cells, NKT cells, macrophages, neutrophils, eosinophils, monocytes, plasmacytoid, and conventional dendritic cells. Additionally, subtypes of B (follicular/ marginal zone/ B1a) cells, T (γδ, helper and regulatory T cells as well as their effector/ memory/ naïve /AIM states) cells, NK cells, NKT cells and cDCs are covered with the panel.

Merging the two panels in one enables for more precise determination of leukocyte populations, reduces the reagent costs, workload as well as analysis time, making the IMPC immuno-phenotyping effort more efficient.

The universality and complexity of this panel should be of interest also beyond the IMPC scope. With minor modifications this panel could be used for immuno-phenotyping of any other mouse tissue, tumors or cells expressing a fluorescent tag.

(PO–12) Adeno-Associated Virus Vectors Enable Efficient Allele Conversion in Mouse Embryos

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Site-specific recombinase technology is a powerful tool with various applications, including mouse transgenesis. This study aimed to investigate the use of adeno-associated viruses (AAV) as a delivery system for Flp and Vika recombinases, which had previously shown inefficient in vitro protein synthesis. The Flp, Vika, Cre, and Dre recombinases were produced as AAV vectors and applied to mouse embryos carrying a transgenic cassette for Cre/Dre/Flp/Vika-dependent expression of GFP protein. The efficiency of gene conversion using AAV vectors was found to be comparable to the traditional methods of protein electroporation or mRNA injection. The results demonstrated that Flp or Vika AAV-based delivery achieved high efficiency, similar to protein electroporation or mRNA injection, while offering the advantages of being non-invasive and reducing animal consumption. This study showcased the versatility of adeno-associated virus vectors and their potential application in transgenic practices. The findings contribute to simplifying the process of generating completely converted mice, thereby minimizing animal usage and advancing the field of mouse transgenesis.

(PO–13) Effect of the number of mice per cage on the amount of body weight lost during overnight fasting, its calculation using an equation and possible influences on IPGTT

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Using mice that have been phenotyped systematically in our center for the international mouse phenotyping consortium (IMPC), we obtained extra data and measured the body weight before and after the 16h fasting previous intraperitoneal glucose tolerance test (IPGTT) of each mouse, as well as the number of mice per cage. The hypothesis was that, regardless of the sex of the mice, more populated cages would lose less body weight from starvation due to better energy conservation because the nesting effect in control C57Bl/6NCrl caged mice.

According to the findings, our hypothesis is correct, as the number of mice in each cage increase, the mice‘s overnight fasting weight tends to decrease. Additionally, we created a model with an equation which is able to predict how much weight a mouse would lose by entering three parameters: initial body weight, sex, and total number of mice per cage. This equation was generated from 760 mice observations. We also provide evidence that the number of animals in each cage may have a slight impact on the glucose concentration during IPGTT, particularly at the 30 minutes time point.

(PO–14) Statistical shape analysis of mouse skull

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Phenotyping the skull of a mouse is an essential aspect of biomedical research, particularly in gene mapping. Phenotyping the mouse skull involves the detailed analysis and quantification of its morphology, including size, shape, and density. This is typically accomplished using imaging techniques, such as micro-computed tomography (micro-CT) and histology, and quantitative analyses, such as landmark-based geometric morphometrics.
Linear registration techniques have long been employed for their ability to align images with a rigid transformation. In our research, we utilise linear registration to establish an initial alignment between mouse skull images. This preliminary alignment provides a crucial starting point for subsequent analyses, reducing errors arising from gross misalignments. However, the mouse skull's complex and intricate features often require more advanced methods to achieve accurate phenotyping. Nonlinear registration techniques come into play to capture intricate shape variations that linear methods alone cannot address. These techniques employ deformable transformations to account for local shape differences, enabling precise mapping of anatomical structures. Registration techniques are commonly used for phenotyping with manual landmarks. Our technique utilise registration to randomly selected reference subject where we labelled skull. Further, we transformed subject labels with transformations produced by the registration method for all subjects (see Figure 1).

Our findings demonstrate that the fusion of linear and nonlinear registration techniques enhances the efficiency and accuracy of mouse skull phenotyping, providing valuable insights for researchers in various disciplines (see Figure 2). This approach can contribute to a deeper understanding of craniofacial development, evolution, and genetic determinants in mouse models, ultimately advancing our knowledge in these critical areas of study.

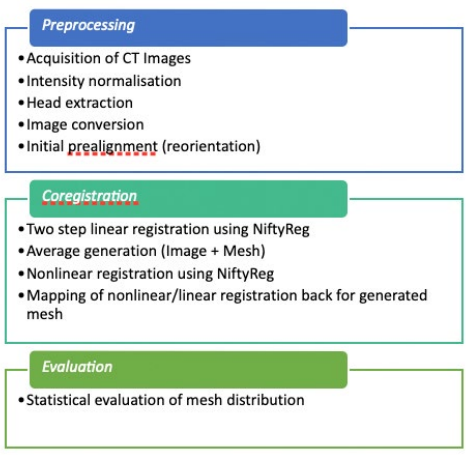

Fig. 1: Shape analysis pipeline

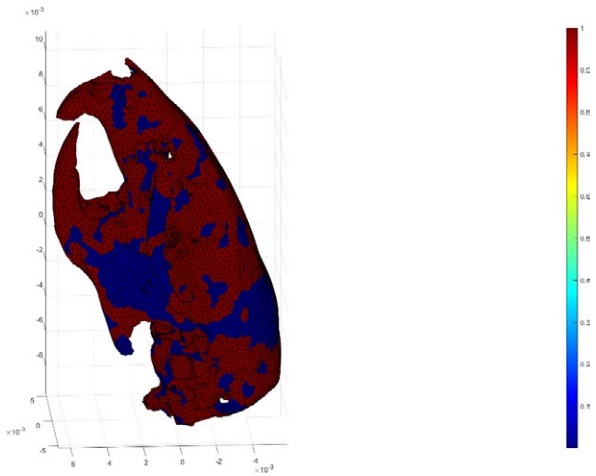

Fig. 2: Statistical difference between male and females (blue p-values < 0.05)

B) Infrastructure poster presentations

(PO-15) Juraj Lábaj: Biochemistry and Haematology Unit (CCP, Phenotyping Module)

(PO-16) František Špoutil: Bioimaging & Embryology Unit (CCP, Phenotyping Module)

(PO-17) Zuzana Nichtová: Cardiovascular Unit (CCP, Phenotyping Module)

(PO-18) Jiří Lindovský: Hearing & Electrophysiology Unit (CCP, Phenotyping Module)

(PO-19) Juraj Lábaj: Histopathology Unit (CCP, Phenotyping Module)

(PO-20) Jana Balounová: Immunology Unit (CCP, Phenotyping Module)

(PO-21) David Pajuelo Regeura: Metabolism Unit (CCP, Phenotyping Module)

(PO-22) Karel Chalupský: Metabolomics Unit (CCP, Phenotyping Module)

(PO-23) Kateryna Pysanenko: Neurobiology & Behaviour Unit (CCP, Phenotyping Module)

(PO-24) Silvia Magalhaes Novais: PDX & Cancer Models Unit (CCP, Phenotyping Module)

(PO-25) Marcela Palková: Vision Unit (CCP, Phenotyping Module)

(PO-26) Dominik Arbon: Models of Infection Diseases (BSL3) (CCP, Phenotyping Module)

(PO-27) Vendula Novosadová: Bioinformatician Unit (CCP, Phenotyping Module)

(PO-28) Petr Nickl: Transgenic and Archiving Module (CCP)

(PO-29) Gizela Koubková: Preclinical testing at the Czech Centre for Phenogenomics

(PO-30) Lucie Dufková: Animal Facility Module (CCP, Vestec)

(PO–15) Biochemistry and Haematology Unit (CCP, Phenotyping Module)

Juraj Lábaj [1], Mariya Glushchenko [1], Eva Štefancová [1], Francisco Machancoses Hernández [1], Jan Procházka [1], Radislav Sedláček [1]

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Clinical Chemistry (the study of the chemical composition of the blood plasma), haematology (the study of the blood cellular components), and urinalysis (analysis of chemical and cellular composition of urine) are integral part of clinical pathology which provides a quantifiable way to assess animal health and to diagnose disease and toxicity. Clinical chemistry analyses of plasma/serum and urine comprise of metabolites, ions, enzymes, and serological quantifications that could be used to assess metabolic and functional abnormalities of different organs of the body. Examination of whole blood for haematology may reveal pathologies or treatments that affect blood cell populations and coagulation.

We use advanced analytical platforms maintained at high standards with methodologies following robust screening protocol by the International Mouse Phenotyping Resource of Standardized Screens (IMPReSS). Furthermore, the Biochemistry and Haematology Unit is a GLP (Good Laboratory Practice) – certified, SUKL (State Institute for Drug Control, ČR) – audited laboratory capable of analyzing samples from pre-clinical studies. The Unit can likewise measure multitude of biomolecules from a single sample using different panels for multiplex immunoassays and tested kits for individual analytes. Multiplexing is done by a bead- and flow cytometry-based assay utilizing Luminex® xMAP® technology in a flexible analyzer.

More information at www.phenogenomics.cz/phenotyping/biochemistry-and-haematology/.

Instrumentation and Technologies:

CLINICAL CHEMISTRY PLATFORMS:
The Beckman Coulter AU480 Clinical Chemistry Analyzer - Electrolytes, enzymes, and organic analytes can be measured as part of a clinical chemistry panel. Available panels include: Liver, kidney, pancreas, inflammation, lipid, cardiac & muscle, anemia, bone and IMPC.
The Siemens CLINITEK Advantus® Urine Chemistry Analyzer - utilizes reflectance spectrophotometry to semiquantitatively analyze urine test strips.

HAEMATOLOGY PLATFORMS:
The Mindray BC-5300 Vet - for measurement of veterinary complete blood count and WBC differentials.
The Siemens Sysmex® CA-560 Automated Blood Coagulation Analyzer - for measuring different blood coagulation parameters via coagulation, chromogenic, or immunoassay methodology (bottom).

IMMUNOASSAY PLATFORMS:
Singleplex ELISA assays or multiplexing using spectrophotometer or the Bio-Plex® 200 Luminex. Samples that can be analyzed include serum/plasma, lavages, urine, milk, culture media, and cell/tissue culture supernatants.

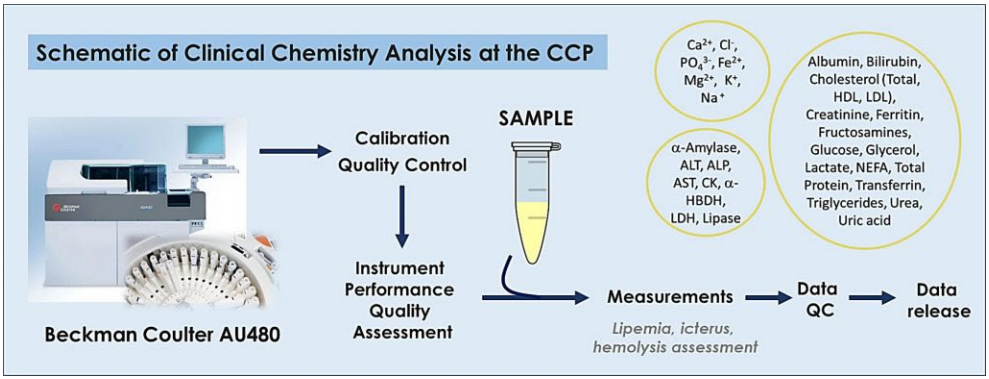

Fig 1. The Beckman Coulter AU480 Clinical Chemistry Analyze

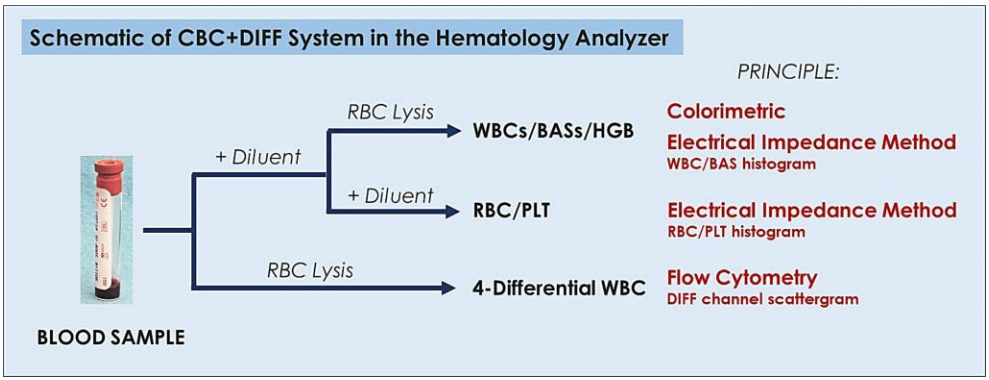

Fig 2. The Mindray BC-5300 Vet

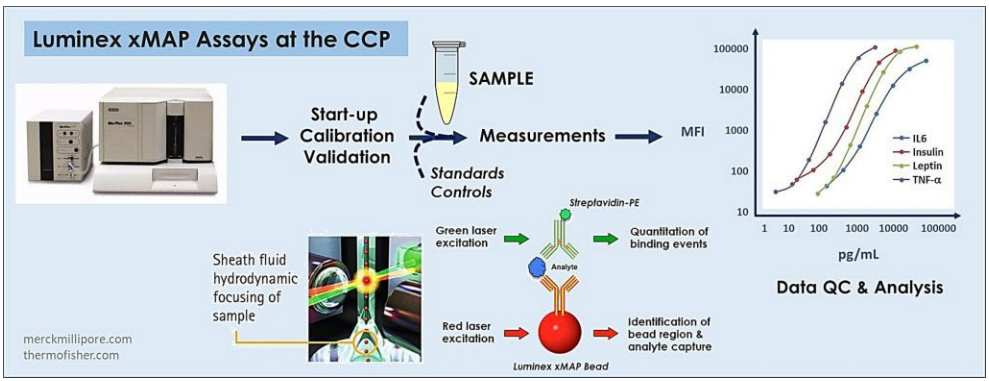

Fig 3. The Bio-Plex® 200 Luminex

(PO–16) Bioimaging & Embryology Unit (CCP, Phenotyping Module)

Michaela Prochazkova [1], Frantisek Spoutil [1], Ivana Bukova [1], Sylvie Dlugosova [1], Barbora Kinska [1], Veronika Martinkova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Siciences, v. v. i. (Biocev), Vestec, CR

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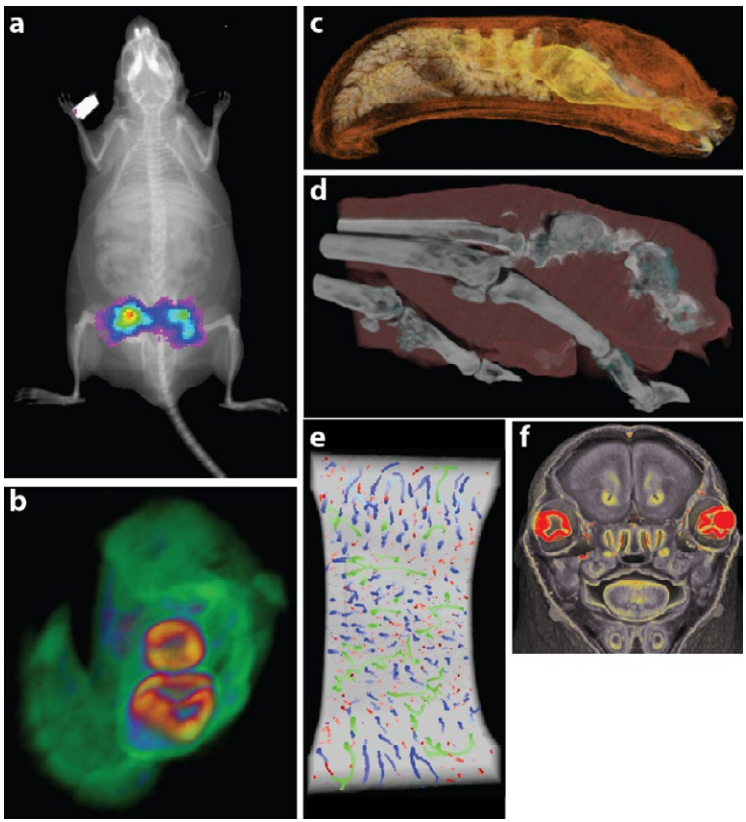
The Bioimaging and Embryology unit is focused on functional morphology projects using state of art 3D imaging technologies of adult mice, rats as well as murine embryos, enamel etc. The anatomical annotation of skeleton dysmorphologies and developmental disorders is a key feature for interpretation of morphology phenotypes. The unit also provides the knowledge base for conditional gene inactivation, embryonic tissue isolation and dissections for OMICs or establishment of primary cell cultures.

The key technological base of our work lies in 3D imaging with µCT which allows visualization not only of mineralized tissues but also of soft tissues with use of appropriate contrast with resolution from 100 to 0.5 µm/vx. The µCT technology is the best cost-effective approach for 3D visualization and the unit provides full data analysis platform. Besides the 3D imaging, the unit is equipped with whole body imaging system for imaging of fluorescence and bioluminescence reporters in mice and rats in-vivo. This technology is very advantageous especially for imaging of cancer models. For non-invasive imaging and cell labelling the set of lentiviral and AAV reporters is available. The physiological processes like inflammation, kidney function or specific enzyme activity can be also non-invasively imaged this way. The unit also provides functional assays on primary cells or their isolation for multiOMICs, offers dissection of embryonic tissues followed by primary cell line or organ culture setup, and delivers immortalized cell lines from KO phenotypes. These approaches can help to substantially accelerate the research of mutants with embryonic lethal phenotypes.



Examples of recent papers:

Structural and functional basis of mammalian microRNA biogenesis by Dicer. Zapletal D et al. Molecular Cell, 2022

Early evolution of enamel matrix proteins is reflected by pleiotropy of physiological functions. Spoutil F et al. Scientific Reports, 2023

Fig. 1: Examples of our outputs: Tumor progression visualized by RFP (a). Teeth developing in kidney capsule (b). Slug anatomy (c). Arthrosis progression in paws (d). Cortical bone porosity (e). Mouse embryo E18.5, frontal section (f). Iodine contrast used in c and f.

(PO–17) Cardiovascular Unit (CCP, Phenotyping Module)

Zuzana Nichtova [1], Jiri Lindovsky [1], Petr Macek [1], Sara Brilhante Viegas Dias [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics BIOCEV – Institute of Molecular Genetics of the Czech Academy of Sciencs

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The cardiovascular system is a fundamental pillar of life. Within CCP, IMG's Cardiovascular Unit, we offer an array of services aimed at uncovering insights into cardiovascular traits in rodent models, particularly mice and rats.
Employing a variety of non-invasive techniques, notably echocardiography, we leverage ultrasound's capabilities to explore the structure and function of heart and vessel systems. This enables us to thoroughly analyze blood flow patterns, chamber efficiency, and more. Our Cardiovascular Unit adeptly navigates through cardiovascular examinations of fetuses, pups, and adults alike, presenting the option of 4D visualization for a comprehensive cardiac evaluation, including strain analysis. Electrocardiography (ECG) permits us to monitor heart electrical activity in conscious or anesthetized rodents. To deepen our understanding of cardiovascular well-being, we incorporate blood pressure measurements.

Additionally, our Cardiovascular Unit embraces challenges to the cardiovascular system. We subject it to controlled physiological stress via exercises, employing treadmill-based assessments to evaluate cardio-metabolic traits. Furthermore, we induce pharmacological stress, often utilizing catecholamines, to simulate pathological scenarios.
Our scope extends further with general sonography services, encompassing gravidity checks, blood flow evaluations across various structures (from eyes to tumors), and even quantification of tumor size and vascularization. Our adeptness in imaging spans diverse anatomical locations, bolstering our capabilities.

A distinctive aspect of our services is the Image-Guided Injection (IGI) technique, facilitated by ultrasound. This innovation enables precise delivery of minute volumes (such as viruses or drugs) into specific organ regions at all developmental stages.
Our pursuit of unravelling cardiovascular traits serves a twofold purpose: illuminating gene functionality and unravelling disease mechanisms. Through these efforts, we contribute to refining therapeutic strategies, holding the potential to enhance both the quality and duration of human lives.

(PO–18) Hearing & Electrophysiology Unit (CCP, Phenotyping Module)

Jiri Lindovsky [1], Kvetoslava Klajblova [1], Miles Raishbrook [1], Jan Prochazka [1], Radislav Sedlacek [1]

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The unit provides electrophysiological methods for functional testing of hearing and vision in mice and rats.
In principal, techniques used are based on recording of electric potentials of sensory pathways evoked by relevant stimuli. Presence or absence and size or form of the evoked potentials is then interpreted as a correlate of activity and functional status of individual structures of the nervous system.

SERVICES
- Auditory Brainstem Response (ABR)
- Electroretinography (ERG)
- Multifocal Electroretinography (mfERG)
- Visual Evoked Potential (VEP)
- Force of isometric muscle contraction
- In development: wireless EEG

DEVICES AND TECHNOLOGIES
Hearing:
- 6 m^3 sound-attenuated chamber
- Tucker-Davis Technologies System 6
- Custom-made scripts for data analysis (Matlab, Java Script)

Vision:
- Roland Consult RETIanimal
- Red IVC cages for dark adaptation (Tecniplast)
- Custom-made scripts for data analysis (Matlab)

Muscle force:
- Digitimer Neurolog System, custom made recording chamber, Matlab.

EEG:
- TSE Neurologger

(PO–19) Histopathology Unit (CCP, Phenotyping Module)

Juraj Lábaj [1], Olha Fedosieieva [1], Hana Holá [1], Antonia Mastrangeli [1], Pavlína Macková [1], Šárka Suchanová [1], Jan Procházka [1], Radislav Sedláček [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

E-mail of the presenting author: juraj.labaj@img.cas.cz

The Histopathology unit is one of the largest units of the CCP Phenotyping Module and provides service for a broad range of research community including users working with non-rodent material. The unit is particularly engaged in experimental pathology. The work flow of the histopathology laboratory covers all procedures from gross morphology through various staining techniques and fluorescent slide scanning to pathology description. Complete necropsy of mouse/rat is performed by veterinary pathologist and all macroscopic findings are documented. Almost all steps in tissue processing and slide preparation are automatized to achieve the highest levels of reproducibility and quality. The lab offers H&E staining done by automated stainer, wide range of special stains and immunochemistry. The microscopic evaluation of histological samples is done by pathologist and complex report with picture documentations is a standard. Most of activities are conformed to Good Laboratory Practices (GLP).

INSTRUMENTATION & TECHNOLOGIES:
Tissue processing: Leica ASP6025 - The most modern vacuum tissue processor

Sectioning fresh specimens: Vibratom Leica 1200 - automated vibrating blade microtome

Slide staining: Multistainer Leica ST5020 in conjuction with Leica CV5030 Coverslipper - an exceptionally versatile stainer-coverslipper workstation; Ventana Benchmark Special Stains - Automated slide stainer for special stains; Ventana Discovery ULTRA - Automated stainer for immunohistochemistry and in situ hybridization

Microscopy and analysis: Carl Zeiss Axio Imager.Z2 - motorized microscope imaging station, capable of both brightfield and fluorescence capture; Leica DM3000 - Semi automated high-throughput brightfield microscope system

Slide scanning:
Carl Zeiss Axio Scan.Z1 - Combined brightfield and fluorescence slide scanner with ability to also scan histotopograms. Equipped with ultra-fast LED fluorescent module and 7 different excitation/emission filters.

ConaPat - Tracking system

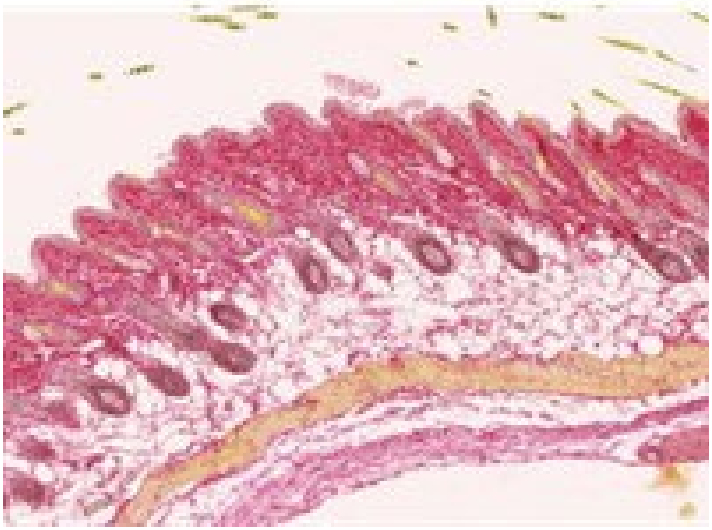

Fig. 1: Skin- Picro Sirius Red

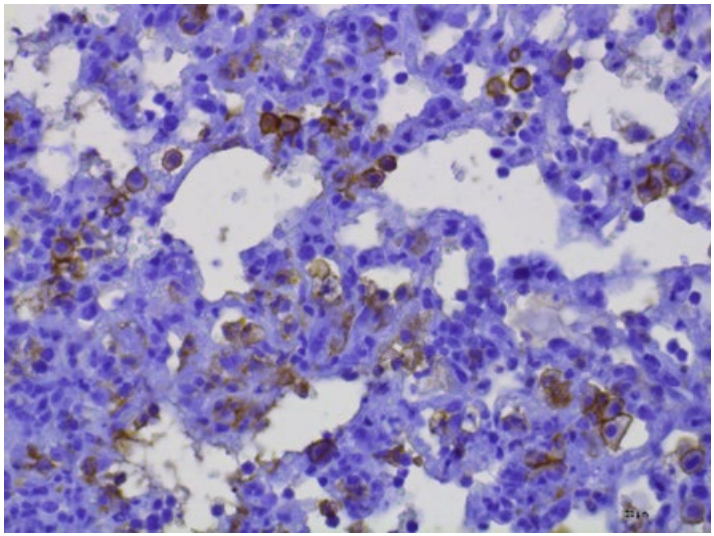

Fig. 2: Lungs - IHC, macrophages

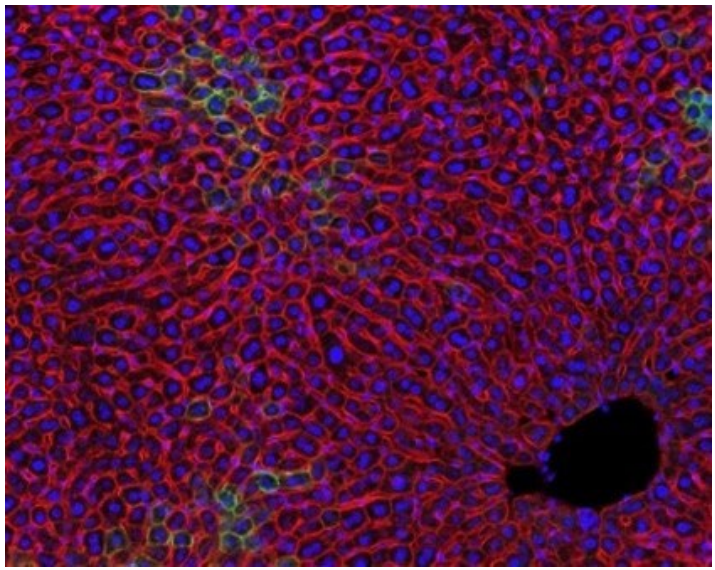

Fig. 3: Liver- Cre

(PO–20) Immunology Unit (CCP, Phenotyping Module)

Jana Balounova [1], Laura Dowling [1], Michaela Simova [1], Veronika Forstlova [1], Carlos Trufen [1], Kamila Krizova [1], Kristina Vicikova [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences

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As an integral part of the terminal screen, immunophenotyping involves characterization of particular immune cell populations in terms of their cellularity and phenotype using multicolor flow cytometry (FCM). The procedures are based on standard immunophenotyping protocols of the Adult and Embryonic Phenotype Pipeline that has been agreed by the research institutions involved: IMPReSS -International Mouse Phenotyping Resource of Standardised Screens. According to these guidelines, we utilize two panels (IMPC Panel A & Panel B) to discriminate various populations of lymphoid and myeloid cells in the mouse spleen or other tissues (peripheral blood, lymph nodes, thymus, bone marrow, peritoneal lavage, intestine). We have developed FCM assays to analyze cell populations in mouse blood, embryonic as well as adult hematopoiesis, thymus and tumor microenvironment. To characterize the PDX models developed at CCP, we have optimized FCM panels to determine human leukocyte populations in humanized mouse strains. Moreover, we can design a suitable FCM panel to detect, characterize or purify cell populations of interest.

Instrumentation & technologies

The Unit is equipped with Cytek Aurora spectral flow cytometer. With 5 lasers (355, 405, 488, 532, 635nm), three scattering channels, 64 fluorescence channels and automated sample loader, the Aurora system is suitable to acquire high dimensional flow cytometry data in hightroughput. The FCM data is then analyzed in FlowJo software and statistically evaluated. Furthermore, the Immunology Unit is equipped with gentleMACS tissue dissociator (Miltenyi Biotec), EasySep cell separation magnet for column-free cell separation (StemCell Technologies), bright field automated cell counter for counting of viable cells (Cellometer Auto T4, Nexcelom Bioscience) and a microplate spectrophotometer - ELISA reader (BioTek Epoch).

(PO–21) Metabolism Unit (CCP, Phenotyping Module)

David Pajuelo Regeura [1], Pavlina Richtarechova [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics at Institute of Molecular Genetics of the Czech Academy of Science

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Rodent models, especially genetically engineered mouse models, are important for discovering gene functions involved in energy metabolism and glucose homeostasis. For first-line phenotyping, we perform intraperitoneal glucose tolerance test, non-invasive body composition, and indirect calorimetry as a starting point for further in-depth and hypothesis-driven studies. Environmental chambers with controlled light:dark regimes, humidity, and temperature allow us to perform cold challenges, thermoneutral studies, or changes in light-dark rhythms while acquiring indirect calorimetric data in mice or rats. Moreover, we could assess the effect of feeding a specific diet (e.g. a high-fat diet) on overall metabolism.

To study glucose metabolism in more detail, we implemented several tests: basal and maximal blood insulin concentrations can be determined during glucose tolerance testing; to assess insulin sensitivity, we perform an insulin tolerance test. Finally, hepatic gluconeogenesis can be checked with a pyruvate tolerance test. These complementary methodologies help explaining possible defects in glucose metabolism caused by genetic modification or specific treatments. Another newly integrated method are lipid tolerance test and telemetry of physiological parameters, such as body temperature at two locations of the body, or real-time measurement of blood sugar levels. These parameters can be measured in home-caged mice or in combination with indirect calorimetry.
The combination of telemetry with indirect calorimetry opens up a wide range of new possibilities for monitoring metabolic functions in real-time and under ad libitum or challenge conditions or, during experiments involving specific treatments with minimum human intervention.

We perform non-invasive body composition analysis based on TD-NMR technology, which provides a fast and precise method to determine lean and fat mass, and free fluids in mice and rats.

The advantages of not having to anaesthetize the animals and the very fast analysis allow repeated measurements of body composition in time series over time. Like all units at CCP, our metabolism services benefit from integration with other units of the center, enabling the systemic and comprehensive characterization of experimental rodent models.

Fig. 1: Body composition analyzer, using the technology quantitative magnetic resonance (TD-NMR), Minispec LF90II, Bruker

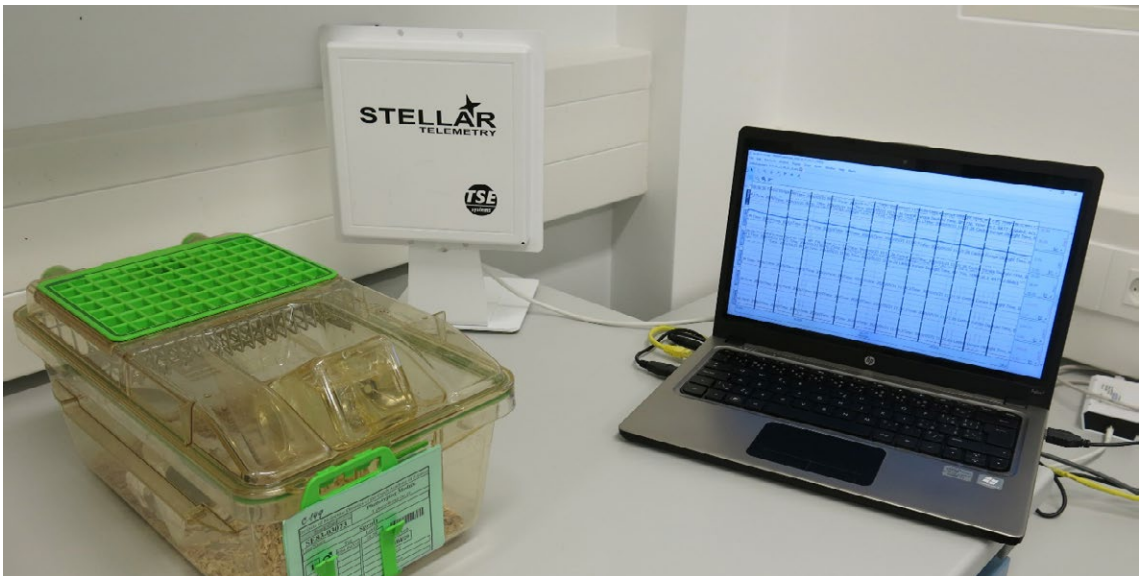

Fig. 2: Telemetry platform compatible with all TSE transponders including: dual temperature, ECG, EMG, EOG, EEG, blood pressure. Stellar antenna, TSE

(PO–22) Metabolomics Unit (CCP, Phenotyping Module)

Klára Dohnalová [1], Kryštof Klíma [1], Evgeniya Biryukova [1], Lukáš Kučera [1], Karel Chalupský [1], Jan Procházka [1], Radislav Sedláček [1]

1. Phenotyping Module, Czech Centre for Phenogenomics, Institute of Molecular Genetics of the CAS, v. v. i

E-mail of the presenting author: karel.chalupsky@img.cas.cz

The Metabolomics unit is expanding the method portfolio of the Metabolic and Clinical Biochemistry Units. The analysis of blood is part of our standard first-line phenotyping. Measuring only a limited number of biochemical markers, increases the risk of missing the physiological impact of a studied gene or a treatment, or the early onset of a disease. Therefore, we implemented metabolomics and lipidomics technology to analyze blood, serum or tissue homogenates that may even give a hint to the mechanistic basis of a disease-relevant phenotype. Using reverse and hilic chromatography we are able to detect and quantify about 300 metabolites. A specific MS/MS lipid library is designed for each lipidomics sample screen and usually consist of over 400 unique lipid species depending of sample type. Additionally, we can also track incorporation of labelled heavy carbon, delivered from 13C glucose, in cell culture samples. Our unit participates in preclinical screening in CCP by targeted detection of experimental compounds and provides stability and pharmacokinetics data. Besides analysis based on liquid chromatography we also provide the mass spectrometry analysis of tissue samples by MALDI imaging. Mass spectrometry imaging is mainly linked with histology and offer analysis of compounds in spatial context, which exceed the possibilities of classical histology. We are able to detect more than three hundreds of molecules on tissue slides. Our metabolomics unit has shown great potential in several biological applications. Discovery of diagnostic biomarkers, drug metabolization and their effects on whole metabolome, and progression of diseases are examples where studying metabolite profiles provided additional value also regarding translation to human disease. Using statistical methods allows to process and compare large data sets. Additional effort is put into the identification of unique metabolites and to map those to specific metabolic pathways which may be an important hint towards the molecular mechanism underlying the function of a gene.

(PO–23) Neurobiology & Behaviour Unit (CCP, Phenotyping Module)

Kateryna Pysanenko [1], Katarina Kanasova [1], Pavel Jina [1], Pavlina Kucerova [1], Jan Prochazka [1], Radislav Sedlacek [1]

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Genetic engineering opens an avenue of research opportunities to probe molecular bases of a variety of human diseases. Neurobehavioural tests using transgenic animal models make it possible to understand genetic mechanisms underlying neurological and psychiatric disorders including, but not limited to, anxiety, schizophrenia, mood disorders, and Parkinson's disease. The Neurobiology and Behaviour Unit employs a number of tests to examine motor abilities, cognitive functions, emotion, sensory processing as well as neurological, and gait impairments in transgenic mice.

Neurobiology and Behaviour module offers standardized primary and secondary phenotype screens based on IMPC (International Mouse Phenotyping Consortium) protocols (https://www.mousephenotype.org/impress). Primary/mandatory screens include modified SHIRPA and dysmorphology evaluation, Open Field, Grip Strength, Acoustic Startle and PPI, Light/Dark Box, and Fear Conditioning.
The Unit also offers more specific secondary/optional screens that comprise tests evaluating animal emotionality and affect (Elevated Plus Maze, Forced Swim Test, Tail Suspension Test), cognitive function (Cued and Contextual Conditioning, Context Discrimination, Spontaneous Alternation, Barnes Maze, Novel Object Recognition), neuromotor abilities (RotaRod, Gait Analysis), pain sensitivity (Hot/Cold Plate, Tail Flick, Plethysmometer, von Frey Test), social preference, and last but not least evaluation of animal cognitive function and circadian activity in more natural conditions in IntelliCages. Oversimplified „impoverished" environments together with stress from human handling may be responsible for substantial heterogeneity in the results of conventional behavioural tests. Social group housing in a large enclosure equipped with multiple gadgets in IntelliCage provides environmental enrichment beyond typically employed protocols. It also eliminates stressful interaction between the animal and the experimenter.

(PO–24) PDX & Cancer Models Unit (CCP, Phenotyping Module)

Silvia Magalhaes Novais [1], Jan Prochazka [1], Radislav Sedlacek [1]

1. Czech Centre for Phenogenomics, Institute of Molecular Genetics of the CAS, 25250, Vestec, Czech Republic

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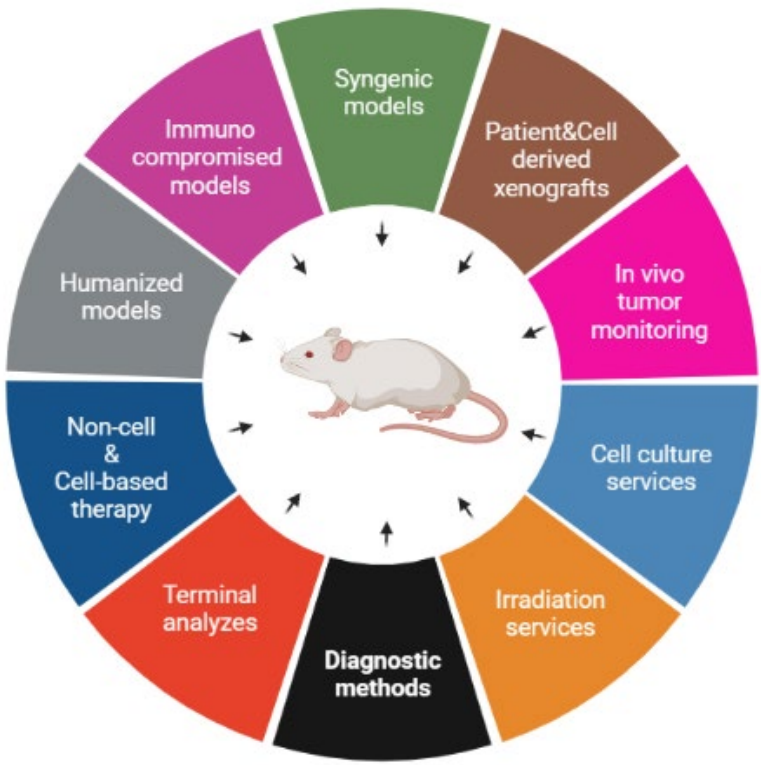
The PDX and Cancer Models Unit is dedicated to creating innovative oncology research models and committed to providing tools that propel effective treatments for cancer. We offer customizable study designs, access to a comprehensive collection of PDX mouse models, cancer cell line xenografted models, and immuno-oncology models to test the effectiveness of novel or existing immunotherapeutic compounds. Moreover, complementing our offerings, our in-house multidomain analysis spans an array of disciplines, including histopathology, hematology, biochemistry, immunology, bioimaging, and metabolomics. These cutting-edge approaches have the transformative potential to elevate any project to unparalleled heights of success. These sophisticated models and applications combine creativity, solid reliability, and a relentless commitment to customer satisfaction, ensuring access to clinically relevant mouse models and precision services.

PARTICIPATION IN PROJECTS:

- Participating as a member in the EuroPDX consortium (https://www.europdx.eu/).
- Research and therapy of myelodysplastic syndrome model with Biocev and 1st Faculty of Medicine (Charles University).
- Gene therapy of mammary and ovarian cancer on orthotopic models with IOCB (Prague).
- Immuno-oncology preclinical research on one side and dual flank mice model with IOCB.
- Testing of immunotherapeutic drugs in mammary, ovarian, and pancreatic cancer models with IOCB.
- Rare lung cancer PDX models establishment with Biocev and General University Hospital in Prague.
- Implantation of telemetric devices for metabolism studies in CCP.
- Development of precise orthotopic cancer models and humanized models for preclinical testing.
- CAR T therapy testing in cooperation with Biocev and IHBT

Hallmarks of PDX & Cancer Models Unit



(PO–25) Vision Unit (CCP, Phenotyping Module)

Marcela Palkova [1], Viktoriia Symkina [1], Kvetoslava Klajblova [1], Jan Prochazka [1], Radislav Sedlacek [1]

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Vision unit is a part of phenogenomic center and it is mainly focused on imaging, analyzing morphological structures and assessing morphological abnormalities in rodent eyes. These primary examinations are routinely performed in all mice coming to our unit. In special cases such as obvious morphological pathology of retina or special requests (e.g. mouse model for the retinopathy, diabetic disease etc.), the function of the retina is proved by electroretinography (ERG).

Additional measurements of the intraocular pressure by rebound tonometer (IcareTonovet plus) provide us important information on the eye function and the health in the mice.

Imaging devices with high image quality and resolution are used to examine the anterior segment (Pentacam), retina (Optical coherence tomography Heidelberg Engineering - OCT) and retinal vascular plexuses (OCT-A Heidelberg Engineering). All procedures are non-invasive, painless and allow long-term studies with repeated examination of eyes.

Pentacam scans the eye from 25-50 different angles and enables to measure many parameters of the cornea and the lens (e.g. surface, form, opacity, thickness and density) for each eye. The OCT scan quantifies reflections of a light beam from individual layers of the retina and composes virtual crosssectional images of the retina. The OCT-A scan enables us to detect and analyze four retinal vascular plexuses (svc - superficial and dvc - deep vascular complex, choriocapillaris and choroid). Each cross-section is evaluated and a variety of parameters are measured, e.g. the thickness and the gross morphology of the retina (retinal layers), form and the position of the optic disc, structure and pattern of the superficial blood vessels and parameters of the blood plexuses, e.g. density, number of blood vessel junctions and endpoints per region. To prove any morphological changes in the retina at different time points of life in mice, the consecutive scans could be done. ERG measures electrical responses of different retinal cell types evoked by light stimulation. This examination enables us to compare/assess the physiological relevance of the morphological abnormalities in the retina for the vision and it is described in more detail in the Electrophysiological section.

Besides covering of the routine IMPC workflow, the unit also collaborated on many other research projects related to vision (1-3). A strong retinal pathology discovered in Fam84b knockout strain initiated a more detailed longitudinal study which has become part of a master thesis and was presented at the conference two years ago. To image degenerative processes of the retina for special projects, we have started to use transmission electron microscopy in a close cooperation with Dr. Z. Nichtova PhD. (Head of Cardiovascular unit) and with a kind help of Imaging Methods Core Facility in Biocev.

1. Szczerkowska et al.: Myopia disease mouse models: a missense point mutation (S673G) and a protein-truncating mutation of the Zfp644 mimic human disease phenotype. 2019, Cell & Biosci 9-21.
2. Krausova et al.: Retinitis pigmentosa–associated mutations in mouse Prpf8 cause misexpression of circRNAs and degeneration of cerebellar granule cells. 2023, Life Sci Alliance 6(6)
3. Lindovsky et al.: OCT and ERG Techniques in High-Throughput Phenotyping of Mouse Vision. 2023, Genes 14(2)

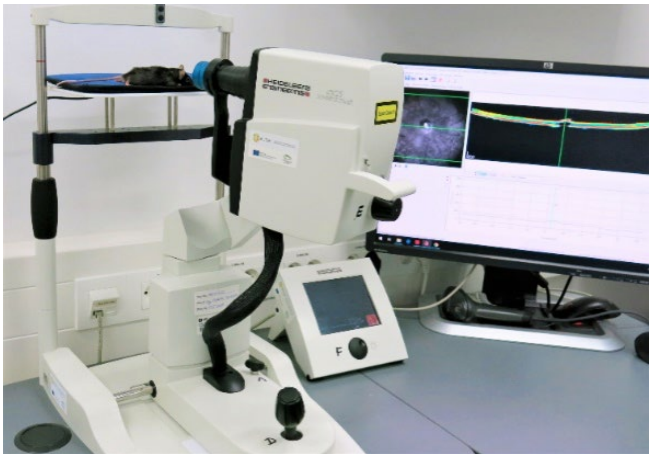

Fig. 1: OCT imaging device

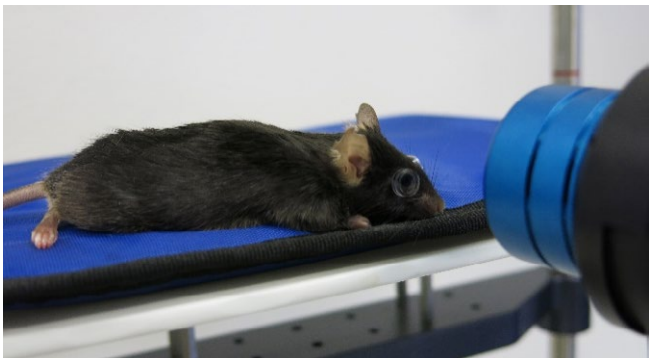

Fig. 2: Non-invasive measurement of retina

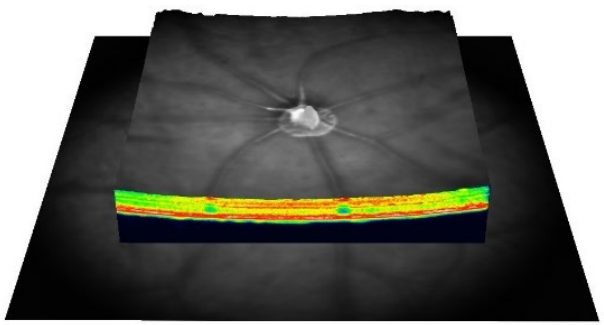

Fig. 3: Representative image of fundus and cross-section of the retina

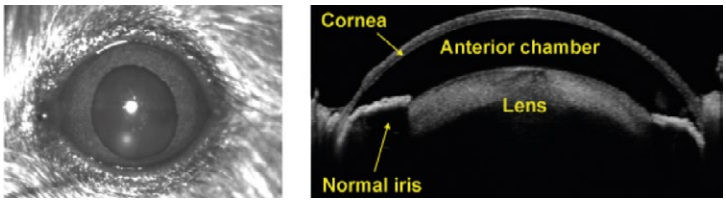

Fig. 4: OCT of the frontal eye-general view

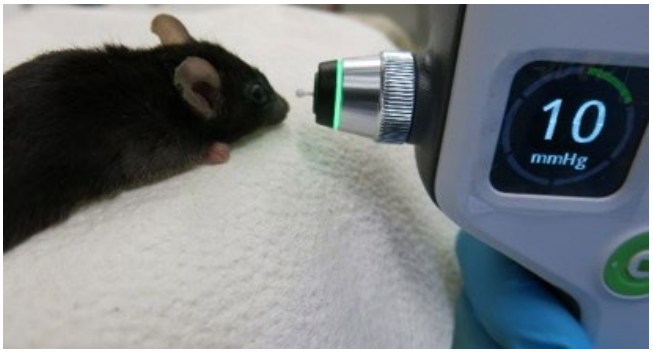

Fig. 5: Non-invasive measurement of intraocular pressure

(PO–26) Models of Infectious Diseases (BSL–3) (CCP, Phenotyping Module)

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The Models of Infectious Diseases unit encompasses an Animal Biosafety Level 3 (ABSL-3) facility, equipped with state-of-the-art technology and adheres to the highest safety standards to support long-term experiments and preclinical testing of antivirals, as well as efficacy studies and drug testing against human pathogens. With a focus on pathogens such as SARS-CoV-2 and Tick-borne encephalitis (TBE), among others, our specialized research encompasses a range of human pathogens. We offer advanced capabilities for customized humanized models, incorporating different gene editing techniques or transient induced expression of viral receptors to create organ-specific sensitized mice. Our expertise extends to transferring in vitro studies into in vivo drug screening, enabling comprehensive analysis of various solutions.

Our facility is staffed with qualified personnel who are trained in a wide range of invasive and non-invasive techniques. This includes administration of compounds via different routes (intravenous, intranasal, intraperitoneal, etc.), terminal and non-terminal blood sampling, organ collection, and histopathological analysis. Additionally, upon request, we provide immunological analysis, lung function and cardiovascular screening, as well as various biochemical, metabolomic, and proteomic studies.

We also offer assistance with experimental design, project plans and facilitating research and collaboration with other units. Our facility aims to contribute to the advancement of scientific knowledge, technology transfer, drug discovery, and the development of therapeutics by providing a safe and comprehensive environment for preclinical studies on human pathogens and antiviral research.

(PO–27) Bioinformatician Unit (CCP, Phenotyping Module)

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Bioinformatics unit of CCP assists other CCP units with computational, statistical, and analytical analyses and provides these services in this field to CCP external customers. The unit focuses, principally, on data analysis, biostatistics, tool and application development and occasional organization of training workshops in biostatistics and programming. We endeavor towards the automation of various interdisciplinary enterprises leveraging such novel approaches as deep learning. The group also maintains a continuous and indispensable effort in integrative bioinformatics as part of its involvement in phenotyping research by large-scale analysis of metabolomics datasets and image analysis. The unit takes care about all phenotyping data including quality control, statistical analysis, their storage and placing them into public web. We are also developing LIMS system and help people with daily routine process automatization.

INSTRUMENTATION & TECHNOLOGIES

For big data analysis, we utilize our own Supermicro 1029GQ-TRT server. This server consists of two Intel Xeon Gold 5120 @ 2.2 GHz processors each with 14 cores, 128 MB RAM, and two SSD drives in RAID 1, each with 240GB memory. For computational acceleration of deep learning/neural network approaches, we use one graphics card NVIDIA Tesla P100 16GB. Especially long-term one threaded tasks are dislocated to MetaCentrum which provides free membership for researchers and students of academic institutions in Czech Republic. Our main used tools in our bioinformatics unit are R, Python.

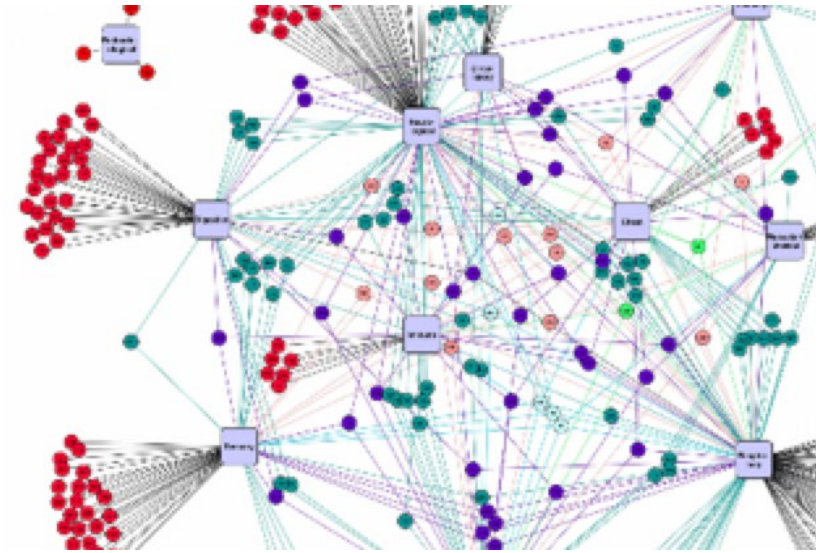

Fig. 1: Scheme of deep learning network

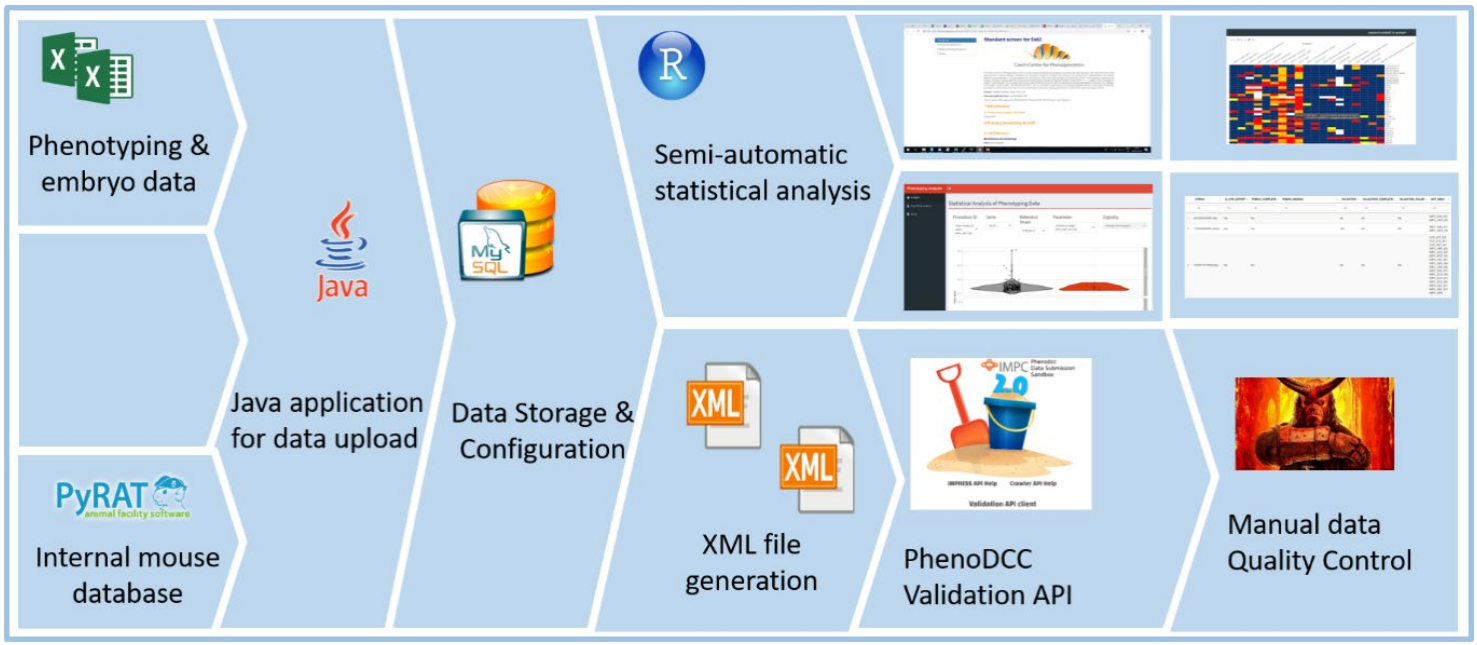

Fig. 2: Phenotyping data processing pipeline

(PO–28) Transgenic and Archiving Module (CCP)

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Transgenic and archiving module is a key part of Czech Centre for Phenogenomics, responsible for generation of novel genetically modified mice and rats using state-of-the-art technologies. TAM consists of subunits for the Genome Engineering & Model Generation and the Genotyping and Breeding, Cryopreservation & EMMA/Infrafrontier Services. Both subunits altogether provide complete service, from the initial gene-targeting design, generation of tools and transgenic rodent models to the genotyping and breeding of desired animal models.

The most commonly used genetic background in CCP is C57Bl/6N, but we are able to generate models on various backgrounds. Vast majority of newly generated mutant rodents are “knock-out” or “knock-in” models based on CRISPR/Cas9 targeting tools and zygote electroporation. Although classical transgenic models generation via pronuclear injection (PNI) of plasmid or BAC DNA is also used. Founder and G1 mice are analyzed to confirm germ line transmission (GLT). The successfully produced mouse/rat lines are cryopreserved (embryo or sperm cryopreservation). Furthermore, we offer mice production with the ES targeting technologies. Routinely we produce models from targeted embryonic stem cells originating from EUCOMM and KOMP repositories. Majority of modifications in these ES cell lines are so called “knockoutfirst” alleles that represent a LoxP-flanked critical exon with LacZ reporter element.
In cooperation with animal facility of CCP we provide consultation, assistance services, and information on the design and use of genetically modified transgenic mice. We also assist in animal rederivation (cleaning of the rodent line), reanimation (creating of the line from frozen embryos or sperms) as well as models import and export using cryopreserves sperm and embryos.

TAM provides services to a broad national and international scientific community. As a member of INFRAFRONTIER, we are contributing with mice generation to the IMPC project that aims to knockout all the mammalian genes. We also represent a Czech node of EMMA (European Mouse Mutant Archive), a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant mouse strains essential for basic biomedical research.

OUR SERVICE COMPRISES:
- Mouse/rat model generation using programmable nucleases (TALEN, CRISPR/Cas9)
- Classical plasmid and BAC transgene generation using PNI (pronuclear injection)
- Mouse model generation using ES cells, including usage of ES cells from EUCOMM and KOMP repositories
- CRE/FLP mediated allele conversions
- Embryo and sperm cryopreservation, and reanimation of strains from frozen material
- Ovary transplantation
- Rederivation/ cleaning of mouse/rat strains
- Genotyping service
- Import/ Export arrangements (together with the animal facility module

Instrumentation & technologies

NEPA 21 type II electroporator (NEPAGENE), micromanipulation microscopes Olympus IX83 equipped with TransferMan4r and XYRCOS (Hamilton Thorne) and Leica DMI6000B with FemtoJet4i. Freezing machine Asymptote EP600 (Grant), MicroePore pinpoint cell penetrator (WPI), sperm analyzer Mouse Traxx (Olympus CX41). Automatic capillary electrophoresis QIAxcel Advanced system.

Projects in selected publications:

- Efficient allele conversion in mouse zygotes and primary cells based on electroporation of Cre protein. Irena Jenickova, Petr Kasparek, Silvia Petrezselyova, Jan Elias, Jan Prochazka, Jana Kopkanova, Michal Navratil, Cyril Barinka, Radislav Sedlacek Methods 2021 Jul 191
- KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype. Kasparek P, Ileninova Z, Zbodakova O, Kanchev I, Benada O, Chalupsky K, Brattsand M, Beck IM, Sedlacek R. PLoS Genet. 2017 Jan 17
- A viable mouse model for Netherton syndrome based on mosaic inactivation of the Spink5 gene. Kasparek P, Ileninova Z, Haneckova R, Kanchev I, Jenickova I, Sedlacek R. Biol Chem. 2016 Dec 1
- Efficient gene targeting of the Rosa26 locus in mouse zygotes using TALE nucleases. Kasparek P, Krausova M, Haneckova R, Kriz V, Zbodakova O, Korinek V, Sedlacek R. FEBS Lett. 2014 Nov 3

(PO–29) Preclinical testing at the Czech Centre for Phenogenomics

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The development of new drugs is an interdisciplinary, time-consuming, and costly process and critically depends on the selection of appropriate and predictive preclinical models. Developing safe and efficacious drugs requires thorough preclinical testing using in vitro, in vivo, and increasingly also in silico approaches. Based on the experiences from high throughput phenotyping of mouse models, the Czech Centre for Phenogenomics (CCP) offers a broad portfolio of highly standardized, state-of-the-art test assays (some in GLP mode) that can be applied in preclinical studies in experimental rodent models reproducing certain features of human disease. Established preclinical tests comprise toxicity studies, hematological, and biochemical testing of samples taken from animals during toxicity studies, determination of active substances, and metabolites in plasma or other biological matrices, histopathology, ECG and echocardiography for effects on cardiovascular functions, body composition analysis, monitoring of energy fluxes, substrate utilization, feeding and drinking behavior, and locomotor activity, as well as various imaging modalities. The CCP has also implemented neurobehavioral testing and established model systems in the field of asthma and lung fibrosis, liver fibrosis, and induced colitis models. Furthermore, we offer efficacy testing in established CDX/PDX models and we can also provide new cancer model development starting with in vivo growth kinetics of the required cell line. Our CDX/PDX modality is strongly supported by the Bioimaging unit including services for in vitro experiments. We can offer genomic modification of provided cell line (e.g. to get luminescent cells or more sophisticated tasks). We can provide therapy testing also on several models for rare diseases using genetically modified animals (e.g. models of Prader-Willi and Angelman syndromes, Netherton syndrome) and also for some human infections – even when the wildtype mice are resistant (e.g. Covid-19 – using various GM mouse models). Further preclinical models are under development.

(PO–30) Animal Facility Module (CCP, Vestec)

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The Animal Facility Module (AFM) of the Czech Centre for Phenogenomics (CCP) located at the BIOCEV research center in Vestec is one of the most progressive animal facilities regarding technologies, high quality laboratory animals and demands on animal health control and welfare.

STRUCTURE OF AFM

The AFM contains four individual, fully separated breeding and experimental barrier areas as well as an autonomous Biological Safety Level - 3 (BSL-3) facility with the strict personnel entry. The AFM holds mice and rats, with the total capacity approximately 24 000 animals. Animals are kept in a controlled SPF (specific pathogen-free) environment in the barriers according to the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA). All animals are housed in the individually ventilated cages (IVC). All procedures with animal are maintained in cage-changing stations. All materials are decontaminated with autoclave or hydrogen peroxide steamer.

Conception of buildings and animal facility management is in accordance with highest world standards for laboratory animals and in compliance with European animal welfare and hygiene recommendations.

AFM SERVICES INCLUDE

Our services include rodent colony management with full integration with other CCP modules.

Our services include:

- housing and husbandry of laboratory rodents,
- rodent colony management,
- comprehensive veterinary care and expertise,
- technical and experimental service,
- rodent health surveillance programme in accordance with FELASA guidelines,
- import and export of animals,
- quarantine of animals,
- personnel training,
- animal ethics,
- project licenses administration.

BSL3 FACILITY

The BSL-3 facility is designed for long-term animal experiments and preclinical testing of antivirals, as well as efficacy studies and testing of other drugs against high-risk biological agents and toxins with a particular focus on SARS-CoV-2. Breeding capacity is: 2 880 mice in 480 IVCs and 168 rats in 56 IVCs for rats.

Overall, the AFM provides an optimal breeding condition for small laboratory rodents and makes animal experiment expertise available for several scientific institutions in the highest feasible standards of the biomedical research.

Fig. 1: BSL-3 facility: All animals are housed in IVCs which are connected with Biosafety level 3 ventilation system

Fig. 2: Pheno barrier: All animals are housed in the individually ventilated cages (IVC). All procedures with animal are maintained in cage-changing stations.

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