

35th VETPHARM-SYMPOSIUM



17th – 18th September 2026



University of Veterinary Medicine, Vienna

Program and Abstracts



Foto: Philipp Jodl/Vetmeduni



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General information

Host and Venue

Pharmacology and Toxicology
Centre of Biological Sciences
University of Veterinary Medicine Vienna
Veterinärplatz 1
1210 Vienna
Austria

@ Vetpharmsymposium2026@vetmeduni.ac.at

 <https://www.vetmeduni.ac.at/pharmakologie/vetpharm/about>

 Vetmeduni, Plenary hall, Building CA, Veterinärplatz 1, 1210 Vienna (see page 59)

Registration

Thursday 17th September starting at 08:30

Organisation

Staff members of the Pharmacology and Toxicology Unit, Centre for Biological Sciences, Vetmeduni

ATF-acknowledgement

ATF-recognised continuing education hours (ATF-Stunden)

Full Symposium Programme (Parts I–III)		11 hours
Part I	3R Workshop <i>Refine. Replace. Rethink.</i>	3 hours
Part II	Scientific Sessions	6 hours
Part III	Specialist Workshop in Veterinary Pharmacology and Toxicology	2 hours

There is no registration fee for this scientific conference.

The organizers would like to extend their sincere thanks to the sponsors and donors (see page 60) for their generous support.

Events

Informal Get-together Wednesday 16th September 2026, 18:00
Gasthaus Napoleon Wien Kagranner Platz 33, 1220 Wien, <https://www.napoleon.wien/>

Guided Tours on Campus Thursday 17th September 2026, 17:45

- Botanical Garden
- Clever Dog Lab
- Clinical Centre for Equine Health and Research
- Diagnostic Imaging
- Ruminant and Camelid Medicine
- VetBiomodels
- VetCore
- VetSim

Dinner / Evening Event Thursday 17th September 2026, 18:45, Building CA

Program

Wednesday, 16th September 2026

18:00



Informal Get-together
(optional)

Thursday, 17th September 2026

8:30

Registration, Coffee & Snacks
Building CA, 1st floor

9:00 – 12:00

Part I:

3R-Workshop: *Refine. Replace. Rethink.*

Building CA, 1st floor, Festsaal

Chair: Bankstahl M., Gotthardt D.

9:00

Welcome

9:05

Ao. Univ. Prof. Dr. Rupert Palme (Vetmeduni Wien):

„Stress hinterlässt Spuren: Nicht-invasive Belastungsbeurteilung beim Tier durch Glukokotikoidmetaboliten im Kot“

9:45

Dr. Isita Sagar (BOKU Wien):

„Physiologically Defined MSC Isolation and Cultivation as a Basis for High-Throughput In Vitro Models“

10:20 – 10:35



Coffee Break

10:35

Dr. Sarah Strauß (MHH):

„Einblicke in die Forschung mit Axolotl und Goldener Radnetzspinne im 3R-Kontext“

11:15

Dr. Theresa Balber (MedUni Wien):

„In-ovo-Modelle als 3R-Ansatz in der präklinischen Forschung und Bildgebung“

11:50

Wrap-up

12:00 – 13:00



Lunch & Registration (continued)
Building CA, 1st floor

13:00 – 17:20 Part II:

Scientific Sessions

Building CA, 1st floor, Festsaal

13:00 Welcome

13:10 – 14:25



Session 1: Neuropharmacology

Chair: Käufer C. (Hannover), Niehaus I. (Stockholm)

13:10

Weiß S, Niehaus I, Ewe A, Aigner A, Stefanis L, Fleming Outeiro T, Richter Assencio F, Feja M (Hannover):
“Nanoparticle-mediated small RNA delivery to modulate alpha-synuclein pathology in Parkinson’s disease models”

13:25

Navarro Ramil L, Krstanović F, Brizić I, Käufer C, Richter F (Hannover):
“Influence of viral infections on neurodegenerative processes in a co-pathology model of congenital infection and synucleinopathy”

13:40

Becker S, Pérez-Pérez D, Buchecker V, Potschka H (München):
“Effects of Cenobamate in young and adult mice with Dravet syndrome”

13:55

Hauser J, Bankstahl JP, Schmitt K, Parmar A, Berndt V, Prchal-Murphy M, Filip T, Zebedin-Brandl EM, Glasenapp A, Leenaars CH, Bankstahl M (Wien):
“Ketogenic interventions as modulators of epileptogenesis in rodent models: A systematic review in progress”

14:10

Egilmez A, Weiß E, Pauletti A, Bröer S (Berlin):
“Astroglia Population Dynamics Throughout Epileptogenesis in the TMEV Model of Epilepsy”

14:25 – 15:05



Session 2: New Drugs and Regulatory Updates

Chair: Wilzopolski J. (Berlin)

14:25

Lüttig A, Emmerich IU (Leipzig):
“New drugs for animals in 2025 and 2026”

14:40

Keynote lecture
PD Dr. Svenja Sander (BVL Berlin):
“Update on Veterinary Medicinal Law – latest European implementations”

15:05 – 15:50



Coffee Break and Poster Walk

Building CA, 1st floor, Festsaal & Festsaal-erweiterung

15:50 – 17:20



Session 3: Transporters and Cancer

Chair: Kuhnert L. (Leipzig), Prchal-Murphy M. (Wien)



15:50

Siegl L, Hamann M, Geyer J (Gießen):
“Inhibition of the canine and feline MDR1 P-glycoproteins by veterinary drugs”

16:05

Breuer J, Leiting S, Karakus E, Geyer J (Gießen):
“Dexamethasone induces human and murine SLC10A6/Slc10a6 gene expression via functional glucocorticoid receptor response elements in their promoters”

16:20

Heilmann K, Siegl L, Hamann M, Geyer J (Gießen):
“Phylogenetic variations in ABC-transporter proteins of birds”

16:35

Fuchs D, Posthaus C, Hungerbühler N, Seehusen F, Guscetti F, Opitz L, Nolff MC, Markkanen E (Zürich):
“Deciphering the STS-landscape – combining spatially defined transcriptomics with clinical outcome data to improve diagnosis and therapy of canine soft-tissue sarcoma”

16:50

Hiesinger A, Klein K, List J, Klampfl T, Kollmann S, Ruelicke T, Hoermann G, Sexl V, Ehl S, Moriggl R, Gotthardt D (Wien):
“Unraveling STAT5B-driven NK-cell leukemia: Mechanisms and Therapeutic Strategies”

17:05

List J, Fryzik P, Klampfl T, Huszarek S, Sippl L, Kudweis P, Hiesinger A, Luesink M, Nierkens S, Chen I, Walter W, Hoermann G, Erfurt P, Sternberg F, Chan WC, Kollmann K, Neubauer HA, Putz EM, Klein K, Sexl V, Belderbos M, Gotthardt D (Wien):
“Ink4a/Arf-deficient mouse model of aggressive NK cell leukemia reveals XPO1 vulnerability”

17:30



Group Photo

Biotope (in front of building CA)

17:45



Guided Tours on Campus

Start: Biotope (in front of building CA)

18:45



Dinner / Evening Event

Building CA, ground floor, Aula

Friday, 18th September 2026

8:30

Coffee & Snacks

Building CA, 1st floor

9:00 – 15:15

Part II:

Scientific Sessions

Building CA, 1st floor, Festsaal

9:00 – 9:45



Session 4: Antimicrobials and Atopic Dermatitis

Chair: Seeger B. (Hannover)

9:00

Hillebrand J, Oltmanns H, Peh E, Kittler S, Meißner J (Hannover):

“Efficacy of four potential antimicrobial substances and bacteriophages against Klebsiella species and Streptococcus uberis from bovine mastitis in vitro”

9:15

Gerdes E, Ammer H (München):

“Opioid signaling through the canine MRGPRX2 receptor”

9:30

Xie T, Dalponte A, Kleuser B, Bäumer W (Berlin):

“Different Roles of S1PR2 and S1PR3 in Mediating Itch and Inflammation Associated with Canine Atopic Dermatitis”

9:45 – 10:30



Session 5: Animal-Based Research – Regulation & Refinement

Chair: Stocker V. (München)

9:45

Wilzopolski J, Hohlbaum K, Rubel D, Schadock I, Neves ML, Schwedhelm P, Bert B, Heintz C (Berlin):

“Sentiment Barometer Regarding the Approval Process for Animal Experiments – Does German Bureaucracy Impact Cross-Border Outsourcing of animal experiments?”

10:00

Kofen M, Munk A, da Silva Carvalho Aguiar MF, Haholu E, Lange C, Reiber M, Hauser J, Parmar A, Bankstahl M, Leenaars C, Bleich A, Potschka H (München):

“Dosing Strategies of Buprenorphine in Laboratory Rodents: A Systematic Scoping Review”

10:15

Schoeniger A, Zipfel I, Schroedl W, Lindner A, Abraham G (Leipzig):

“Development and validation of a diagnostic assay for porcine stress marker detection”

10:30 – 11:05



Poster Walk and Coffee Break

Building CA, 1st floor, Festsaal & Festsaal Erweiterung

11:05 – 12:05



Session 6: Innovative Model Systems for Neurodevelopment and Neurological Diseases

Chair: Kollmann K. (Wien), Steinmetz L.K. (Hannover)

11:05

Ducray AD, Ben Chaabene R, Jamaati A, Capstick M, Mevissen M (Bern):
“In a wireless world: Is PARP1 a key player in the development of iPSC-derived dopaminergic neurons?”

11:20

Alonso Jiménez S, Ben Chaabene R, Ducray AD, Mevissen M (Bern):
“Nogo-A in Parkinson's Disease: Insights from hiPSC-Derived Dopaminergic and Mitochondrial Profiling”

11:35

Haiber LM, Cirkse K, Kühne BA, Hupfeld J, Duven M, Fischer A, Schenke M, Richter Assencio F, Weiß S, Altenbuchinger M, Seifert N, Zacharias HU, Konzok S, Gerold G, Seeger B (Hannover):
“A Human iPSC-Derived Neurosphere Model of Venezuelan Equine Encephalitis Virus Infection to Study Its Effects on the Developing Human Brain”

11:50

Fischer A, Haiber LM, Kotzur R, Lemke C, Fulde M, Seeger B, Bröer S (Berlin):
“Investigating Prenatal Listeria-Induced Neurodevelopmental Disturbance in a Human iPSC Neurosphere Model”

12:05 – 12:25



Past and Future
Meike Mevissen

12:25 – 12:40



Poster and Presentation Award, Closing Remarks

12:40 – 13:30



Lunch Break

13:30 – 15:00



Faculty Meeting („Hochschullehrer-Versammlung“)
Building CA, 1st floor, Großes Sitzungszimmer

13:30 – 15:15

Part III:

Specialist Workshop in Veterinary Pharmacology and Toxicology

Building CA, 1st floor, Festsaal

Chair: Schmitt K. (Wien), Stocker V. (München)

13:30

Dr. Inka Kuhlmann (MSD, Schwabenheim):
„Zielgerichtete Forschung in der Arzneimittelindustrie – Karriereperspektiven für Tiermediziner“

14:00

Mag. med. vet. Werner Höllriegl (MedUni Wien):
“Preclinical efficacy and safety evaluation of systemic AAV gene therapy products.”

14:30

Dr. Michelle Wegrad (BVL Berlin):
“Borderline Products - Navigating the borderline between veterinary medicinal products and other goods”

15:15

End of Symposium

Abstracts: Oral presentations

Weiß et al.

Nanoparticle-mediated small RNA delivery to modulate alpha-synuclein pathology in Parkinson's disease models

Sandra, Weiß^{1,2}, Isabell, Niehaus^{1,2}, Alexander Ewe³, Achim Aigner³, Leonidas Stefanis⁴, Tiago Fleming Outeiro⁵, Franziska Richter Assencio^{1,2}, Malte Feja^{1,2}

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³Rudolf-Boehm-Institute for Pharmacology and Toxicology, Clinical Pharmacology, Faculty of Medicine, University of Leipzig, Germany

⁴First Department of Neurology, Eginition Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece

⁵University Medical Center Göttingen, Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, Göttingen, Germany

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, misfolding and accumulation of alpha-synuclein (aSyn), a disease-associated protein implicated in neuronal dysfunction and degeneration. Small RNA-based strategies represent a promising therapeutic approach to modulate dysfunctional molecular pathways associated with aSyn pathology. However, their clinical translation remains challenging due to RNA instability, inefficient cellular uptake, and difficulties in achieving targeted neuronal delivery.

Here, we developed and characterized a nanoparticle-based platform for the delivery of small RNAs targeting aSyn-associated pathology in an *in vitro* PD model. Tyrosine-modified polyethylenimine was complexed with small RNA molecules and combined with liposomes to generate polymeric nanoparticles suitable for neuronal delivery. In doxycycline-regulated SH-SY5Y cells overexpressing human wildtype aSyn, nanoparticle-mediated delivery of siRNA targeting aSyn mRNA (*SNCA*) significantly reduced mRNA levels, while exhibiting low cytotoxicity, demonstrating the feasibility of this platform for small RNA-based modulation of aSyn expression.

Building on these results, we are currently investigating microRNA (miRNA) replacement therapy as a strategy to normalize aSyn-dependent molecular alterations. Emerging evidence indicates that miRNAs are dysregulated in PD patient brains and contribute to post-transcriptional regulation of PD-associated pathways. In particular, miR-34b/c is reduced in PD-relevant brain regions and targets *SNCA*, suggesting that its loss may promote aSyn accumulation and downstream pathological changes. To identify aSyn-dependent transcriptomic alterations and assess their modulation by miRNA therapy, we performed mRNA and miRNA sequencing in differentiated SH-SY5Y cells with doxycycline-regulated aSyn expression. Differential expression, pathway and network analyses will be performed to identify dysregulated genes, miRNAs and regulatory networks, with a particular focus on mitochondrial function, oxidative stress, autophagy and mitophagy. This approach may provide a foundation for future miRNA replacement therapies aimed at modulating disease-relevant molecular pathways and improving neuronal survival in PD.

Navarro Ramil et al.

Influence of viral infections on neurodegenerative processes in a co-pathology model of congenital infection and synucleinopathy

Lucas Navarro Ramil^{1,3}, Fran Krstanović², Ilija Brizić², Christopher Käufer^{1,3}, Franziska Richter^{1,3}

¹*Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Hannover, Germany*

²*Centre for Proteomics, Faculty of Medicine in Rijeka, Croatia*

³*Center for Systems Neuroscience (ZSN), Hannover, Germany*

Neurodegenerative diseases such as Parkinson's disease (PD) represent one of the greatest medical challenges associated with demographic change. PD is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, as well as the accumulation of misfolded α -synuclein protein. In addition to genetic factors, environmental factors are increasingly recognized as pivotal drivers. In particular, viral infections in early childhood are suspected of triggering long-term neuroinflammatory processes that, in interplay with genetic predispositions, could contribute to the development of neurodegenerative diseases. The most common congenital viral infection is caused by the herpesvirus cytomegalovirus (CMV), which establishes a latent, lifelong infection. The objective of this project was to investigate the impact of neonatal murine CMV (MCMV) infection on neurodegenerative processes using a two-stage approach: First, in collaboration with leading international CMV experts (University of Rijeka, Croatia), we investigated whether neonatal MCMV infection induced long-term neuropathological alterations in wild-type C57BL/6 mice. We observed a significant increase in α -synuclein immunoreactivity as well as increased densities of myeloid (Iba1⁺) and astrocytic (GFAP⁺) cells in the hippocampus and cortex 5 months post-infection, suggesting α -synuclein accumulation and chronic neuroinflammation in various brain regions, thereby supporting the central hypothesis of the project. Building upon these findings, we are currently evaluating whether MCMV acts as a "second hit" to exacerbate the development of synucleinopathy in the transgenic Thy1-aSyn mouse model of PD. Behavioral, molecular, and immunological analyses are being combined to elucidate the interplay between viral persistence, neuroinflammation, and protein aggregation. Preliminary results from this second stage will be presented. Our findings provide a translationally relevant contribution to research on risk factors for neurodegenerative diseases and may reveal novel approaches for prevention and therapy.

Becker et al.

Effects of Cenobamate in young and adult mice with Dravet syndrome.

Becker Selina¹, Pérez-Pérez Daniel¹, Buchecker Verena¹, Potschka Heidrun¹

¹Institute of Pharmacology, Toxicology, and Pharmacy, Ludwig-Maximilians-Universität München, Munich, Germany

Dravet Syndrome (DS) is a severe developmental and epileptic encephalopathy. Seizure onset is within the first year of life, and various seizure types can occur during the progression of the disease, along with behavioral and physical impairments. It is caused by a mutation in the SCN1A gene, which encodes the voltage-gated sodium channel (VGSC) Nav1.1 subunit. This VGSC subunit is preferentially expressed in GABAergic neurons. Despite currently available antiseizure medications (ASMs), seizures are frequently refractory and require multimodal management. Sudden unexpected death presents a high risk in patients with DS. All of this showcases the need for novel therapeutic approaches. VGSC modulators are generally considered to be contraindicated; however, preferential inhibition of the persistent sodium current may be beneficial. Cenobamate (CNB) combines this mechanism of action with allosteric modulation of GABA_A receptors. Clinical data in patients with DS is limited and controversial, especially in pediatric patients, suggesting a possible age-dependent benefit. In this study, the effects of CNB on the phenotype (seizure and behavior) across different developmental stages in a DS mouse model were evaluated.

Mice carrying the *Scn1a*^{A1783V/+} mutation (Dravet mice) and their wild-type littermates (WT mice) were used. The optimal route of administration for CNB (5 and 10 mg/kg) was determined by comparing oral gavage (n = 11) and drinking solution (n = 13) in young (postnatal day (pnd) 25-39) and adult (postnatal week (pnw) 14-16) Dravet mice. Potential adverse events were monitored, and the seizure frequency was evaluated using video recordings. Furthermore, the effects of CNB on seizure activity and behavioral parameters were tested in young (pnd 25-39, n = 74) and adult mice (pnw 14-16, n = 58). An electrode and a telemetric transmitter were implanted in adult animals for electroencephalogram (EEG) analysis. Dravet mice were randomly assigned to Vehicle or CNB (10 and 30 mg/kg) groups. WT mice treated with the same compounds (young) or receiving vehicle administrations (adult) served as controls for behavioral studies. Seizure parameters were analyzed from video or video-EEG recordings, and behavioral studies comprised the open field, social interaction, and saccharin preference tests. A modified Irwin test was applied to evaluate tolerability.

CNB was well tolerated across both routes of administration and doses. However, a wide variation in voluntary CNB intake and the lack of differences in the frequency of adverse events led to the selection of oral gavage for subsequent experiments. In young animals, we observed a comparable seizure frequency, duration, and intensity across all experimental groups. Moreover, behavioral changes observed in Dravet mice were not reversed by CNB administration. Preliminary data suggests no alteration in seizure and behavioral parameters by CNB administration in adult mice.

In conclusion, CNB did not show an improvement or worsening in the seizure and behavioral phenotypes of DS mice. Since effectiveness in patients with DS is generally observed with a multimodal therapy. It would be of interest to further delineate the compound's effectiveness spectrum by assessing dose-dependency and/or combinations with other ASMs.

Funding: This work was supported by Angelini Pharma S.p.A, Rome, Italy

Hauser et al.

Ketogenic interventions as modulators of epileptogenesis in rodent models: A systematic review in progress

Jana Hauser¹, Jens P. Bankstahl², Katharina Schmitt¹, Amisha Parmar¹, Victoria Berndl¹, Michaela Prchal-Murphy¹, Thomas Filip³, Eva Maria Zebedin-Brandl⁴, Ayлина Glasenapp⁵, Cathalijn H. Leenaars⁵, Marion Bankstahl^{1,5}

¹*Department of Biological Sciences and Pathobiology, Centre of Biological Sciences, Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria*

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³*VetBioModels, University of Veterinary Medicine, Vienna, Austria*

⁴*Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria*

⁵*Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany*

Background: Epilepsy is a common chronic neurological disease characterized by recurrent unprovoked seizures. While anti-seizure medications effectively control seizures in many patients, a substantial proportion remains pharmacoresistant, and no clinically approved therapies prevent epileptogenesis. Preclinical evidence suggests that the ketogenic diet (KD) may modulate epileptogenesis, making it a promising candidate for further investigation. This systematic review aims to compile and evaluate the existing evidence on the effects of the KD and alternative ketogenic treatments on epileptogenesis in rodent models.

Methods: The research question was defined according to the PICO format. The protocol, developed according to the SYRCL guidelines, defined the search strategy and specific inclusion and exclusion criteria and was preregistered in PROSPERO. The search encompassed three main components (epilepsy, ketogenic treatments, and rodents) and was conducted in PubMed, Web of Science, and PsycINFO, resulting in a total of 3,836 records. These records underwent title and abstract screening, followed by full-text screening in Rayyan, with each study screened by at least two independent reviewers at every stage.

Preliminary results and discussion: Three main model categories were identified: kindling (43%), genetic models with epilepsy development (26%), and insult-driven models (31%). The earliest included studies were published in 1999, with the number of studies increasing over time across all model types. Out of the 64 included studies, 48% were performed in mice and 52% in rats. While most studies used only male animals (53% of rat studies and 82% of mouse studies), both sexes were included in 38% and 11%, respectively. Sex was not reported in 5-6% of studies. The three main treatment regimens were KDs with different ketogenic ratios (52%), fatty acid treatments (20%), and fasting/caloric restriction (12%). Ketosis, defined as a significant increase in ketone bodies compared with controls, was assessed in 58% of experiments and evident in 92% of those experiments. Based on the findings to date, the systematic review highlights that study designs were only partially suitable for distinguishing anti-epileptogenic from anticonvulsant effects, complicating interpretation of the findings. Furthermore, the heterogeneity of therapeutic regimens, combined with a small number of studies per regimen, limits the feasibility of meta-analysis and makes it difficult to assess whether potential anti-epileptogenic effects of the KD can be reproduced by alternative ketogenic therapies.

Egilmez et al.

Astroglia Population Dynamics Throughout Epileptogenesis in the TMEV Model of Epilepsy

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¹*Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

²*Einstein Center for Neurosciences (ECN), Berlin, Germany*

Background: Astrocytes are the most abundant glial population of the central nervous system and are indispensable for ion and neurotransmitter buffering, synaptic homeostasis, neuroinflammatory signaling and blood-brain barrier integrity. Astrocyte dysfunction is increasingly implicated in epilepsy, a chronic disorder affecting approximately fifty million people worldwide. Reactive astrogliosis is a consistent hallmark of epileptic tissue, yet the temporal dynamics of astrocyte proliferation, morphological transformation, and maturation during viral epileptogenesis remain poorly understood.

Aim: We asked how the astroglia population changes over the course of epileptogenesis, and whether these changes are attributable to viral encephalitis per se or specifically to the occurrence of acute seizures, with the goal of defining the window in which astrocytes could be therapeutically targeted before chronic epilepsy is established.

Methods: We used the Theiler's Murine Encephalomyelitis Virus (TMEV) mouse model, which recapitulates human infection-induced epilepsy with acute symptomatic seizures, a seizure-free latent period, and subsequent chronic epilepsy. C57BL/6 mice were stratified into uninfected controls, infected non-seizing and infected acutely seizing animals, with acute seizure burden defined by behavioral scoring, and continuous telemetric EEG. This design separates the effect of infection from the effect of acute seizures within the same cohort. Cells proliferating during the acute phase were birth-dated with bromodeoxyuridine (BrdU) and their fate followed into the acute (7 dpi), latent (28 dpi) and chronic (90 dpi) phases, while Ki67 labelling captured the proliferation ongoing at each of these respective timepoints. Astroglia identity, maturation state and morphology were assessed in the hippocampal hilus using GFAP and S100 β immunohistochemistry.

Results: Cell proliferation increased in all infected animals at 7 dpi; however, only seizing animals maintained proliferation above control levels through 90 dpi and generated a substantial cohort of GFAP⁺BrdU⁺ cells born during the acute phase. Ki67 staining further showed that proliferative activity peaked earlier, within the seizure window. Total GFAP⁺ density normalized across groups by 90 dpi, yet the GFAP⁺/BrdU⁺ cells born during the acute phase persisted and constituted a disproportionately large population in seizing animals, leaving it compositionally distinct despite an apparently overall normal cell density. Cell fate was likewise changed by seizure occurrence: Astrocytes that were generated during the acute phase acquired the maturation marker S100 β more frequently in seizing animals at all investigated timepoints, and increasingly so by 90 dpi, whereas this proportion declined in non-seizing animals. Hypertrophy was strictly seizure-dependent and sustained at 28 and 90 dpi, while astrocytes in non-seizing mice returned to control size once infection resolved.

Conclusions: Seizures during viral encephalitis do not simply amplify the astroglia reaction to infection; they redirect it. Infection-driven astrogliosis resolves, whereas seizures generate astrocytes that persist, remain hypertrophic and are preferentially channeled toward a mature S100 β ⁺ identity, leaving a chronic population that is compositionally altered even where the overall cell density appears normal. This remodeling is established early and settled by the time the latent period ends, identifying the acute-to-latent transition as the window in which astroglia contributions to epileptogenesis might be therapeutically addressed.

This study is funded by a grant from the Else Kröner-Fresenius-Stiftung to Sonja Bröer (2022 EKEA.132).

Lüttig et al.

New drugs for animals in 2025 and 2026

Anika Lüttig¹, Ilka Ute Emmerich¹

¹*Institut für Pharmakologie, Pharmazie und Toxikologie, Veterinärmedizinische Fakultät der Universität Leipzig*

In 2025 and the first half of 2026, the following innovations in non-immunological veterinary drugs enriched the German veterinary drug market:

1. Products containing active ingredients approved for veterinary use for the first time.

Seven new active ingredients received approval for small animals: the two Janus kinase inhibitors *atinvicitinib* (Numelvi®) and *ilunocitinib* (Zenrelia®), for the treatment of pruritus associated with allergic or atopic dermatitis; the serotonin reuptake inhibitor *fluoxetine* (Fluoxevet®, Reconcile®) for the adjunctive treatment of behavioral disorders caused by separation anxiety; the selective ghrelin receptor agonist *capromoreline* (Eluracat®) for weight gain in cats suffering from loss of appetite or unintentional weight loss due to chronic conditions; and the anti-anemic agent *molidustat* (Varenzin®) for the treatment of non-Furthermore two new monoclonal antibodies for the treatment of pain associated with osteoarthritis in dogs (*izenivetmab*; Lenivia®) and cats (*relfovetmab*; Portela®).

2. Products that have significantly enriched the veterinary pharmaceutical market, particularly due to their new dosage form or method of administration.

The first medicated diet for cats with chronic kidney disease, containing the active ingredient *benazepril* (Vikaly®), is now available.

3. Active ingredients that have been approved for other animal species.

The authorization of six active substances has been extended to cover additional animal species: A *dexamethasone* (Dexafast®) preparation is now approved for use in goats; a new product containing *sodium salicylate* (Dophacyl Avi®) for chickens has been launched; *omeprazole* (Omeprazol TriviumVet®) has been authorized for dogs; a premix containing *praziquantel* (Prazivetin®) for fish (golden bream); and *amprolium* (Surricoxx®) for ducks and guinea fowls.

Sander

Update on Veterinary Medicinal Law – latest European implementations

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The European regulation on veterinary medicines (VMPs) became applicable on 28 January 2022 and mandated the European Commission to adopt further supplementary rules. The main goals of the regulation were to harmonise the rules for VMPs, reduce the administrative burden, and strengthen the EU's action to combat antimicrobial resistance.

The supplementary rules include further specifications regarding the manufacturing, labelling, packaging, distribution and use of VMPs, as well as pharmacovigilance and the collection of use and sales data on antimicrobials. Most of the supplementary regulations have now been implemented. For example, harmonised pictograms for the packaging and labelling as well as a common logo for the retail of VMPs at a distance were introduced, and specifications concerning use and sales data have been in place since 2022. Further restrictions on the use of antimicrobials in animals in order to obtain treatment options of for certain human infections and the use of antimicrobials in accordance with Articles 112–114 ('cascade use'), have also come into force. Specific regulations for the use of VMPs under the 'cascade' principle for food-producing aquatic and equine species are under the way.

Considerable efforts have been made over the past decade to draft, introduce and implement new European legislation on VMPs. Now that the new legislation has been applied for almost five years, only a few loose ends remain, and many emerging questions have been clarified by the Commission. However, ongoing efforts are needed to finalise implementation at the European and national levels.

Siegl et al.

Inhibition of the canine and feline MDR1 P-glycoproteins by veterinary drugs

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P-glycoprotein (also known as MDR1 or *ABCB1*) is an ATP-dependent efflux transporter that mediates the transmembrane transport of numerous endogenous and exogenous compounds. By transporting these substances across cellular membranes, P-glycoprotein significantly affects the absorption, distribution and elimination of drugs.

This study investigates the interaction of selected compounds from licensed veterinary medicinal products, particularly antiparasitic drugs, with the MDR1 transporter in dogs and cats. The inhibitory potential of the compounds was evaluated by determining half-maximal inhibitory concentration (IC_{50}) values through *in vitro* assays, including rhodamine 123 transport experiments performed in MDR1-overexpressing HEK293 cells. Understanding the extent of MDR1 inhibition is crucial for predicting potential drug–drug interactions and ensuring the safe and effective use of drugs in veterinary medicine. Within the group of antiparasitic drugs, ivermectin, eprinomectin, spinosad and emodepside were shown to interact with the MDR1 P-gp of both dogs and cats. These compounds exhibited the lowest IC_{50} values (3–5 μ M) and were identified as the most potent inhibitors of MDR1-mediated rhodamine 123 efflux. Additionally, loperamide and cyclosporin A demonstrated a strong interaction with the MDR1 P-glycoproteins of both species with IC_{50} values of 2–4 μ M. In contrast, IC_{50} values for MDR1 P-glycoprotein inhibition could only be determined for the cat transporter for milbemycin oxime, erythromycin, diltiazem and quinidine, indicating species differences.

In conclusion: Transport studies in HEK293 cells stably expressing the canine and feline MDR1 P-glycoproteins made it possible to directly compare the inhibitory potencies of selected veterinary drugs, revealing significant species differences in their interaction profiles.

Breuer et al.

Dexamethasone induces human and murine *SLC10A6/Slc10a6* gene expression via functional glucocorticoid response elements in their promoters

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The sodium-dependent organic anion transporter SOAT (gene symbol *SLC10A6* in humans, *Slc10a6* in animals) is a specific uptake carrier for sulfated, biologically inactive steroid hormones that are present at quite high concentrations in plasma, namely dehydroepiandrosterone sulfate (DHEAS), pregnenolone sulfate, and estrone-3-sulfate. After their cellular uptake, these molecules serve as precursors for the intracrine synthesis of active estrogens and androgens and thus play a role for the overall regulation of steroid responsive cells and organs.

Despite its potential relevance for local steroid hormone availability, the transcriptional regulation of *SLC10A6/Slc10a6* remains poorly understood. In the present study, we analyzed the transcriptional regulation of the murine *Slc10a6* gene and evaluated whether a comparable regulatory mechanism is present in the human *SLC10A6* gene.

We first demonstrated that treatment of murine NIH3T3 fibroblasts with the glucocorticoid dexamethasone significantly increased *Slc10a6* mRNA expression in a time- and concentration-dependent manner. Consistently, transient transfection experiments using a specific *Slc10a6* promoter construct linked to a luciferase reporter gene revealed enhanced promoter activity after dexamethasone stimulation. Further promoter analyses revealed a putative glucocorticoid response element within this region.

In the next step, alignment of the active murine promoter region with the human *SLC10A6* promoter identified a corresponding regulatory region. In human HeLa cells, dexamethasone also induced *SLC10A6* mRNA expression. Additionally, elevated luciferase activity was observed for the aligned human promoter region after dexamethasone stimulation. Within this promoter region, a putative glucocorticoid receptor (GR)-binding site was identified. Deletion of this GR-binding site abolished the dexamethasone-induced increase in promoter activity.

In conclusion: Our findings suggest that glucocorticoid-dependent regulation of *Slc10a6/SLC10A6* expression is conserved between mouse and human. We identified corresponding dexamethasone-responsive regions within the murine *Slc10a6* and human *SLC10A6* promoters and provide evidence that putative glucocorticoid receptor-binding sites are functionally required for the dexamethasone-induced activation of both promoters. Future studies are needed to determine the extent to which glucocorticoid-mediated upregulation of *Slc10a6/SLC10A6* expression contributes to the overall clinical effects of glucocorticoids by modulating intracrine steroid regulation.

Heilmann et al.

Phylogenetic variations in ABC-transporter proteins of birds

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The family of ABC transporter proteins is widely expressed across a range of tissues and organs, observed in a multitude of species. Despite their structural similarities, they facilitate the transmembrane transport of a diverse range of substrates. In humans ABCB1 (syn. MDR1 or P-glycoprotein) is expressed in a multitude of organs, particularly the small intestine, liver, kidney, and brain. It transports mainly xenobiotics and therefore plays an important role for drug safety. In contrast, ABCB4 (syn. MDR3) is specifically located in the liver and catalyzes the export of phospholipids in the biliary tract. Considering the structural similarities observed among these carriers (ABCB1 and ABCB4), a gene duplication of ABCB1 is proposed as a potential origin for ABCB4. In mammals, ABCB1 and ABCB4 are considered gene homologs. However, in birds, alternative and partly conflicting nomenclatures are provided in the NCBI and Ensemble databases. Genes with high homology to ABCB1 and/or ABCB4 are either designated as ABCB1-like / ABCB1LA or ABCB4-like. Even more interesting, in some species belonging to the Neoaves clade, only one single ABCB1/ABCB4 gene ortholog can be found with no clear homology preference for ABCB1 or ABCB4. To gain further insight into this matter, the related gene clusters and protein sequences were subjected to detailed phylogenetic analysis. Furthermore, the mRNA expression pattern for the above-mentioned homologs over different organs in chicken, zebra finch and canary were analyzed. As a result, chicken ABCB1 is comparable to human ABCB1 and chicken ABCB1LA is comparable to human ABCB4 in its organ expression pattern. The zebra finch and canary ABCB1 seem to be more like human ABCB1 than ABCB4 based on RNA expression in different organs. The different carriers ABCB1 and ABCB4 of the human, ABCB1 and ABCB1LA of the chicken, ABCB1 of the zebrafish and ABCB1 of the canary were cloned and HEK293 cells were stably transfected for functional characterization. However, only for human ABCB1 and ABCB4 protein expression could be detected. This was despite all cells were positive for bird mRNA expression of the respective construct, indicating lack of protein translation of these bird proteins in human HEK293 cells.

Fuchs et al.

Deciphering the STS-landscape – combining spatially defined transcriptomics with clinical outcome data to improve diagnosis and therapy of canine soft-tissue sarcoma

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Soft-tissue sarcomas (STS) are a heterogeneous group of mesenchymal tumours affecting dogs across breeds and anatomical sites. While human STS are classified into more than 100 molecularly defined entities, that inform diagnosis and therapy, canine STS classification relies primarily on morphology and lacks subtype-specific markers. As we cannot accurately distinguish between subtypes, canine STS are largely managed using a uniform therapeutic approach, which is in strong contrast to human data showing that subtypes matter very much when it comes to therapy. Consequently, up to 30% of canine patients develop fatal disease despite surgical treatment. To improve therapy of canine STS, a more accurate molecular classification is needed to inform on patient outcome and identify subtype-specific therapeutic options. However, limited molecular datasets linked to clinical outcomes have hindered development of such a classification.

Here we performed a tissue-resolved analysis of tumour and matched adjacent normal tissue from archival FFPE specimens comprising 331 tumours from 323 canine patients. Laser-capture microdissection was combined with RNAsequencing to characterize tumour subtypes, identify key markers to improve diagnosis and assess subtype-specific targets for canine STS.

Multimodal profiling revealed a diverse molecular STS landscape highlighting already defined canine STS subtypes as unique molecular entities, while also revealing a diverse cloud of fibroblastic tumours as well as two undefined novel subtypes. In addition, on average 10% (3 – 33%) of cases were reclassified based on their molecular profile. While all tumour samples shared enrichment in E2F targets and G2M checkpoint, cluster specific analysis revealed individual enrichment of subtype-specific features as well as markers and targets for improved diagnosis and novel therapeutic options.

These findings provide the first comprehensive molecular characterization of the canine STS landscape, highlight the diversity of canine STS as well as novel subtypes and diagnostic challenges, and identify candidate biomarkers for diagnostic refinement and targeted approaches. Linkage to clinical data will further illuminate shared and subtype-specific features to assess prognosis and guide clinical decision making.

Hiesinger et al.

Unraveling STAT5B-driven NK-cell leukemia: Mechanisms and Therapeutic Strategies

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Natural Killer (NK) cells are cytotoxic lymphocytes essential for early host defense and, in rare cases, the origin of highly aggressive malignancies. NK-cell leukemias have a very poor prognosis and limited treatment options, highlighting the need for novel therapeutic strategies and adequate preclinical models. NK-cell functions are tightly regulated by cytokines, such as IL-2 and IL-15, which are potent activators of STAT5. Gain-of-function mutations of STAT5B, in particular STAT5B^{N642H}, have been reported as oncogenic drivers in several T-cell and NK(T)-cell malignancies and are associated with highly aggressive forms.

To study the role of hyperactive STAT5B in NK cells, we developed a STAT5B mutant NK cell-specific mouse model that effectively mimics indolent NK-LGLL (NK-large granular lymphocytic leukemia). Notably, upon aging, approximately 33% of these mice further developed late-onset aggressive NK-cell leukemia, providing a unique *in vivo* system to study NK-cell transformation.

Our experiments also revealed that STAT5B^{N642H} drives NK-cell senescence, potentially acting as a barrier to leukemic transformation. However, prolonged *in vitro* culture allowed some NK cells to evade senescence and acquire disease-initiating potential, suggesting that senescence escape may drive aggressive disease progression.

We now aim to dissect the senescence-to-transformation process using our novel mouse model. This model enables us to study the paradoxical role of STAT5B in both enforcing NK-cell senescence and promoting NK-cell malignancy and identify novel therapeutic targets and strategies. We believe that our work will expand our knowledge of the oncogenic mechanisms of STAT5B in NK-cell leukemia.

List et al.

Ink4a/Arf-deficient mouse model of aggressive NK cell leukemia reveals XPO1 vulnerability

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Aggressive NK cell leukemia (ANKL) is a rare, highly lethal malignancy with limited therapeutic options and preclinical models. Through molecular characterization of a pediatric EBV-negative ANKL case harboring homozygous *CDKN2A* deletion, we identified genetic disruption of the *CDKN2A/INK4A-ARF* axis as a candidate driver of NK cell leukemogenesis. Supported by recurrent *CDKN2A* alterations in additional ANKL cases, we investigated the functional consequences. *Cdkn2a* loss promoted senescence escape and drove sustained proliferation of primary murine NK cells and, upon transplantation, induced aggressive, serially transplantable NK cell leukemia that molecularly and immunophenotypically recapitulated human ANKL. High-throughput drug screening identified XPO1 as a therapeutically actionable vulnerability in leukemic NK cells, which exhibited elevated XPO1 expression. The clinically approved XPO1 inhibitor Selinexor exhibited potent anti-leukemic activity *in vitro* and *in vivo* across murine and human ANKL models. Together, we present a clinically relevant ANKL model that facilitates pre-clinical therapeutic discovery, positioning XPO1 inhibition as a promising strategy for ANKL.

Hillebrand et al.

Efficacy of four potential antimicrobial substances and bacteriophages against *Klebsiella* species and *Streptococcus uberis* from bovine mastitis *in vitro*

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In the need of alternatives for mastitis therapy due to emerging antimicrobial resistances and limited antibiotic agents, antimicrobial potential of four promising substances and bacteriophages (phages) was examined: Polyvinylpyrrolidone-iodine (PVP-I), N-acetylcysteine (NAC), Methylglyoxal (MGO), and N-chlorotaurine (NCT).

Minimal inhibitory concentrations (MICs) of the substances (n=6) and common antibiotic agents (n=1) against *Klebsiella* species (spp.) and *Streptococcus* (S.) *uberis* isolates from bovine mastitis were determined with broth microdilutions in accordance with CLSI standards. Antibacterial efficacy of five different phages was evaluated with planktonic killing assays (n=3). The biocompatibility of substances and phages was examined with primary bovine mammary epithelial cells (pbMECs), which were isolated from udders of dairy cows obtained from an abattoir. The impact of substances and phages on viability and proliferation of pbMECs was measured with neutral red and crystal violet assays (n=5).

Although *Klebsiella* spp. isolates were not susceptible to examined concentrations of penicillins, macrolides, and lincosamides, all four substances revealed an antimicrobial effect: MICs of PVP-I were 5 mg/mL (*Klebsiella* spp.) and 4 mg/mL (*S. uberis*), NAC 5 mg/mL (*Klebsiella* spp.) and 1.75 mg/mL (*S. uberis*), NCT 1.5 mg/mL (*Klebsiella* spp.) and 1 mg/mL (*S. uberis*) and MGO with 0.4 mg/mL (*Klebsiella* spp.) and 0.14 mg/mL (*S. uberis*). Out of five investigated phages, vB_KpnS_LmqSRe28-2 was most efficient and completely inhibited growth of seven *Klebsiella* spp. strains over 24 hours with a multiplicity of infection (MOI) of 10. Analysis of cytotoxicity data indicates significantly reduced viability and proliferation of pbMECs after treatment with the substances at MIC level and one step below, while phage vB_KpnS_LmqSRe28-2 had no significant influence.

Growth of bacterial isolates was inhibited by all four substances and phage vB_KpnS_LmqSRe28-2, despite resistance to commonly used antibiotic agents. Biocompatibility testing will be extended to tissue with precision cut bovine udder slices (PCBUS) and infection models with pbMECs and PCBUS will assess capability for use in infected cells and tissue. With positive findings, the substances and phages could be considered as promising alternatives for mastitis treatment in dairy cattle.

Gerdes et al.

Opioid signaling through the canine MRGPRX2 receptor

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The Mas-related G protein-coupled receptor member X2 (MRGPRX2) mediates drug-induced pseudo-allergic reactions and is proposed to contribute to the pathogenesis of atopic dermatitis, making it a potential therapeutic target. Although initially described for primates, functional orthologs have been identified in other mammals, including the dog. While human MRGPRX2 (hMRGPRX2) activation by opioids with morphinane structure is well-characterized, this study investigated whether morphine-induced histamine release in dogs may similarly be mediated via canine MRGPRX2 (cMRGPRX2).

Sequence analysis revealed significant structural divergence at the N-terminus of the canine receptor, which is much longer in the dog (117 amino acids; AA) than in man (28 AA). Apart from the N-terminus, sequence homology was approximately 65%, demonstrating lower conservation compared to classical opioid receptors (> 90 %). Despite this divergence, molecular docking simulations identified a conserved binding pocket for endogenous peptide agonists (substance P, cortistatin-14, and PAMP-12) across both species. To our surprise, docking simulations with morphine revealed different interaction points within the binding pocket. Additionally, while hMRGPRX2 showed no stereoselective preference between morphine enantiomers, cMRGPRX2 models exclusively bound the levo-enantiomer.

Functional assays including ERK 1/2 phosphorylation confirmed that hMRGPRX2 is activated by both levo- and dextro-morphine, as well as the synthetic agonist compound R-Zinc. In contrast, cMRGPRX2 was activated by both morphine enantiomers, but remained unresponsive to R-Zinc.

These findings indicate that while cMRGPRX2 shares functional endpoints with its human ortholog, it possesses distinct structural characteristics and altered ligand-binding preferences. Both the similarities and differences in activation profile make it an interesting target to study further, for its possible involvement in atopic dermatitis which is a common pathology witnessed in veterinary practice.

Xie et al.

Different Roles of S1PR2 and S1PR3 in Mediating Itch and Inflammation Associated with Canine Atopic Dermatitis

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Sphingosine 1-phosphate (S1P) has been found to regulate inflammatory skin diseases such as canine atopic dermatitis (CAD). However, the roles of its receptors S1PR2 and S1PR3 in mediating CAD-associated itch and inflammation remain poorly understood. We hypothesized that the two receptors differentially regulate itch and inflammation in CAD.

To investigate itch-related mechanisms, murine sensory dorsal root ganglion (DRG) neurons were isolated, and calcium imaging was conducted to measure their responses to pruritogens. Additionally, Neuronal responses in DRG were compared between wild-type and S1PR2 knockout (KO) mice. In vitro, a 3D canine epidermal equivalent model stimulated with IL-4/IL-13 was used to mimic CAD-like conditions and evaluate the inflammatory response after exposure to antagonists. In vivo, a chronic allergic contact dermatitis (ACD) model was induced in BALB/c mice using toluene-2,4-diisocyanate (TDI). Mice were topically pre-treated, followed by five consecutive TDI challenges, and corresponding itch behaviors and ear swelling were monitored. Furthermore, a CAD model was established in beagles by house dust mite (HDM) sensitization under a blinded, cross-over design. Dogs were topically pre-treated, followed by HDM challenge. Itch behaviors and scores of skin lesion were evaluated.

The S1PR2 modulates the itch response as JTE013 (an S1PR2 antagonist) significantly reduced sensory neuron activation in response to S1P in mice. Responses to S1P, CYM5520 (S1PR2 agonist), histamine and capsaicin were significantly lower in S1PR2 KO mice compared to wild-type controls. Notably, topical administration of JTE013 significantly reduced itch events in dogs with AD. S1PR3 mainly modulates inflammatory responses as TY52156 (S1PR3 antagonist) decreased CXCL8 expression induced by IL-4/IL-13 in the 3D canine epidermal equivalent model. Topical treatment of TY52156 significantly suppressed the ear swelling induced by TDI challenges in the murine ACD model and reduced the skin lesion scores in dogs following HDM exposure in the CAD model. Our study indicates that S1PR2 predominantly mediates itch, whereas S1PR3 primarily modulates inflammation in allergic dermatitis.

Wilzopolski et al.

Sentiment Barometer Regarding the Approval Process for Animal Experiments – Does German Bureaucracy Impact Cross-Border Outsourcing of animal experiments?

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Introduction

In recent years the number of animal experiments in Germany has fallen steadily. The exact causes of this trend remain unclear. The research community, in particular, has suggested that the complex authorisation process for animal experiments in Germany is leading to research projects being relocated abroad and could thus contribute to the decline.

Description

We investigated these issues in more detail as part of an online survey of researchers involved in animal experiments. The survey aimed, on the one hand, to assess current attitudes towards the authorisation process and, on the other, to identify possible reasons for the reduction in animal experiments. Furthermore, potential solutions for improving the existing authorisation process were examined. The survey, conducted in early 2026, received a high response rate with over 3,000 participants.

Initial analyses reveal a high level of dissatisfaction with the current authorisation process. 94 % of respondents criticise the high level of bureaucracy, and 85 % the lack of compatibility with the framework conditions of their research.

Furthermore, 82 % state that the effort involved in writing an animal experiment application has increased, and 75 % report a significant increase in the duration of the approval process. The median processing time reported is approximately 120 working days from the submission of the application to the final regulatory decision.

39 % of respondents stated that they are or have been involved in projects in which animal experiments were carried out abroad. The most frequently mentioned reasons for this are time savings in the authorisation process (63 %), the expertise of the collaborating partners (63 %) and less bureaucratic burden (50 %). Almost all respondents (97 %) see room for improvement in the current approval process. In particular, they would like to see shorter processing times (92 %), shorter animal project applications (82 %), fewer rounds of questions (78 %) and harmonisation of the application form (69 %).

Conclusions

The high level of interest and clear dissatisfaction highlight the relevance of the issue. The main focus is on implementation issues, such as long processing times and extensive application forms. Additionally, there is a risk that bureaucracy might drive German researchers and industry to perform their animal experiments abroad. Based on the available data, the identified suggestions for improvement should be systematically reviewed and integrated into future political and administrative decisions.

Kofen et al.

Dosing Strategies of Buprenorphine in Laboratory Rodents: A Systematic Scoping Review

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Buprenorphine is a widely used opioid for managing postoperative pain in mice and rats. Pharmacokinetic studies suggest that maintaining therapeutic plasma concentrations for sufficient pain reduction may require frequent administration. Different administration strategies, including oral self-administration or use of slow-release drug formulations, are increasingly sparking interest in refined dosing practices. However, short dosing intervals and high-dose application raise concerns about potential adverse drug effects. Given these considerations, this systematic scoping review aimed to identify the most common dosing practices in the use of buprenorphine, as well as the reported analgesic efficacy and adverse events.

This review was based on two convenience samples derived from previously published reviews by Aulehner et al. 2022 and King et al. 2023. In this study, we conducted a follow-up search applying the same search strategies to capture studies published in more recent years. Two trained reviewers conducted an independent screening of title/ abstract and full text, followed by data extraction on study and animal characteristics, surgical procedures, analgesic dosing strategies, analgesic efficacy, and adverse events.

A total of 372 studies were included in the data extraction. The preliminary results presented are based on studies identified by King et al. (k=331). The two anaesthetics most commonly used in combination with buprenorphine were isoflurane (67.5 %) and ketamine/ xylazine (15.8 %). Buprenorphine was most commonly administered subcutaneously. 21 % of the studies did not specify the route of administration. The proportion of studies reporting preoperative administration of buprenorphine increased over time from 30.8 % in 2009 to 48.0 % in 2024. In this sample, 24.8 % of studies reported repeated buprenorphine administration. In 2024, dosage ranges for standard (non-sustained release) formulations ranged from 0.01 to 1 mg/kg in rats and from 0.01 to 0.6 mg/kg in mice. The use of sustained-release buprenorphine formulations increased from 0 % in 2009 to 27.5 % in 2024, with dosages ranging from 0.5 to 1.2 mg/kg in rats and 0.05 to 5 mg/kg in mice.

Based on the preliminary findings of this systematic review, the limited evidence on efficacy and tolerability, combined with the substantial variability in buprenorphine doses used across studies, underscores the need for standardized evaluation of dosing regimens. This will provide a stronger evidence base for dosing recommendations in laboratory rodents and contribute to the refinement of animal research.

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Schoeninger et al.

Development and validation of a diagnostic assay for porcine stress marker detection

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Rising global demand for pork has driven the intensification of pig production. To maximize efficiency and reduce costs, husbandry and transport practices are optimized, compromising animal health through chronic stress. While some stress biomarkers (e.g., cortisol, alpha-amylase) have been identified, the high individual variability and the lack of a standardized panel limit the accurate assessment of stress in pigs, emphasizing the need for new biomarkers. Cyclophilin A (CypA) has emerged as a promising candidate. CypA is a ubiquitously expressed intracellular enzyme involved in protein folding/trafficking and T-cell activation. Due to its increased presence in extracellular body fluids in response to factors such as oxidative damage, hypoxia or infection, CypA can be regarded as a potential biomarker for stress, inflammation, and tissue injury. This study therefore aims to develop a sandwich ELISA-based detection method for the quantitative determination of CypA in serum/plasma obtained from different pig breeds.

To generate a CypA-specific capture antibody, a synthetic peptide unique to CypA and absent in related cyclophilin isoforms was designed and used to immunize rabbits. Anti-CypA antibody from antisera was affinity-purified and diluted in carbonate buffer (pH 9.5) for microplate coating. A recombinant CypA standard was used to validate assay performance and to define the working concentration range. Detection was performed using a commercially available and in-house HRP-conjugated anti-CypA antibody. Signal was developed with TMB substrate and quantified spectrophotometrically at 450 nm. Preliminary results indicate that the ELISA reliably detects CypA with high sensitivity, reaching a detection limit in the low nanomolar to subnanomolar range. Therefore, the assay is expected to facilitate the monitoring of stress-induced physiological changes in pigs. Currently, the stability of the CypA standard is being evaluated, and the assay conditions are being optimized.

Ducray et al.

In a wireless world: Is PARP1 a key player in the development of iPSC-derived dopaminergic neurons?

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The rapid expansion of wireless technologies has increased exposure to radiofrequency electromagnetic fields (RF-EMF), prompting concerns about potential health effects. While no conclusive evidence links RF-EMF to neurodevelopmental disorders or neurodegeneration, possible cognitive effects remain under investigation.

Here, we examined the role of poly(ADP-ribose) polymerase 1 (PARP1) in neuronal development and its interaction with RF-EMF exposure using wild-type (WT) and PARP1-knockout (KO) human induced pluripotent stem cell-derived dopaminergic neurons. PARP1 expression declined between differentiation and maturation in wild type cells, supporting its role in early neurodevelopment. PARP1 deficiency enhanced the dopaminergic phenotype and synaptophysin expression without affecting neuronal maturity, and selectively altered spike timing and burst properties during early and late differentiation. In contrast, RF-EMF exposure did not affect neuronal phenotype, maturation, synaptic integrity, electrophysiological activity, or the proteome in either genotype. Proteomic analyses further showed that genotype-dependent differences far exceeded any RF-EMF-related effects. Future studies should assess later developmental stages and disease-relevant models.

Jiménez et al.

Nogo-A in Parkinson's Disease: Insights from hiPSC-Derived Dopaminergic and Mitochondrial Profiling

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Nogo-A, a myelin-associated protein, is known for limiting the central nervous system's capacity for self-repair by inhibiting axonal regeneration. However, emerging evidence points to broader roles in synaptic regulation, myelin maintenance, and mitochondrial homeostasis—dysfunctional processes in Parkinson's disease (PD). Understanding the role of Nogo-A in dopaminergic neuron survival may provide key insights into PD pathophysiology and highlight novel therapeutic targets.

We examined the effects of Nogo-A on neuronal development and neurodegeneration using wild-type (WT) and Nogo-A-knockout (KO) human induced pluripotent stem cell (hiPSC)-derived dopaminergic neurons. To model PD-related stress, cells were exposed to 6-hydroxydopamine (6-OHDA). Viability, oxidative stress, mitochondrial function, and electrophysiology were assessed using protein analysis, functional assays and high-density multi-electrode array (HD-MEA).

Preliminary data suggest that Nogo-A deletion may enhance dopaminergic differentiation, with KO cultures exhibiting a higher baseline proportion of TH-positive cells and an apparent upregulation of LMX1A and TH. Conversely, KO cells appeared to express lower levels of synaptophysin alongside higher levels of high-molecular-weight alpha-synuclein across conditions. At baseline, KO neurons showed a trend toward increased mitochondrial superoxide production and elevated general oxidative stress, despite general mitochondrial integrity remaining preserved. While 6-OHDA reduced cell survival and heightened oxidative stress to a similar extent in both genotypes, KO neurons displayed subtle shifts in mitochondrial functional parameters under stress compared to WT cells.

These findings suggest that Nogo-A influences dopaminergic homeostasis, synapsis and mitochondrial dynamics. Although the absence of Nogo-A does not grant direct neuroprotection against acute 6-OHDA toxicity, its influence on baseline differentiation and oxidative homeostasis highlights complex molecular pathways that warrant further study.

Haiber et al.

A Human iPSC-Derived Neurosphere Model for Venezuelan Equine Encephalitis Virus Infection to Study Its Effects on the Developing Human Brain

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Venezuelan equine encephalitis virus (VEEV) is a re-emerging neurotropic arbovirus that targets the developing human brain and can lead to miscarriage, stillbirth, or long-term neurological sequelae, including cognitive impairment. The mechanisms underlying these outcomes remain poorly understood.

Human stem cell-based in vitro models with defined developmental neurotoxicity endpoints have been established as useful tools for mechanistic hazard identification in brain development. In this study, we apply this framework and use human induced pluripotent stem cell (iPSC)-derived neurospheres to analyse VEEV-induced neurodevelopmental impairments and to identify mechanistically anchored targets for intervention.

We show that both neuronal and glial cells within neurospheres are susceptible to VEEV infection and that infection consistently impairs neurite outgrowth, a neurodevelopmentally relevant endpoint linked to network formation and later cognitive function. To investigate the underlying mechanisms, we performed multi-omics analyses, which indicate a global translational shutoff and downregulation of cytoskeleton-related pathways, in particular Rho GTPase signaling, accompanied by reduced secretion of neurite-associated proteins.

These observations support a role for disrupted neurite outgrowth and migration as a central mechanistic axis of VEEV-associated neurodevelopmental toxicity and point to a reduced availability of secreted migration-associated factors as a potential target for therapeutic modulation. Overall, our work characterises iPSC-derived neurospheres as a human-relevant platform to study virus-brain interactions and offers mechanistic clues for strategies aimed at reducing long-term neurological sequelae.

Fischer et al.

Investigating Prenatal *Listeria*-Induced Neurodevelopmental Disturbance in a Human iPSC Neurosphere Model

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Vertical transmission of viral, bacterial, and parasitic pathogens during pregnancy can result in infection of the developing central nervous system of the fetus. These gestational infections may result in miscarriage and stillbirth or in neurodevelopmental disorders later in life. *Listeria monocytogenes* represents a key example, as prenatal exposure has been associated with persistent neurological impairments. However, the mechanisms underlying long-term consequences remain poorly understood. Suitable animal models are limited for many pathogens, as numerous agents are host-specific and cannot be readily modeled in mice. In addition, conventional two-dimensional cell culture systems are insufficient for investigating complex brain developmental processes. To address these limitations, three-dimensional models derived from human induced pluripotent stem cells (iPSCs) are gaining increasing relevance. We used human iPSC-derived neurospheres predominantly composed of neural stem cells (NSCs) along with neuronal and glial cell populations. Their developmental stage approximates that of the early fetal forebrain, rendering them suitable for analyzing key developmental processes. Infection with *L. monocytogenes* was shown to reduce neurite outgrowth in neurospheres significantly, detectable at various time points post-infection, which was particularly pronounced in younger neurospheres, and during the acute infection phase. Furthermore, gene expression analyses indicated that infection influenced the differentiation of neural progenitor cells toward a neuronal identity, whereas no significant differences were observed for glial markers between infected and uninfected neurospheres. Preliminary results of Multi-Electrode Array measurements suggested potentially elevated network activity in infected neurospheres, warranting further in-depth analysis. Overall, these findings establish a viable methodological and conceptual foundation for subsequent investigations into the disruption of early brain development by bacterial infections, specifically *Listeria monocytogenes*.

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Abstracts: Poster presentations

P01 Berndl et al.

Standard opioid dosages may not provide continuous analgesic coverage in C57BL/6J mice

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Background and methods: Analgesic refinement is an essential part of the 3R principles, directly supporting both animal welfare and scientific validity. We conducted a comparative analysis of buprenorphine (BUP), butorphanol (BUT), and tramadol (TRA) in healthy adult C57BL/6J mice (n = 21 per sex and group). Drugs were administered either as a single subcutaneous injection or continuously via drinking water at dosage regimens derived from published literature or current guidelines. Following acclimatization and baseline measurements, we evaluated serum pharmacokinetics (LC-MS/MS) alongside thermal antinociceptive efficacy (tail immersion test). To assess tolerability and side effects, we monitored core body temperature, neurobehavioral responses (Irwin test), grimace scale, body weight, as well as food and water consumption. Furthermore, changes in spontaneous home-cage behavior, including voluntary wheel running, nesting, burrowing and grooming were recorded. Finally, hematological and biochemical parameters were measured, and selected organs underwent histological analysis.

Results and discussion: Subcutaneous injection resulted in fast plasma elimination with very short half-lives ($t_{1/2}$ = 1.9 h for BUP, 0.84 h for BUT, 0.76 h for TRA). Consequently, antinociceptive efficacy was restricted to 1 h post-injection for BUT and BUP, whereas TRA failed to induce significant antinociception. Side effects such as hyperthermia and Irwin-test parameter alterations (Straub tail, increased irritability, and impaired grip strength) were seen across all drugs. During administration via drinking water, plasma concentrations remained consistently below estimated therapeutic thresholds, producing no antinociceptive effects, despite achieving target dosage intake. Yet, mice still showed reduced voluntary wheel-running activity (BUP and TRA), and reduced nesting and grooming scores (BUT and TRA). Notably, TRA might have impaired liver function, indicated by elevated ALT and low thrombocyte count.

Conclusion: These findings suggest that currently recommended opioid dosages do not provide continuous, effective analgesic coverage in C57BL/6J mice. For subcutaneous injections, extended- or sustained-release formulations appear advisable. For voluntary oral administration, higher target dosages (specifically for BUP and BUT) or multimodal regimens should be investigated.

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P02 Borojević et al.

INK4a and ARF in NK-cell senescence and leukemia

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Natural Killer (NK) cells are effector lymphocytes controlling tumors and microbial infections. Age-related NK-cell dysfunction contributes to a reduced host defense with accumulating age, called immunosenescence. Senescence is a physiological event limiting the proliferation of damaged cells, thereby functioning as a barrier to malignant transformation. We have limited knowledge of senescence and neoplastic drivers in NK cells and how it progresses to an aggressive NK-cell leukemia, a serious disease with limited treatment options. New pre-clinical mouse models of NK-cell leukemia are required to develop novel therapeutic approaches.

We have established an NK-cell senescence model and could show that senescent NK cells upregulate p16^{INK4a} and p19^{ARF} and that loss of the *Ink4a/Arf* locus allows leukemic transformation of NK cells and outgrowth of stable NK cell lines. Transplantation of *Ink4a/Arf* KO NK cells into immunocompromised mice leads to the development of NK-cell leukemia.

We propose that INK4a and ARF have pivotal roles in NK-cell senescence and postulate that the development of NK-cell leukemia is only promoted when both p16^{INK4a} and p14/19^{ARF} are lost concurrently, allowing for the accumulation of additional mutations in NK cells. This study aims to explore the individual functions of p16^{INK4a} and p19^{ARF}, as well as their cooperative roles, in i) NK-cell senescence both *in vitro* and *in vivo* in mice, ii) NK-cell transformation, and iii) validation of the role of INK4a and ARF in human NK-cell senescence.

Additionally, we seek to establish a senescence signature for mouse NK cells to precisely define and characterize senescent mouse NK cells, and to identify pathways implicated in NK-cell senescence.

We will use NK-cell *in vitro* cultures and genetically modified mouse models, aging experiments, flow cytometry, CRISPR-Cas9-mediated manipulation of NK cells and multiplex arrays to study NK-cell senescence and leukemia development. Given the challenges posed by NK-cell malignancies, our project could establish a pre-clinical NK-cell leukemia mouse model, providing crucial resources for screening new therapies.

P03 Cordel et al.

Potential therapeutic benefit of combining cephalexin and loratadine in the treatment of MRSP infections in dogs – an in vitro and ex vivo investigation

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Among veterinary pathogens, *Staphylococcus (S.) pseudintermedius* is a leading pathogen in canine pyoderma, a common and often recurrent skin infection.

The clinical utility of cephalexin, a first-generation cephalosporin and mainstay of systemic therapy for deep pyoderma, is increasingly limited by the widespread emergence of methicillin resistant *S. pseudintermedius* (MRSP), which exhibits resistance not only to methicillin but also to other beta-lactam antibiotics, including cephalexin. The global rise in antibiotic resistance highlights the critical need for innovative therapeutic strategies in the management of bacterial infections.

In our recent in vitro study, using agar dilution assay, we observed an enhancement of cephalexin's activity against MRSP when combined with loratadine, a second-generation antihistamine. This finding suggests a potential repurposing of loratadine as a non-antibiotic adjuvant to overcome resistance mechanisms and improve treatment outcomes. To assess the feasibility of topical application, thereby enabling localized drug delivery and potential synergistic effect with cephalexin, we investigated the cutaneous penetration of loratadine using an ex vivo Franz diffusion cell model with canine skin. Quantification of loratadine in both the receptor compartment and within skin layers was performed via validated high-performance liquid chromatography (HPLC).

The minimal inhibitory concentration (MIC) of each agent alone and in combination was determined by using agar dilution assay according to Clinical and Laboratory Standards Institute (CLSI M07-A9). Dose Reduction Index (DRI), Fractional Inhibitory Concentration of loratadine relative to cephalexin ($FIC_{\text{loratadine}\rightarrow\text{cephalexin}}$), and the FIC index (FIC_i) were calculated to evaluate the synergistic and antagonistic potential of the combination. All MRSP strains exhibited a dose-reduction effect when the combination was applied (DRI). Regarding the $FIC_{\text{loratadine}\rightarrow\text{cephalexin}}$, 80% of the strains (4/5) demonstrated a synergistic interaction. The FIC_i values were less than 0.5 in 40% of the isolates (2/5), which confirms a synergistic effect.

After 24h, 0.04% of loratadine was detected in the acceptor medium, indicating that loratadine permeates through the skin. On average, 93.81µg/cm² of loratadine was recovered from the canine skin following 30 hours of topical application, corresponding to 0.32% of the applied dose.

These results support the hypothesis that loratadine is a promising substance that can act synergistically with cephalexin and, due to its ability to permeate canine skin, could be suitable for additional topical therapy in the treatment of MRSP infections.

P04 Dalponte et al.

Evaluating Phage–Antimicrobial Combination Strategies Against *Pseudomonas aeruginosa*

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Infections caused by *Pseudomonas aeruginosa*, especially chronic infections, are often challenging to manage. This is mainly due to the organism's ability to form biofilms and its pronounced resistance to many antimicrobial agents, both naturally and through acquired mechanisms. For this reason, there is increasing interest in treatment approaches that can improve antimicrobial activity while potentially reducing reliance on conventional antibiotics. Bacteriophages, particularly when applied together with supportive antimicrobial compounds, may offer a promising alternative or complementary strategy.

The objective of this study was to determine whether bacteriophages applied individually, combined as a phage cocktail, or used together with additional antimicrobial substances such as antibiotics or antiseptics can produce enhanced antibacterial activity against *P. aeruginosa*. A key focus was to assess whether the tested combinations produced additive or synergistic antibacterial effects.

Two lytic bacteriophages (JG003 and PTLAW1) were examined both separately and in combination. Their antibacterial activity was further tested alongside the antibiotic marbofloxacin and the antiseptic agents octenidine and polyhexanide. A checkerboard assay was used to evaluate the interactions between phages and the tested compounds. Bacterial growth was followed over a 20-hour period by measuring optical density at 600 nm. The resulting growth curves were analyzed to assess whether the observed effects were synergistic, additive, or antagonistic. In addition, the impact on biofilms was investigated by assessing cell viability and biofilm biomass using live/dead staining and fluorescence microscopy.

The combined use of the two phages led to a stronger reduction in bacterial growth than either phage alone, as shown by a significantly decreased area under the growth curve and a lower final bacterial density. Microscopic analysis of biofilms supported these findings, revealing a higher proportion of non-viable cells and a decrease in total biofilm biomass after treatment with the phage cocktail. When phages were combined with marbofloxacin, a synergistic antibacterial effect was observed, resulting in markedly reduced bacterial growth and endpoint density. A comparable synergistic response was also detected when phages were combined with octenidine at the tested concentration. In contrast, the addition of polyhexanide did not further improve antibacterial activity under the conditions tested here.

The effectiveness of phage-based antibacterial treatment can be strongly affected by the antimicrobial substances used in combination with phages. Screening phage interactions with antibiotics and antiseptics in a systematic way can help to identify beneficial combinations and may contribute to the development of optimized treatment strategies. Such approaches could support more targeted and antibiotic-sparing therapies for infections caused by *P. aeruginosa*.

P05 Freymüller et al.

Efficacy, Adverse Effects, and Tolerability of Antiseizure Medications and Alternative Treatments in Canine Idiopathic Epilepsy: A Systematic Analysis

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More than a decade has elapsed since treatment strategies for canine idiopathic epilepsy (IE) were last evaluated in a systematic review. This review aimed to provide an updated assessment of the evidence for antiseizure medications (ASMs) and alternative therapeutic interventions in dogs diagnosed with IE.

A systematic search of PubMed, CAB Direct, Frontiers, and Google Scholar was performed. Study selection was conducted in accordance with PRISMA guidance and recommendations from the Cochrane Handbook for Systematic Reviews of Interventions. Risk of bias was evaluated using RoB 2 for randomized controlled trials and ROBINS-I for non-randomized studies, while the methodological quality of case reports and case series was assessed using the CARE Guidelines. Studies were grouped by design, and relevant clinical outcomes were extracted. Meta-analysis was undertaken when sufficiently comparable data were available.

Fourteen studies assessing ASMs and eighteen investigating alternative therapies fulfilled the inclusion criteria. Direct comparison between studies was restricted by substantial variation in study design, patient characteristics, treatment protocols, outcome definitions, and risk of bias. Adjunctive cannabidiol, medium-chain triglyceride supplementation, and transcranial magnetic stimulation were associated with improvements in seizure control. Phenobarbital, potassium bromide, and imepitoin also showed therapeutic efficacy consistent with the conclusions of earlier reviews.

This systematic review offers an updated overview of both conventional and alternative treatments for canine IE. Although the evidence base for alternative interventions has grown, the overall methodological quality of the available literature remains inconsistent. Larger, adequately powered clinical trials using standardized outcome measures are needed to improve evidence-based treatment recommendations and guide individualized mono- and polytherapy approaches.

P06 Gamper et al.

CDK8 at the mitotic spindle: A novel vulnerability in leukemia

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Background: Cyclin-dependent kinase 8 (CDK8) is being explored as a therapeutic target across multiple cancer types. In B cell acute lymphoblastic leukemia (B-ALL), kinase-independent functions of CDK8 are critical for leukemic maintenance¹. In contrast, pharmacological inhibition of CDK8 kinase activity shows pronounced anti-proliferative effects in acute myeloid leukemia (AML)². This highlights a possible context-dependent and divergent role of CDK8 in leukemia, underscoring the need for a better understanding of CDK8's functions and novel therapeutic strategies with CDK8 inhibitors.

Aims: The aim of this study is to dissect kinase-dependent and kinase-independent functions and to identify novel roles of CDK8 in leukemia, by mapping the CDK8 interactome as well as transcriptomic patterns. Ultimately, this work aims to provide a rational for mechanism-based combination strategies targeting CDK8 and its functional network in leukemia.

Methods: The generation of a novel mouse model expressing wild-type CDK8 (wt), kinase-inactive CDK8^{D173A} or lacking CDK8 (ko) allowed us to establish bone-marrow derived stable leukemic cell lines expressing BCR-ABL^{p185+}. The impact of CDK8 on cell proliferation and disease latency was analysed by *in vitro* and *in vivo* experiments. The combination of RNA sequencing and CDK8 co-immunoprecipitation (Co-IP) followed by mass spectrometry (MS) and Western Blot was used to unravel CDK8 downstream mechanisms and identified novel CDK8 interactors. Findings were validated in human AML cell lines and combinatorial drug treatments.

Results: The lack of significant effects of CDK8^{D173A} expression and of CDK8 loss on normal hematopoiesis supports the feasibility of therapeutically targeting CDK8, suggesting selective vulnerability of malignant cells while sparing normal hematopoietic functions. Notably, CDK8^{D173A} and CDK8 ko BCR-ABL^{p185+} cell lines displayed reduced proliferative capacity and resulted in a significantly prolonged disease latency *in vivo*. RNA sequencing of stable BCR-ABL^{p185+} cell lines uncovered both kinase-dependent and -independent functions of CDK8. Integration of CDK8 Co-IP MS and transcriptomic data revealed a novel direct involvement of CDK8 in mitotic spindle organization and centrosome function, validated in human AML cell lines and underlining a general novel CDK8 kinase dependent mechanism. Pharmacologic agents targeting mitotic processes in combination with CDK8 inhibition highlighted anti-proliferative and apoptotic effects in our models.

Summary: This project advances our understanding of CDK8's versatile roles by establishing a mechanistic link between the transcriptional CDK and centrosome regulation, using novel leukemia models. Our findings support combinatorial strategies that pair CDK8 inhibition with agents targeting mitotic processes, with applicability extending beyond BCR-ABL^{p185+} disease to AML subtypes driven by diverse oncogenic programs.

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2. Lee, J. C., Liu, S., Wang, Y., Liang, Y. & Jablons, D. M. MK256 is a novel CDK8 inhibitor with potent antitumor activity in AML through downregulation of the STAT pathway. *Oncotarget* **13**, 1217–1236 (2022).

P07 Huszarek et al.

CDK4/6 inhibition releases CDK6-mediated suppression of NK Cell cytotoxicity

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Natural killer (NK) cell-based immunotherapies are actively being explored for cancer treatment; however, their efficacy is limited by functional impairment within immunosuppressive tumor microenvironments and heterogeneous patient responses. Novel strategies to enhance NK-cell cytotoxicity are therefore urgently needed. Cyclin-dependent kinases 4 and 6 (CDK4/6), key regulators of G1–S cell cycle progression, are clinically targeted by inhibitors such as Palbociclib, used in cancer therapy. While the immunomodulatory effects of CDK4/6 inhibition on T cells are well studied, their impact on NK-cell function remains unclear.

Here, we investigated the role of CDK4/6 inhibition and loss in regulating NK-cell effector function. In-vitro cytotoxicity assays demonstrated that treatment with three distinct CDK4/6 inhibitors significantly enhances NK cell-mediated killing, with this increased cytotoxicity persisting overtime. Gene set enrichment analysis of treated human NK-cell lines revealed consistent upregulation of the NK cell-mediated cytotoxicity pathway, further validated at protein levels.

Using NK cells isolated from wildtype (WT), CDK4-deficient, and CDK6-deficient mice, we further dissected the individual contributions of CDK4 and CDK6. Loss of CDK6 - but not CDK4 - was essential for enhanced NK-cell cytotoxicity. Importantly, Palbociclib-treated anti-CD19 CAR-NK92 cells exhibited enhanced killing of target cells. In-vivo, CDK6-deficient mice displayed reduced tumor burden following B16F10 or EO771 tumor challenge. Notably, adoptive transfer of Palbociclib-treated NK92 cells in NSGhIL15 mice enhanced tumor control, highlighting the therapeutic promise of CDK4/6-modulated NK-cell therapy.

Our findings identify CDK6 as a critical negative regulator of NK-cell cytotoxicity and highlight CDK4/6 inhibition as a promising strategy to potentiate NK cell-based cancer immunotherapies.

P08 Janßen et al.

***In vitro* antibacterial efficacy of nitrogen- and oxygen-enriched argon-based Cold Plasma (CP) against Canine Infectious Ulcerative Keratitis (CIUK) related pathogens**

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CIUK is a common ophthalmic disease caused by various pathogens requiring prompt treatment to preserve corneal integrity and vision. Current therapy relies on topical antibiotics but is limited by the need for pathogen-specific antibiotics and restricted application. CP has emerged as a promising alternative due to its broad-spectrum antibacterial activity combined with wound-healing properties. Previous studies demonstrated antibacterial efficacy of argon cold plasma, primarily mediated by reactive oxygen and nitrogen species. Therefore, this study investigated whether the addition of oxygen and nitrogen to argon CP enhances antimicrobial efficacy and established primary corneal cell models for future assessment of plasma cytocompatibility.

Staphylococcus (St.) pseudintermedius, *Pseudomonas (Ps.) aeruginosa* and *Streptococcus (Sc.) canis* were treated with CP generated from 100 % argon and five argon-based gas mixtures (G1-G5) containing increasing concentrations of oxygen and nitrogen (0.5-2.5 % each). Samples were exposed to CP for 30 sec., 2 min. and four consecutive 30-second treatment cycles separated by 30-second pauses and with distances of 3 mm and 6 mm reflecting clinically feasible treatment distances. Antibacterial efficacy was assessed by quantifying colony-forming units per ml (CFU/ml). For later cell compatibility studies, primary bovine corneal endothelial cells, keratocytes, and epithelial cells were cultured and characterized by immunocytochemistry. Growth curves were established to verify normal cell proliferation to ensure the suitability of cells for subsequent experiments.

Argon and gas mixture CP reduced CFU/ml compared with untreated controls. Increasing treatment duration (4 x 30 sec., 2 min.) resulted in greater bacterial reduction compared with a single 30 sec. treatment. Compared with pure argon, gas mixtures demonstrated higher antibacterial efficacy against *Sc. canis* and *St. pseudintermedius*, with G5 representing the most effective gas mixture. Treatment distance had no major impact on bacterial reduction, with comparable results observed at 3 and 6 mm. *Ps. aeruginosa* exhibited a different response pattern: at 3 mm distance, prolonged treatment duration and increasing concentrations of oxygen and nitrogen were associated with higher bacterial growth. In contrast, this effect was not observed at a treatment distance of 6 mm. As for cell culture, all corneal cell types displayed characteristic growth patterns consistent with their respective characteristics and were successfully identified by immunocytochemistry.

In conclusion, supplementation of argon plasma with oxygen and nitrogen enhanced antibacterial efficacy against Gram-positive CIUK-associated pathogens, whereas *Ps. aeruginosa* exhibited a distinct response pattern. Gas mixture G5 showed the most promising antimicrobial activity and may represent a suitable candidate for further investigations. Future studies are required to evaluate corneal cell compatibility following plasma treatment and to validate efficacy in clinically relevant models.

P09 Karakus et al.

Localization and Functional Characterization of the Orphan Carrier SLC10A5: Insights from in vitro Studies and the *Slc10a5* Knockout Mouse Model

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Abstract

SLC10A5 is classified as a member of the solute carrier family 10 (SLC10), which was originally annotated based on sequence similarity to the bile acid transporters NTCP and ASBT. However, the physiological function of this phenomenon remains to be fully elucidated. In the present study, the role of SLC10A5 in bile acid metabolism was investigated using a comprehensive combination of in vitro, ex vivo, and in vivo approaches, including heterologous expression systems, a plasma membrane-targeted SLC10A5–NTCP chimeric construct, primary hepatocyte uptake studies, *Slc10a5*^{−/−} mice, targeted bile acid profiling, gene expression analyses, microbiome sequencing, and untargeted metabolomics. SLC10A5 was predominantly localized to intracellular compartments and failed to mediate detectable bile acid transport in a variety of experimental systems. These findings are consistent with the observation that membrane targeting of SLC10A5 did not result in transport activity. However, primary hepatocytes isolated from *Slc10a5*^{−/−} mice exhibited reduced taurocholate uptake, suggesting an indirect role in hepatic bile acid handling. In vivo, *Slc10a5* deficiency did not induce significant metabolic abnormalities under basal conditions; however, it resulted in pronounced alterations in bile acid composition, including shifts in secondary bile acids, conjugation patterns, and bile acid hydrophobicity. These alterations were accompanied by substantial remodeling of the gut microbiota, characterized by depletion of Verrucomicrobia and enrichment of Clostridiales and Lactobacillales. However, these alterations did not result in significant changes to the total bile acid pool size or the core FXR-dependent feedback pathways. Interestingly, female mice exhibited selective transcriptional adaptations involving *Nr1h4* (FXR), *Abcb11* (BSEP), and *Cyp7b1*. Collectively, these findings demonstrate that SLC10A5 does not function as a classical plasma membrane bile acid transporter. Instead, SLC10A5 appears to act as an intracellular regulator of bile acid homeostasis, potentially through post-transcriptional modulation of NTCP-dependent bile acid uptake and intracellular bile acid processing. The resulting adaptations in bile acid composition, gut microbial ecology, and FXR-dependent signaling identify SLC10A5 as a previously unrecognized component of the host–microbiome–bile acid axis and provide new insight into the regulation of enterohepatic homeostasis.

P10 Kieron et al.**Synthetic Cyclotide T20K as a Biophysical Sensitizer in High-Risk Myeloid Leukemia**

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Acute Myeloid Leukemia (AML) presents a major clinical challenge due to extreme genetic heterogeneity and rapid chemoresistance. In our research, we investigate the plant-derived, cyclic peptide T20K as a novel "biophysical sensitizer"—a molecule that physically strains leukemic cells to uncover hidden metabolic vulnerabilities and boost combination therapies.

Utilizing its unique amphiphilic cyclic cystine knot (CCK) structure, T20K selectively targets phosphatidylethanolamine-rich membranes, permeabilizing both plasma and mitochondrial membranes. This physical disruption triggers p53-dependent pro-apoptotic signalling, suppresses ATM-mediated DNA repair, activates key transcriptomic stress and inflammatory networks, and causes a structural collapse of inner mitochondrial membrane machinery. Once T20K induces baseline organelle stress, it exposes subtype-specific metabolic vulnerabilities that we exploit through two distinct paradigms:

- **The "Pincer Attack":** In *KMT2A*-rearranged cells—which depend on hyperactive protein synthesis—combining T20K with the RNA Polymerase I inhibitor BMH-21 simultaneously strikes mitochondria and nucleoli, resulting in strong global synergy.
- **The "Stress Trap":** In the same *KMT2A*-rearranged model, combining T20K with the autophagy inhibitor Spautin-1 blocks the cell's ability to clear T20K-damaged organelles, driving a lethal bioenergetic collapse. Conversely, t(8;21) cells exhibit an additive effect.

These data demonstrate that T20K-mediated membrane stress can bypass oncogenic adaptation, offering a targeted, precision-medicine strategy for high-risk AML.

P11 Mayer et al.

Linking Seizure Burden and Anti-Seizure Drug Response to Neurodegeneration and Cerebral Glucose Metabolism in Experimental Epilepsy

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Background and purpose: Pharmacoresistance in epilepsy has been associated with both structural damage and altered cerebral glucose metabolism. However, the relationship between neurodegeneration, metabolic dysfunction, and anti-seizure drug response remains incompletely understood. This study investigates cerebral glucose metabolism and neurodegeneration in relation to individual phenobarbital (PB) responsiveness in the BLA post-status epilepticus (SE) rat epilepsy model.

Methods: Epileptic rats (n=12) underwent continuous EEG-video monitoring to determine spontaneous recurrent seizure (SRS) frequency during a pre-drug baseline period, PB treatment, and a post-drug period. Six non-epileptic rats served as controls, and PB plasma concentrations were determined during treatment. Positron emission tomography (PET) imaging with fluorodeoxyglucose ([¹⁸F]FDG) was performed in epileptic rats (n = 9, one drop-out) and controls (n = 6) to assess regional cerebral glucose metabolism *in vivo*. Neurodegeneration was quantified in NeuN- and Nissl-stained brain sections by quantification of neuronal density in the hippocampal hilus and semi-quantitative scoring of neuronal loss in hippocampal subregions and piriform/entorhinal cortex.

Results: Epileptic rats exhibited considerable interindividual variability in SRS frequency and response to PB, with five rats becoming seizure-free during treatment. PB plasma concentrations were negatively correlated with SRS frequency during treatment ($r = -0.8516$, $p = 0.0008$). Higher SRS frequency was associated with lower neuronal density in the dentate hilus during the pre-drug ($r = -0.693$, $p = 0.0014$), PB treatment ($r = -0.689$, $p = 0.0016$), and post-drug periods ($r = -0.609$, $p = 0.0073$). Similarly, higher SRS frequency correlated negatively with hippocampal [¹⁸F]FDG SUV during the pre-drug ($r = -0.631$, $p = 0.018$), PB treatment ($r = -0.571$, $p = 0.036$), and post-drug periods ($r = -0.661$, $p = 0.013$). The severity of hippocampal neurodegeneration correlated negatively with hippocampal [¹⁸F]FDG SUV ($r = -0.542$, $p = 0.049$), with a similar trend for neurodegeneration in the piriform cortex ($r = -0.489$, $p = 0.078$).

Conclusions: Higher seizure burden was associated with lower neuronal density in the dentate hilus and reduced hippocampal glucose metabolism, while more pronounced hippocampal neurodegeneration was associated with lower [¹⁸F]FDG uptake. These findings link seizure burden, neuronal loss, and metabolic dysfunction in experimental epilepsy and support further evaluation of [¹⁸F]FDG-PET as an *in vivo* marker of epilepsy-associated neuropathology and disease activity.

P12 Merten et al.**Establishment of a Primary Neuronal Culture Derived from Mito-QC Mice and Investigation of Mitophagy in the Thy1-aSyn (Line 61) Mouse Model**

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In Parkinson's disease, alpha-synuclein pathology coincides with mitochondrial dysfunction, contributing to dopaminergic neuron loss in the substantia nigra. To maintain cellular homeostasis healthy cells clear dysfunctional mitochondria via mitophagy. We hypothesize that alpha-synuclein pathology directly impairs mitophagy, resulting in the accumulation of damaged mitochondria which promotes neurodegeneration. To test this, we quantified mitophagy-related proteins in the Thy1-aSyn (Line 61) mouse model, which develops alpha-synuclein pathology accompanied by microgliosis and motor deficits. Our analyses revealed alterations in mitophagy-related protein levels consistent with impaired mitophagy compared with wild-type mice. We aim to crossbreed Thy1-aSyn and mito-QC mice, which inhabit a fluorescent reporter system that enables discrimination between mitochondria and mitolysosomes based on the differential pH sensitivity of the fluorophores. This model enables us to dissect the interplay of alpha-synuclein pathology and mitophagy. To investigate mitophagy at the cellular level, we established a primary neuronal culture derived from mito-QC mice. The primary cultures successfully expressed the reporter and formed functional neuronal networks. Future experiments will include primary neuronal cultures from Thy1-aSyn/Mito-QC to validate therapeutic impact of mitophagy on alpha-synuclein pathology. Taken together, this newly established model is an important foundation for future in vivo studies regarding mitochondrial dysfunction in Parkinson's disease. This approach will provide a powerful tool to investigate disease mechanisms and evaluate mitophagy-modulating therapeutic strategies while supporting the principles of the 3Rs.

P13 Niedergeses et al.**PeriMyelinTox: Developing an *in Vitro* Assay for Human Peripheral Demyelination**

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Current *in vitro* test batteries contain a critical data gap surrounding peripheral neurotoxicity. Along with the European Partnership for the Assessment of Risks from Chemicals (PARC), project PeriMyelinTox aims to develop a human-relevant assay for detecting chemical-induced peripheral myelin toxicity.

Human iPSC-derived Schwann cells (SC) and sensory or motor neurons (SN/MN) are co-cultured in 3D spheroids for 1-3 weeks, allowing myelin formation. Relative myelin content is then quantified by comparing confocal immunofluorescence images of neuronal and myelin-specific markers such as neurofilament or β III-tubulin and myelin basic protein. Using semi-automated software analysis, relative myelin content may then be compared across substances alongside positive and negative demyelinating controls (hexachlorophene and acetaminophen, respectively) to make statements regarding the potential myelin toxicity of each tested substance.

Both co-cultures (MN-SC and SN-SC) underwent in-depth characterization during platform development, including identification of physical sphere growth (size) over time and repeated mappings of myelin onset and stability. In this way, crucial timepoints in myelin expression, optimal testing window, and culture stability were identified. Additionally, the effects of potentially pro-myelinating substances were successfully identified using the same quantification system with the goal of increasing assay sensitivity by optimizing myelin content.

As a final step, best operating protocols for each individual assay (MN-SC vs. SN-SC) are presently being confirmed, after which, both versions will be validated with a blinded, standardized compound set from the European Joint Research Center in a cumulative, multi-institute work package from PARC. In conclusion, the PeriMyelinTox platform provides a novel, human-relevant, replacement approach for peripheral neurotoxicity testing in next-generation risk assessment with a wide range of potential applications for further studies.

P14 Parmar et al.

Multimodal analgesia for post-operative pain in mice and rats: A systematic review in progress

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Background: Clinical studies in humans show superiority of multimodal over single-agent analgesia for postoperative pain, but respective evidence in laboratory rodents remains unclear. We systematically reviewed existing studies in mice and rats to synthesize evidence on multimodal analgesia in post-operative pain.

Methods: A systematic review (SR) protocol was created according to the SYRCLE guidelines and preregistered on OSF Registries. After searching in PubMed, Web of Science, and CAB International Digital Library and subsequent deduplication, 3,085 records were screened in two phases (title/abstract; full text) by two independent reviewers per article using predefined inclusion and exclusion criteria, yielding 63 full texts for data extraction.

Preliminary results and conclusion: Twenty-three studies (36.5%) originated from North America, followed by 21 (33.3%) from Europe, with the earliest ones published in 1994 (rats) or 2010 (mice). The number of studies investigating rats was significantly higher than those focusing on mice (42 vs. 21; $\chi^2(1) = 7$, $p = 0.008$). With 88%, the use of males was predominant in rat studies, but less common in mouse studies (43%). Both sexes were used in only 4.8% of rat studies but in 33.3% of mouse studies. Sprague-Dawley rats (66.7%) and C57BL/6 mice (42.9%) were the most commonly used strains. Group sizes ranged from 3 to 50 animals/group or remained unclear in four rat and three mouse studies. Across the 63 studies, 81 unique experiments were identified (54, rats; 27, mice), of which the majority used the plantar incision model (63%, rats; 33.3%, mice), while procedure-specific models (e.g., laparotomy, craniotomy) were used in the other experiments. Investigated drugs, drug class combinations, and dosage regimens varied considerably. For instance, in rats, morphine doses ranged from 0.01 to 7 mg/kg or paracetamol from 10 to 500 mg/kg. While the combination of NSAID and opioid was the most frequently investigated regimen in mice (44.4% of experiments), it was far less common in rats (16.6%). Outcomes from individual experiments indicate superiority of multimodal treatment over monotherapy in rats (38/54) and mice (15/27), although findings were inconclusive in 8/54 and 2/27 experiments, respectively. However, conclusions were seldom (18.5% of rat and 33.3% of mouse experiments) based on the use of pain surrogate markers from more than one category (e.g., physiological, biochemical, stimulus-evoked and spontaneous behaviors). An on-going risk of bias assessment, followed by meta-analysis (if feasible), will provide quantitative estimates of the effectiveness of multimodal versus monotherapy. Overall, the current body of evidence is heterogeneous and methodologically inconsistent, limiting the development of evidence-based recommendations on multimodal analgesia.

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P15 Stauffenberg et al.**Cold Shock Protein RBM3 as a Neuroprotective Target:
Therapeutic Potential of RBM3-Inducing ASOs**

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Reducing cell death is a key goal of drug development for acute and chronic neurodegenerative diseases. Therapeutic hypothermia is neuroprotective, but systemic cooling carries potentially fatal adverse effects, making the molecular pathways of the cold-shock response a focus of research. Here we use an antisense oligonucleotide (ASO)-based approach to induce expression of the cold shock protein RBM3 (RNA-binding motif 3) at normal body temperature. RBM3 preserves synaptic plasticity, exerts anti-apoptotic effects, and prevents neuronal loss, making it a promising therapeutic target across neurodegenerative diseases. We assessed the safety of sustained RBM3 overexpression in transgenic mice and evaluated the neuroprotective efficacy of the RBM3-inducing ASO in a kainic acid (KA)-induced excitotoxic injury model. Safety was evaluated in mice lacking the poison exon in the RBM3 gene, resulting in significantly higher RBM3 levels across multiple organs compared to wild-type controls, with a significant increase in disease-relevant regions such as the hippocampus. Necropsy revealed no visible organ pathology, and the modified Irwin screen did not detect behavioral side effects at 6 and 12 months of age. Seven days after KA injury, histological analysis showed a significant, model-specific increase in hippocampal pyramidal cell death, reflected in higher neurodegeneration scores in Fluoro-Jade C-stained sections. In contrast, RBM3 ASO-treated mice were protected from neurodegeneration. Therapeutic upregulation of the cold-shock protein RBM3 at normothermia represents a promising strategy to mimic the benefits of systemic hypothermia without its adverse effects.

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P16 Steinmetz et al.**Biocompatibility of N-Acetylcysteine-containing bone cement with potential as an antibiotic alternative**

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Antibiotic-loaded polymethyl methacrylate (PMMA) bone cement is widely used in joint replacement surgery to prevent periprosthetic joint infections. However, the increasing prevalence of antibiotic-resistant pathogens highlights the need for alternative antimicrobial strategies that reduce the dependence on conventional antibiotics. N-acetylcysteine (NAC) has recently demonstrated antibacterial activity against *Staphylococcus pseudintermedius*. However, whether therapeutically relevant concentrations of NAC can be released from PMMA bone cement while maintaining osteoblast biocompatibility remains unknown.

Osteoblasts were exposed either to NAC or to extraction media obtained from NAC-modified PMMA bone cement at different concentrations. Cell viability and proliferation were assessed after 24 hours using the neutral red uptake assay and crystal violet staining, respectively. NAC release from PMMA bone cement was quantified by high-performance liquid chromatography (HPLC) over a period ranging from 1 h to 56 days. Furthermore, the expression of NF- κ B, procaspase-3, and cleaved caspase-3 was analysed by Western blot to investigate inflammatory signalling and apoptosis induction in osteoblasts following exposure to NAC or NAC-containing bone cement extracts. Exposure to NAC or NAC-containing extracts resulted in a dose-dependent decrease in cell viability and proliferation, with pronounced cytotoxic effects observed at higher concentrations, whereas lower concentrations were well tolerated. Western blot analysis confirmed the expression of NF- κ B and procaspase-3 in osteoblasts treated with NAC or NAC-containing extracts. Despite reduced cell viability at higher concentrations, no evidence of early caspase-3-dependent apoptosis was detected. HPLC analysis revealed a time-dependent release profile of NAC from PMMA bone cement.

In conclusion, these findings demonstrate that the biocompatibility of NAC is highly concentration dependent. While higher NAC concentrations impair osteoblast viability and proliferation, lower concentrations were well tolerated. These findings support the further development of NAC-loaded PMMA bone cement as a potential antibiotic-sparing strategy for the prevention of implant-associated infections.

P17 Stocker et al.

Preventive Effects of Sigma-1 Modulation by E1R and Fenfluramine in the Kainate Mouse Model of Temporal Lobe Epilepsy

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Purpose: Sigma-1 regulates homeostasis, neuronal excitability, and synaptic transmission through interaction with ion channels and neurotransmitter receptors. It is considered a promising target candidate for antiseizure and antiepileptogenic therapies. This study examined whether treatment with Fenfluramine, which has a potential mechanism of action involving Sigma-1, and E1R, a selective positive allosteric Sigma-1 modulator, impacts epileptogenesis after induced status epilepticus (SE) through effects on spontaneous recurrent seizures (SRS).

Method: Male C57BL/6JRj mice received intraperitoneal E1R (100 mg/kg, n=12), Fenfluramine (15 mg/kg, n=8), or Vehicle (saline 0.9%, n=21) injections for 14 days post-SE. During the chronic phase following drug withdrawal, two main electrographic patterns were examined: hippocampal paroxysmal discharges (HPDs) and high-voltage sharp waves (HVSWs).

Results and Conclusion: HPDs occurred in 63% of Fenfluramine-treated animals and 88% of vehicle-treated animals (5/8 vs. 7/8), with a lower median seizure frequency (0-4 vs. 0-7.5 seizures per hour) and a lower mean cumulative duration (9.61 vs. 42.67 s/recording day). Similarly, HVSWs occurred in 75% of Fenfluramine-treated animals compared with 88% of vehicle-treated animals (6/8 vs. 7/8), with a lower median frequency (25.75 vs. 32.75 HVSWs/hour) and a lower cumulative duration of HVSWs (11.45 vs. 14.05 min/recording day).

Overall, Fenfluramine treatment did not show a pronounced impact on SRS. Nevertheless, an effect cannot be excluded and could potentially be observed with a larger sample size.

HPDs were observed in 50% of E1R-treated versus 85% of vehicle-treated animals (6/12 vs. 11/13) with a lower median seizure frequency (0-3 vs. 0-7 seizures per hour) and mean cumulative duration (10.71 vs. 16.35 s/recording day) in the E1R group.

Similarly, HVSWs occurred in 58% of E1R-treated animals versus 78% of vehicle-treated animals (7/12 vs. 11/13), with a lower median frequency (9.75 vs. 29.0 seizures/hour) and mean cumulative duration (9.84 vs. 14.98 min per recording day). E1R treatment suggests potential evidence for disease modification, based on observed differences in HPD and HVSF frequency and duration.

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P18 Verdier et al.

Pharmacokinetic profile of free and total ropivacaine after intraperitoneal instillation in anaesthetised dogs

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Objective To evaluate the pharmacokinetics of two doses of intraperitoneal ropivacaine in anaesthetised dogs.

Study design Randomised clinical trial.

Animals A total of 20 dogs undergoing ovariectomy/ovariohysterectomy.

Methods Dogs were anaesthetised and randomised to be given 1 (group R1) or 3 (group R3) mg kg⁻¹ of ropivacaine intraperitoneally, diluted with 0.9% NaCl to a total volume of 0.8 mL kg⁻¹. Before abdominal wall closure, solution aliquots were instilled over the ovarian pedicles and, if applicable, the uterine stump. Venous blood was sampled 2 minutes before and 5, 10, 15, 30, 45, 60, 120 and 240 minutes after instillation. Cardiovascular signs of toxicity were noted. Plasma concentrations of free and total ropivacaine were measured using ultra high performance liquid chromatography–mass spectrometry. Plasma protein binding (PPB) was determined by rapid equilibrium dialysis. Pharmacokinetic parameters were derived by a noncompartmental analysis, and the maximum total and free ropivacaine plasma concentrations (C_{max}) were compared between groups using an independent samples t-test. Data are presented as mean or median (95% confidence intervals). Significance was set at $p < 0.05$.

Results One dog in R3 was excluded. Total ropivacaine C_{max} were 0.27 (0.21–0.33) and 0.67 (0.43–0.92) mg L⁻¹ at 15 (8.64–30.35) and 5 (1.77–8.23) minutes (T_{max}) for R1 and R3, respectively, and different between groups ($p = 0.005$). Free ropivacaine C_{max} were 0.02 (0.02–0.03) and 0.06 (0.03–0.09) mg L⁻¹ at 10 (4.67–15.32) and 5 (0–19.13) minutes (T_{max}) for R1 and R3, respectively, and C_{max} was different between groups ($p = 0.015$). Median ropivacaine PPB for all dogs was 92.5 (89.7–95.2) %. No signs of cardiovascular toxicity were noted.

Conclusion and clinical relevance Although R3 had a greater total and free ropivacaine C_{max} than R1, C_{max} remained below toxic levels in all dogs.

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P19 Weiß et al.

Increased Cell Proliferation Fails to Prevent Biphasic Neuronal Progenitor Cell Depletion After Encephalitic Seizures

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Background: Acquired epilepsy following central nervous system (CNS) infections represents a major clinical challenge, yet the cellular mechanisms linking neuroinflammation, seizures, and long-term hippocampal remodeling remain poorly understood. The adult dentate gyrus retains a population of neural stem cells capable of generating new neurons throughout life, and disruption of this process has been implicated in epilepsy-associated circuit alterations. However, it remains unclear whether infection-induced seizures primarily affect stem cell maintenance, lineage commitment, or later stages of neuronal maturation. Using a viral encephalitis model, we previously observed an early impairment of hippocampal neurogenesis after Theiler's Murine Encephalomyelitis Virus (TMEV) infection. Here, we longitudinally dissected cellular changes across the neurogenic lineage and investigated whether seizure suppression or reduction of inflammation could prevent these alterations.

Methods: Male C57BL/6J mice (7–8 weeks) were intracortically infected with the Daniel's strain of TMEV. Acute seizures occurred in 35–55% of infected animals. Mice were sacrificed at 7 (n = 28), 28 (n = 14), and 90 (n = 58) days post infection (dpi). Adult hippocampal neurogenesis was assessed by immunohistochemistry using Nestin, BrdU, Doublecortin (DCX), and NeuN. To examine the specific contribution of seizures or inflammation to changes in neurogenesis, an additional group of mice (n = 35) received levetiracetam (1000 mg/kg) or ibuprofen (50 mg/kg) twice daily from 3–7 dpi.

Results: Seizing mice exhibited a striking biphasic alteration of the neuronal progenitor pool, with marked depletion of DCX-positive neuronal progenitors at 7 dpi, transient recovery to control levels at 28 dpi, and repeated depletion during the chronic phase (90 dpi). In contrast, the number of Nestin+ neural stem cells remained unchanged after acute seizures. Despite preserved stem cell numbers and increased overall cell proliferation (BrdU+), the fraction of Nestin+ and DCX+ cells undergoing proliferation was almost completely abolished during the acute phase. BrdU-labeled mature neurons (BrdU+/NeuN+) generated during the acute phase were not significantly altered, whereas glial proliferation increased in seizing mice. Neither levetiracetam-mediated seizure suppression nor treatment with the anti-inflammatory drug ibuprofen was sufficient to restore neuronal progenitor dynamics.

Conclusions: Encephalitis-induced seizures induce a long-lasting, biphasic disruption of neuronal progenitor dynamics that occurs despite preserved neural stem cell numbers and elevated overall cell proliferation. The near-complete arrest of the fraction of proliferating stem and progenitor cells within the neuronal lineage suggests a lineage-specific impairment rather than stem cell depletion. Pharmacological targeting of seizures or inflammation during the acute phase alone in this model is insufficient to restore adult hippocampal neurogenesis.

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P20 Wilzopolski et al.

Investigating TRP Channels in Zebrafish (*Danio Rerio*) Nociception

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The involvement of transient receptor potential (TRP) channels, particularly TRPA1 and TRPV1, in mammalian nociception is well established. Notably, the expression and nociceptive functions of these channels are conserved across a wide range of animal species. Zebrafish (*Danio rerio*) express orthologs of the mammalian receptors (*trpa1a*, *trpa1b*, and *trpv1*), predominantly in sensory neurons. Furthermore, commonly used TRP agonists elicit nocifensive behaviors in both larval and adult zebrafish. However, the precise mechanisms by which these channels contribute to nociception in zebrafish remain incompletely understood.

The objective of this project is to investigate the role of TRP channels in zebrafish nociception.

To evaluate algogen-induced nociception, we established a behavioral swimming assay and performed confocal as well as fluorescence imaging of brain activity in 5 days post-fertilization (dpf) zebrafish larvae. TRP channel mRNA expression was assessed during early larval development using RT-qPCR.

Our initial findings confirmed the expression of *trpv1*, *trpa1a*, and *trpa1b* in 5 dpf larvae, supporting the suitability of zebrafish at this life stage as a model for investigating TRP channel function and nociceptive processes. Behavioral analyses demonstrated that cinnamaldehyde, a TRPA1 agonist, and acetic acid, a TRPV1 agonist, induced nocifensive swimming responses in 5 dpf larvae. These responses were attenuated by TRP channel inhibitors. Moreover, preliminary confocal imaging data revealed that stimulation with a TRPA1 agonist activated a distinct cluster of cells within the cerebellum. Further studies are required to determine whether these cells are directly involved in TRPA1-mediated nociceptive processing.

Overall, the developed zebrafish models represent promising tools for investigating TRP channel signaling pathways involved in nociception. Future work will focus on refining the molecular characterization of TRP-mediated signaling mechanisms, including the establishment of primary zebrafish neuronal cultures to enable targeted manipulation and detailed analysis of individual neurons.

P21 Zalpanow et al.**Characterization of neuroprotective effects mediated by prolyl endopeptidase-inhibitory compounds for the treatment of Parkinson's disease**

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Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by a progressive loss of dopaminergic neurons in the substantia nigra, which causes the signature motor deficits of this movement disorder. The deposition of abnormally folded alpha-synuclein (aSyn) protein in Lewy bodies is thought to contribute to the pathogenesis of PD. Furthermore, mitochondrial dysfunction leading to oxidative stress is implicated in neuronal cell death, and dopaminergic neurons are specifically vulnerable to oxidative stress due to the toxic dopamine metabolism. In addition to motor symptoms, non-motor symptoms such as depression, sleep disturbances and cognitive impairment develop early in disease progression. There is currently no treatment to alleviate or halt neurodegeneration in PD, but reduction of aSyn pathology and oxidative stress are promising therapeutic targets.

Here, we investigated whether a prolyl endopeptidase (PREP) inhibitor, previously shown to reduce aSyn pathology and to improve mitochondrial function, is neuroprotective in *in vivo* and *in vitro* models of aSyn pathology. Mice overexpressing human wild-type aSyn (Thy1-aSyn, line 61) were used to establish primary neuronal cultures on multi microelectrode arrays (MEA) to measure neuronal firing and viability. Oxidative stress, induced by a stable peroxide, reduced neuronal firing, which was alleviated by adding the PREP inhibitor, suggesting neuroprotective effects. These findings were further supported by an *in vivo* study using Thy1-aSyn transgenic mice. Treated for one month via intraperitoneal implanted osmotic minipump loaded with the PREP inhibitor, transgenic mice exhibited less progression of motor deficits, enhanced short-term memory, and reduced hyperactivity. Importantly, the compound reduced aSyn pathology and microglia reactivity in the substantia nigra.

These results indicate that PREP-inhibitors hold promise as neuroprotective agents in PD, warranting further investigation to validate and expand upon these initial findings.

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Two *ex vivo* udder models, one pathogen: Comparing early host responses to *Streptococcus uberis* Infection

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Background: Mastitis in dairy cattle is a major global challenge, causing significant economic losses and animal welfare concerns; inappropriate use of antibiotics can contribute to antibiotic resistance. To study early host–pathogen interactions and provide controlled platforms to test potential pharmacological interventions, two *ex vivo* udder models were established. Udder tissue from healthy dairy cows was collected post-mortem. In the precision-cut bovine udder slice (PCBUS) model, tissue cores were sectioned into 350–450 µm slices, cultured for a 48-hour resting period, then infected with *Streptococcus uberis* (10⁸ CFU/ml) for 6 hours. In the isolated perfused bovine udder (IPBU) model, udders were perfused via the external pudendal arteries under near-physiological conditions and udder quarters were infected with *Streptococcus uberis* (10⁸ CFU/ml) for 6 hours. Gene expression of cytokines (*TNFα*, *IL-1β*, *IL-6*, *IL-10*), chemokines (*CCL20*, *CXCL8*), and antimicrobial effectors (*LAP*, *S100A9*) was assessed by RT-qPCR, and protein levels of *IL-6* and *IL-1β* were measured by ELISA. Tissue viability was monitored by MTS assay (PCBUS) or metabolic activity during perfusion (IPBU).

Results: In both models, an upregulation of inflammatory markers was detected at 6 hours post-infection, reflecting an early host inflammatory response to *Streptococcus uberis*. RT-qPCR demonstrated inflammatory gene induction at 6 hours post-infection in both models, with different expression patterns between systems. In PCBUS, *CCL20*, *IL-1β*, *IL-10* and *TNFα* were highly significantly upregulated ($P \leq 0.01$) and *LAP* was significantly increased ($P \leq 0.05$). In the IPBU model, *CXCL8* was strongly significantly upregulated ($P \leq 0.01$); several genes upregulated in PCBUS showed similar non-significant trends in IPBU. ELISA confirmed elevated *IL-1β* protein in both models ($P \leq 0.05$), whereas *IL-6* protein did not differ between infected and control groups in either model. Overall, these data suggest that the PCBUS model shows a stronger early transcriptional response after 6 hours, while the IPBU response is more moderate

Conclusion: Both *ex vivo* systems capture early host responses and provide complementary tools for mechanistic mastitis research: the perfused udder preserves organ-level physiology, while the PCBUS system allows standardized, higher-throughput experimentation and a platform for preclinical testing of non-antibiotic therapeutic strategies, supporting the reduction of *in vivo* animal experiments.



By public transport

Address: Veterinärplatz 1, 1210 Vienna

Stop: Josef-Baumann-Gasse or Veterinärmedizinische Universität (bus only)

⇒ from Wien Hauptbahnhof:

U1 heading to Leopoldau, get off at Kagraner Platz,

change to tram **26/27** heading to Strebersdorf and get off at Josef-Baumann-Gasse/
Veterinärmedizinische Universität.

Another alternative:

U1 heading to Leopoldau, get off at Kagran,

change to bus **27A** heading to Hermann-Gebauer-Straße and get off at
Veterinärmedizinische Universität

or change to tram **25** heading to Floridsdorf and get off at Josef-Baumann-Gasse.

⇒ from Vienna International Airport (Schwechat):

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Take a train of the ÖBB category RJX, RJ, IC or REX from Vienna Airport (Schwechat) to Vienna Main Station (Hauptbahnhof).

Tickets can be bought at the airport or at the train station.

For the onward journey from Vienna Central Station to Josef-Baumann-Gasse, please see the directions above.

Option 2:

A bus (Vienna Airport Lines, Line VAB3) goes from Vienna International Airport straight to Wien Donauzentrum (via Vienna International Center VIC)/U1 station Kagran without the need to change (duration of the journey approximately 40 minutes).

The bus stations are directly in front of the arrival hall at the airport, tickets can be bought online or at the airport.

A one-way ticket is 11.00€

<https://www.viennaairportbus.com/en/dam/jcr:51909e23-1a93-4b92-94ee-96cca42695b6/fahrplan-vab3-bis0409.pdf>

This line only operates every hour.

⇒ from Westbahnhof train station:

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The **replacement bus line 26E** will connect the Floridsdorf station (S+U) with Josef-Baumann-Gasse starting September 14, 2026.

Extensive track renewal work will take place along Donaufelder Straße in autumn 2026. The work will be carried out between the Fultonstraße and Josef-Baumann-Gasse stops.

<https://www.wienerlinien.at/modernisierungen/baustelleninformation#september-bis-dezember-linien-25-26-27-donaufelder-strae>

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Please use replacement bus **26E**, the **U1** or **S-Bahn** services as alternatives and allow a little extra time for your journey.

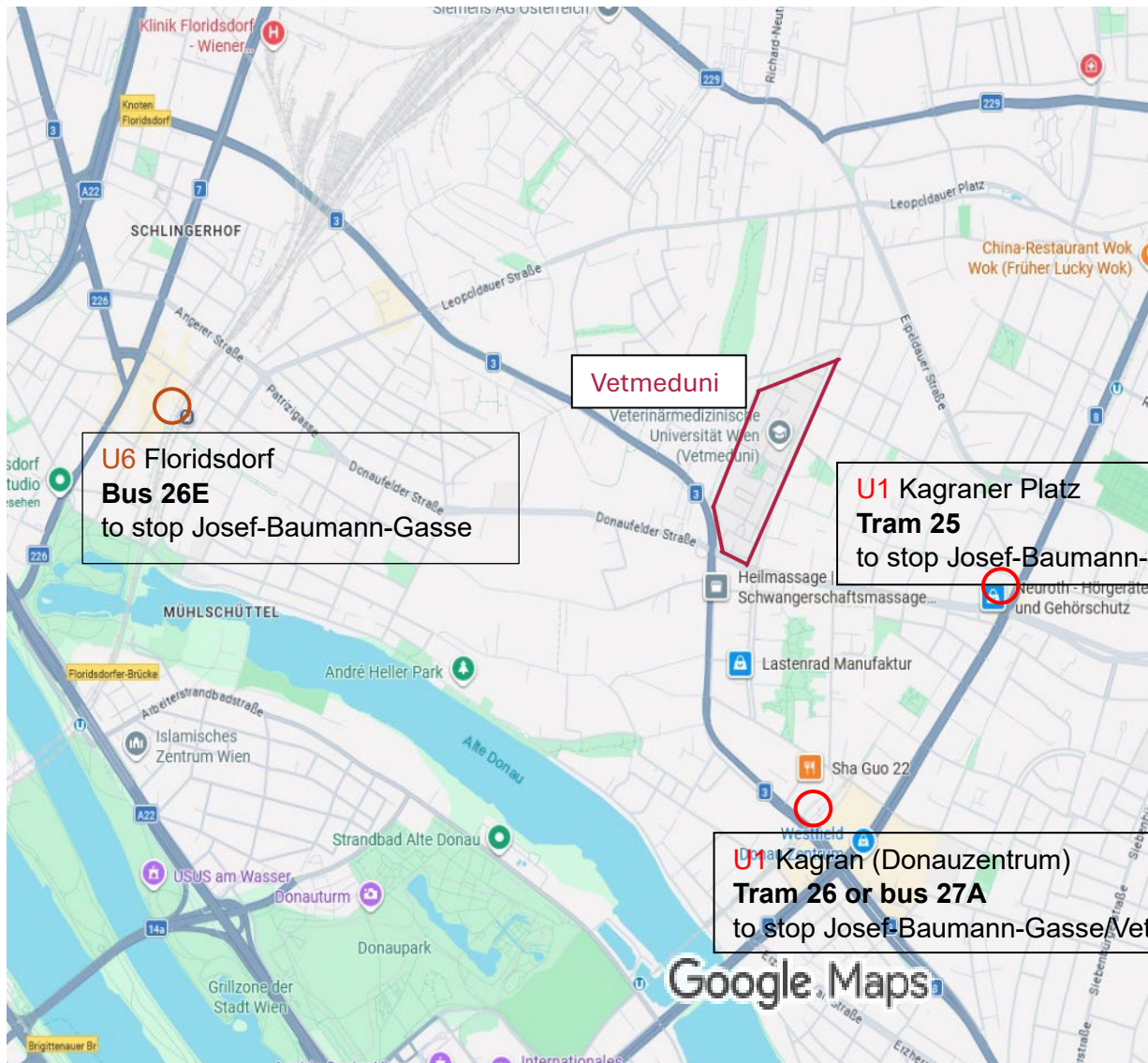
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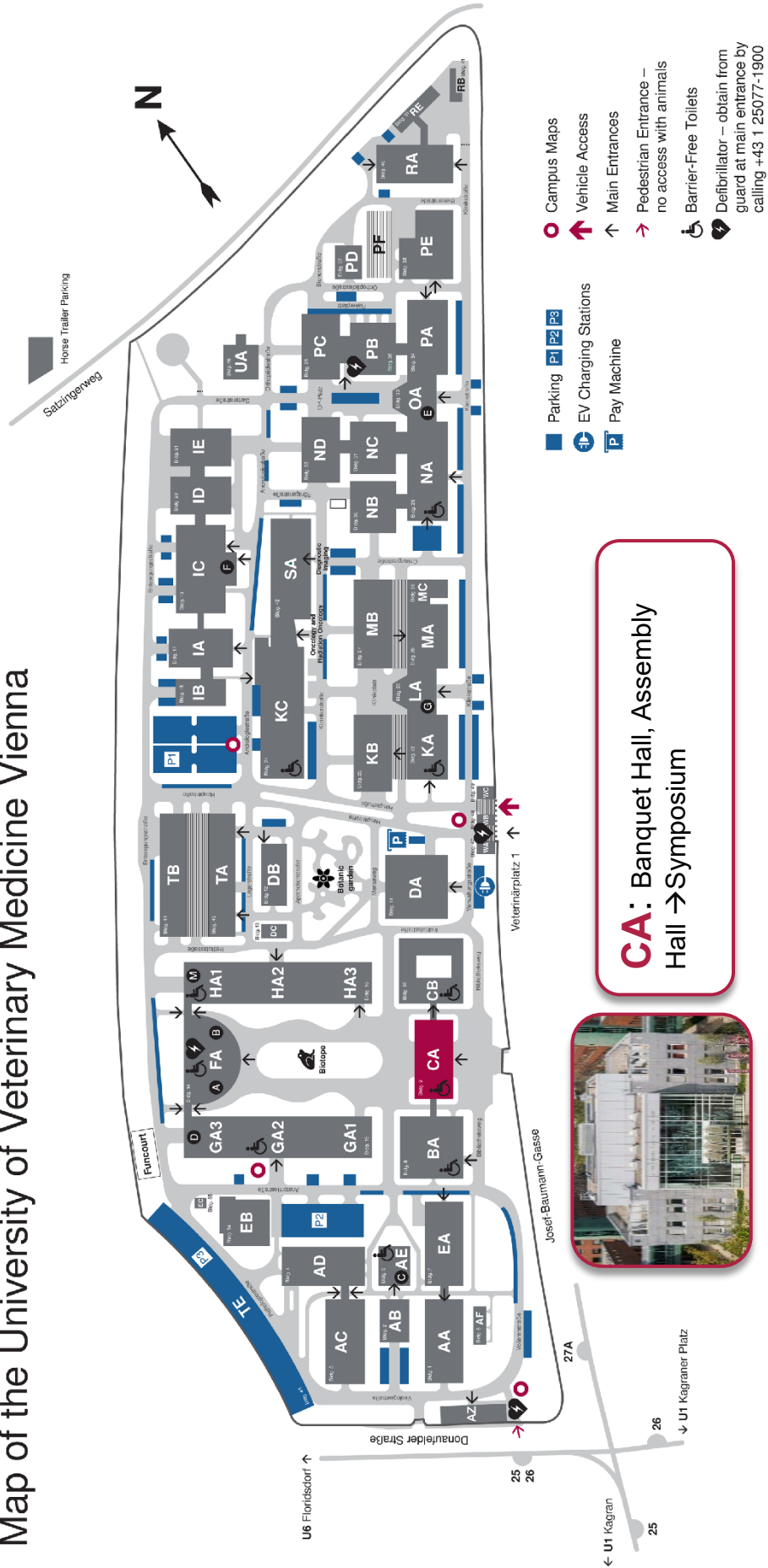
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