

STP 44th Annual Symposium

TOXICOLOGIC NEUROPATHOLOGY: BASICS AND BEYOND

Poster Setup and Presentation Times

Poster Presentation Times

(Please plan to attend your posters during the following times)

Sunday, June 22 (Welcome Reception).....	6:00 PM–6:30 PM
Monday, June 23.....	10:30 AM–11:00 AM and 3:00 PM–3:30 PM
Tuesday, June 24.....	10:00 AM–10:30 AM
Wednesday, June 25.....	10:00 AM–10:30 AM

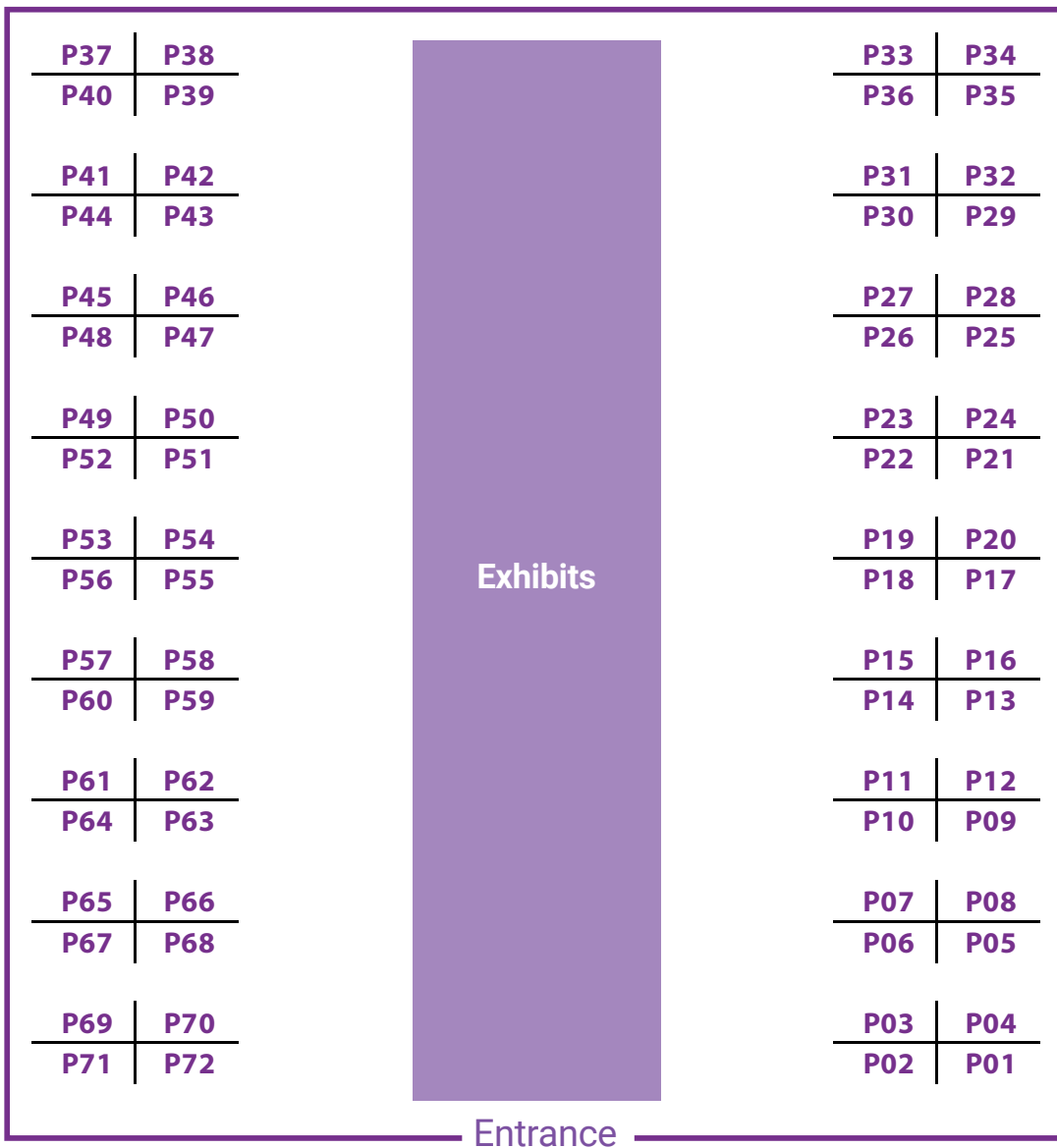
Poster Setup

Sunday, June 22..... 12:00 Noon–5:00 PM

Poster Teardown

Wednesday, June 25..... 1:00 PM–2:00 PM

If your poster is not removed before 2:00 PM, it will be removed and placed outside the Exhibit Hall for pickup.



STP 44th Annual Symposium

TOXICOLOGIC NEUROPATHOLOGY: BASICS AND BEYOND

Poster Presentation Index

Poster Categories

Young Investigator Award Candidate.....P01–P16
General Pathology/Toxicologic Pathology..... P17–P33, P71
New Technologies.....P34–P41

Oncology/Carcinogenesis P42–P47
Biomarkers P48–P52
Systemic/Organ-Specific Toxicologic Pathology P53–P70

- P01 Techniques to Study Chimerism at the Tissue Level in Humanized Mice**
Arin Cox, Esha Banerjee, Jillian Verrelle, Charles-Antoine Assenmacher, Enrico Radaelli
- P02 Characterization of a Novel Murine Model of Chronic Typhoid Fever**
Allysa L. Cole, John S. Gunn
- P03 Severe Cerebellar Dysplasia and Congenital Hydrocephalus Associated with Mice Lacking Hsd17b7 in Wnt1 Cell Lineages**
Bayla Bessemer, Rolf Stottmann
- P04 Revised Clinicopathologic Reference Intervals and Urinalysis Data for Virscio's African Green Monkeys (*Chlorocebus sabaeus*)**
Elizabeth Hines, Nadine R. Swierzawski, Kevinette Wattley, Amanda Murti, Rahul Dange, Matthew S. Lawrence
- P05 Neurologic Deficits in a Three-Year-Old Australian Shepherd with Disseminated *Cryptococcus* of the Central Nervous System**
Jingyi Li, Shakirat Adetunji
- P06 Atopic Dermatitis Modulates AHR Ligands in the Intestine: An Explanation for the Skin-Gut Axis in the Atopic March**
Katelin L. Davis, Estefania Claudio-Etienne, Derron A. Alves, Elisabeth Meyer, Benjamin Schwarz, Karen Laky, Pamela A. Frischmeyer-Guerrero
- P07 Telomere Attrition as a Key Link Between Prenatal Heavy Metal Exposure and Developmental Neurotoxicity: A Scientometric and Mechanistic Mapping Review**
Vanitha Thuraiarasu, Mohd Hasni Ja'afar
- P08 Development of an HTLV-1 mRNA Vaccine Using a New Zealand White Rabbit Model**
Emily King, Joshua Tu, Victoria Maksimova, Susan Smith, Ramon Macias, Xiaogang Cheng, Tanmayee Vegesna, Lianbo Yu, Lee Ratner, Patrick Green, Stefan Niewiesk, Justin Richner, Amanda Panfil
- P09 A Multiple Instance Learning Approach for Detecting Foci of Cellular Alteration in the Liver**
Erica Ramaker-Erlanson, Lise Bertrand, Jogile Kuklyte, Ajaz Ahmad, Hope Williams, Akshat Singh, Pierre Moulin
- P10 Poster Withdrawn**
- P11 Utility of Prechronic Histologic Lung Lesions as Early Markers of Chemical-Induced Carcinogenesis in Rodent Toxicology Studies**
Mallikarjun Bidarimath, Mark F. Cesta, Kathryn Konrad, Shawn Harris, Keith Shockley, Helen Cunny, Robert Sills, Sonika Patia

- P12 Defining Homeostatic TGF β Responses and Their Evolution in Mammary Tumorigenesis Using a Novel TGF β Pathway Reporter Mouse**
Zachary Millman, Yu-an Yang, Lalage Wakefield
- P13 Early-Life 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Exposure Induces Long-Term Neurotoxicity in Adult Zebrafish (*Danio rerio*)**
Ji-Hang Yin, Katharine Horzmann
- P14 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo)-Induced Developmental Toxicity and Neurotoxicity in the Zebrafish Model Following the Early-Life Exposure**
Ji-Hang Yin, Katharine Horzmann
- P15 Combined Liver-Specific Deletions of RNA-Binding Proteins, ZFP36L1 and ZFP36L2, Attenuate Carbon Tetrachloride-Induced Hepatotoxicity**
Rahul Kumar, Sonika Patial, Yogesh Saini
- P16 Immunohistochemical Evaluation of Ketamine-Induced Neurotoxicity in Neonatal Sprague-Dawley Rats as a Model for Pediatric Anesthesia**
Simone Canesi, Camilla Recordati
- P17 Spontaneous Accumulation of Basophilic Granular Material in Neurons of Vagal/Nodose Ganglion and Superior Cervical Sympathetic Ganglion of Cynomolgus Monkeys**
Ahamat Aboulmali, Armando R. Irizarry Rovira
- P18 Hemangioma in the Urinary Bladder of a Cynomolgus Monkey Used as a Control Animal in a Toxicity Study**
Ahamat Aboulmali, Armando R. Irizarry Rovira
- P19 Total Western Diet Exacerbates Colonic Proliferative Lesions in Pirc Rats**
Allison C. Boone, Ricardo Cortes, Xuying Zhang, Cameron Grant, Willie Cunningham, Krishna Latha Kanchi, Peter Pediaditakis, Thai-Vu Ton, Arun Pandiri
- P20 Spontaneous Findings in the Reproductive System of Male Mice**
Andressa Varella Gonsioroski, Justin D. Vidal
- P21 Poster Withdrawn**
- P22 Impact of *Plasmodium* spp. Infection on Spleen Weights and Absolute White Blood Cell, Lymphocyte, and Basophil Counts in Captive-Bred Laboratory Cynomolgus Macaques**
Cory Howard, Bonnie Harrington
- P23 The Trigeminal Pathway in Drug-Induced Peripheral Neuropathy**
Deepa B. Rao, Aditi Namputhiripad

STP 44th Annual Symposium

TOXICOLOGIC NEUROPATHOLOGY: BASICS AND BEYOND

- P24 Cerebrospinal Fluid Collection-Induced Nerve Fiber Changes in the Monkey**
Fei Zhou, Lin Zhu, Xi xing Zhao, Tian sheng Zhou, Xi hua Wang, Dong yang Nie
- P25 Foresight V2: Development and Performance of an AI Decision Support Tool for the Detection of Inflammatory Cell Infiltration in Rodent Brain for Nonclinical Toxicological Studies**
Hope Williams, Mark Smith, Jogile Kukyte, Lise Bertrand, Yvette Tien, Andrew Fink, Pierre Moulin
- P26 Effects of Excessive Fructose on Kidney in SDT Fatty Rats**
Kaoru Toyoda, Tadakazu Takahashi, Yuki Tanaka, Yusuke Suzuki, Toshiyuki Shoda, James K. Chambers, Kazuyuki Uchida
- P27 Outcome Assessment of Non-Matrix-Assisted Trimming Utilizing Anatomic Landmarks for Reliable Canine Brain Sampling in Nonclinical General Toxicity Studies**
Molly L. Liepnieks, Charlotte Hollinger
- P28 Procedure-Related Findings Associated with Intravenous Injection in Non-Human Primates Utilized in Preclinical Toxicity Studies**
Nicholas Vetter, Charlotte Hollinger, Keith Nelson
- P29 An Evaluation of scAAV-Mediated Combinatorial Gene Therapy in an Equine Model of Post-Traumatic Osteoarthritis**
Parvathy Thampi, Jaiden H. Oropallo, Katie A. Seabaugh, Brigita Fiske, Jennifer N. Phillips, Lester Suarez-Amaran, Joshua C. Grieger, C. Wayne McIlwraith, Constance R. Chu, R. Jude Samulski, Laurie R. Goodrich
- P30 Organ Weight Data Interpretation in Toxicology Studies**
Sherry Morgan, Sarai Milliron
- P31 Metastatic Malignant Granular Cell Tumor in the Distal Reproductive Tract of a Control Wistar-Han Rat**
Sasmita Mishra, Vimala Vemireddi, Steven Ploch, Carine Kolly, Tanasa S. Osborne, Pamela Blackshear
- P32 Anti-Drug Antibody (ADA)-Associated Hepatotoxicity in Rats Administered an Anti-SARS-CoV-2 Antibody**
Vikas Kulshreshtha, Rabih Slim, Joel D. Kantrowitz, Dharani Ajithdoss
- P33 Historical Control Incidence of Göttingen Minipig Microscopic Background Lesions in Drug Safety Assessment: 2014–2024**
Yung-Tien Tien, Loni Schumacher, Timothy Carlson, Keith Nelson
- P34 Safety Assessment of a 1940 nm Tm:YAP Laser for Fractional Skin Ablation in a Swine Model**
Yuval Ramot, Michal Steiner, Udi Vazana, Rotem Nahear, Neria Suliman, David J. Friedman, Salman Noach, Abraham Nyska
- P35 Development and Evaluation of a Deep Learning-Based Tool for Interpretable Abnormality Detection in Preclinical Toxicology Whole Slide Images**
Shima Nofallah, Syed A. Javed, Matthew Bronnimann, Robert Egger, Jacqueline Brosnan-Cashman, Bahar Rahsepar
- P36 Using Microinjection to Test Developmental Toxicity of Pharmaceuticals in Zebrafish**
Hannah J.T. Nonarath, Kayla Frost, Rebecca Kohnken, Rita Ciurlionis
- P37 Utilizing Spectral Entropy to Enhance Neurotoxicity Prediction in iPSC-Derived 3D Neural Cultures**
Mei-Lan C. Chu, Julien G. Roth, Serah Kang, Tomomi Kiyota, Kimberly A. Homan
- P38 Utilizing Cynomolgus Macaque Induced Pluripotent Stem Cells (iPSCs)-Derived Neurons as a Viable Tool for Regulatory and Investigative Neurotoxicology in NHP Models**
Morteza Roodgar, Rishitha Golla, Lucia Ramirez, Munmun Nandi
- P39 A Technique for Half Brain Fixation-Perfusion in Macaques**
Nathan Crilly, Kristina Peterson, Amanda Johnson, Elizabeth Aguirre, Lois Colgin, Anne Lewis
- P40 Poster Withdrawn**
- P41 Interpretable Predictions of Toxicological Liver Abnormalities Through Multiple Instance Learning Algorithms Applied to Digital Whole Slide Images**
Shima Nofallah, Syed A. Javed, Limin Yu, Bahar Rahsepar, Kyathanahalli Janardhan, Melissa Dsouza, Matthew Bronnimann, Thomas Forest
- P42 Atypical Grade II Canine Meningioma: A Comparative Approach to Understanding Human Brain Tumors**
Heather Yee, Julie Bedwani, Shakirat Adetunji
- P43 Glucose-Starvation-Adapted Cancer Cells Obtained Resistance to Alpelisib by an Increase of YAP Through Fatty Acid Oxidation**
Hyun-ki Hong, Yeseul Yang, Na-Yon Kim, Yongbaek Kim
- P44 Upper Urinary Tract Urothelial Cell Carcinoma in Two Macaques**
Jazz Q. Stephens, Mark J. Hoenerhoff, Sarah Corner, Sarah Coe
- P45 Tumor-Derived VEGFA Limits Prostate Cancer Growth and Osteolysis in a Mouse Model of Bone Metastasis**
Nathan K. Hoggard, Arthur W. Meehan, Makahla M. Schnarre, Noriko Kantake, Jonathan A. Young, Dario Palmieri, Michael F. Tweedle, Blake E. Hildreth III, Thomas J. Rosol
- P46 Ovarian Teratoma in a rasH2 (Wild Type) Mouse**
Santhipriyadarsini Sridharan, Molly H. Boyle
- P47 Two-Year Carcinogenicity Study of SJP-0132, a TRPV1 Antagonist, in Rats**
Yoshinori Yamagiwa, Kotaro Yamada, Yuichi Kuroiwa, Yuko Yamaguchi, Akiko Inoue, Gen Suzuki, Ikuyo Atsumi
- P48 Whole Brain In Vivo Assessment of Brain Development in a Rat Model of BPA Exposure**
Alex D. Edmondson, Bradley Wright, Joel Levoy, Elizabeth Fugate, Diana Lindquist

STP 44th Annual Symposium

TOXICOLOGIC NEUROPATHOLOGY: BASICS AND BEYOND

- P49 Elevated Serum Neurofilament Light Chain (NfL) Levels Associated with Drug-Induced Phospholipidosis in Dorsal Root Ganglion (DRG) Neurons**
Pankaj Kumar, Erin Vaughan, Stuart Foster, Samuel Ayres, Magali Guffroy
- P50 Neurofilament Light Chain as a Good Indicator for Monitoring the Neurodegenerative Changes in SOD1^{G93A} Rats**
Tomoya Sano, Tetsuaki Hiyoshi, Yasushi Masuda, Masato Nakashima
- P51 Serum N⁶, N⁶-Dimethyl-L-arginine (SDMA) Concentrations in Rhesus Macaque (*Macaca mulatta*) Models for Renal Transplantation**
JodiAnne T. Wood, Kendall E. Clark, Paul A. Vue, Donald Szlosek, Chelsea D. Landon, Rebecca L. Bacon, Francis J. Sun, Lee-Ronn Paluch, Alyson R. Guy, Colleen E. Thurman, Rebecca K. Tierce, Valerie M. Wong
- P52 Clinical Pathology Standard Parameters in Nude Rats**
Anes Bendrimia, Alexandre Bidaut, Raafat Fares, Myriam Defontis
- P53 Cytological Bone Marrow Cell Differential Counts and Morphological Findings in the Common Marmoset (*Callithrix jacchus*)**
Myriam Defontis, Alexandre Bidaut, Raafat Fares
- P54 Placental Pathology in Per- and Polyfluoroalkyl Substance (PFAS) Gestational Exposure**
Alix D. Rogers, Julie F. Foley, Catherine A. Picut
- P55 Thyrotoxicity in the Developing Male Reproductive Tract**
Alix D. Rogers, Kyle Takayama, Jessica Kees, Jaime Mesnard, Catherine A. Picut
- P56 Intraneuronal Mutant Protein Accumulation in an Alpha-1-Antitrypsin Deficiency Mouse Model**
Amy Flis, Kelly Keefe, Tatyana Taksir, Hong Ma, Caroline Morel, Christian Mueller, Can Kayatekin, Dinesh Bangari
- P57 Drug-Induced Bone Marrow Effects Resembling Hypereosinophilic Syndrome (HES) in Dogs**
Bhanu P. Singh, Kevin Holsapple, Jacob Jabbour, Eric Lansdon, Frances Clemo, Jim Hartke
- P58 Drug-Related Brain Vacuolation as an Unusual Presentation of Neurotoxicity in Sprague-Dawley Rats**
Megan Wilichinsky, Bhanu Singh, Alok K. Sharma, Mark Butt, Shelly Moores, Anne Carey, Sandra Chang, Luke Muhumuza, Kate Bowenkamp, Adrian S. Ray, Anne Chester, Leigh Ann Burns Naas
- P59 Epigenetic Alterations Associated with Trichloroethylene (TCE): Developmental and Transgenerational Effects in Zebrafish (*Danio rerio*)**
Carlos E. B. Lopes, Taylor A. Yenrick, Maryam Hariri, Katharine A. Horzmann
- P60 Variation in Canine Neurohypophyseal Histology Associated with Sectioning Plane**
Charlotte Hollinger, Rachel Stoops, Molly Liepnieks
- P61 Urinary Bladder Background Findings in Beagle Dogs: Detrusor Myopathy Remains an Infrequent but Important Finding**
Charlotte Hollinger, Maria Bates, Judit Markovits, Sarah Coe, Nick Vetter, Keith Nelson
- P62 Overview and Interspecies Comparison of the Neuroanatomy and Neurophysiology of the Caudate Nucleus**
Cindy E. Fishman, Stephen D. Cahalan
- P63 Toxicological Assessment of Combined Exposure to PFOA and Ethanol Using Human Renal Proximal Tubule Organoids**
Ji-Seok Han, Min Heui Yoo, Wan-Jung Im, Heejin Park, Jae-Woo Cho, Byoung-Seok Lee, Yong-Bum Kim
- P64 Organophosphate Toxicity in a Four-Year-Old Dog**
Julie Bedwani, Jonathan Samuelson
- P65 Poster Withdrawn**
- P66 Retrospective Review of Histopathology Findings in Cynomolgus Monkeys in Powder Inhalation Toxicology Studies**
Predrag Novakovic, Joseph Younan, William Lee, Kevin McNally, Lev Kolodzieyski, Stephen Groom
- P67 Histopathologic Characterization of Eyes from a Laser-Induced Primate Glaucoma Model**
Rahul B. Dange, Bibiana Iglesias, Elizabeth Hines, Richard Bouffard, Matthew S. Lawrence
- P68 Histological Evaluation of Neuronal Necrosis and Microvasculopathy in a Neonatal Rat Model of Hypoxic-Ischemic Encephalopathy**
Shanny H. Kuo, Kylie Corry, Olivia Brandon, Elizabeth Nance, Thomas R. Wood, Jessica M. Snyder
- P69 Immunohistochemical Characterization of Bilateral Basal Nuclei Lesions in Beagle Dogs Using Novel Neurotransmitter Marker Panels**
Stephen D. Cahalan, Farzana Noor, Leslie A. Obert, John M. Kreeger, Cindy E. Fishman
- P70 Bioavailable Iron Coal Dust-Induced Oxidative Damage and Histopathological Changes in Rat Lung**
Sanvidhan G. Suke, Prasad Shrekar, Shubhangi Pingle, Rajpal Singh Kashyap
- P71 INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions—An Update – 2025**
Emily Meseck, Victoria Laast, Stacey Fossey, John Vahle, Alys Bradley, Matt Jacobsen, Ute Bach, Rupert Kellner, Thomas Nolte, Susanne Rittinghausen, Shim-mo Hayashi, Junko Sato, Katsuhiko Yishizawa

Abstracts

P01 Techniques to Study Chimerism at the Tissue Level in Humanized Mice

Arin Cox, Esha Banerjee, Jillian Verrelle, Charles-Antoine Assenmacher, Enrico Radaelli

University of Pennsylvania, Philadelphia, PA, USA.

Abstract

OBJECTIVE: Understanding the origin and distribution of different cell populations in humanized mice is critical for interpreting pathologic changes in these models. The methodological work presented here illustrates the validation of various labeling strategies and protocols to differentiate between specific mouse and human components in FFPE samples from humanized mice.

METHODS: Broad-spectrum (HLA-A, Ku80, and human mitochondria/hMito) and narrow-spectrum (CD40, CD86, CD11b/c) IHC markers against human and mouse were applied to a tissue microarray of normal and neoplastic human tissues and to archival samples of lymphoreticular tissues from human, normal C57BL/6 mouse, and humanized NOD mouse to determine the spectrum of recognized cell types. Tissues were graded for staining intensity and assessed for marker quality and cross-reactivity with other species.

RESULTS: HLA-A was found to be the most useful marker out of the three broad-spectrum markers due to its membranous staining, but Ku80 is a useful alternative when nuclear staining is preferred or when HLA-A is not expressed on a cell of interest. HMito had limited utility in many tissues due to its granular cytoplasmic staining pattern, but its strong expression in most neoplasms and in skeletal muscle may position it as a good choice for certain patient-derived xenografts. While most CD markers were species-specific, human CD11b and CD11c were also cross-reactive with mouse and dog.

CONCLUSION: This work provides a valuable starting reference for pathologists and investigators working with humanized models and seeking to add additional markers or alternative IHC options to the complex landscape of chimeric tissues.

P02 Characterization of a Novel Murine Model of Chronic Typhoid Fever

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¹The Ohio State University, Columbus, OH, USA. ²Nationwide Children's Hospital, Columbus, OH, USA.

Abstract

Salmonella enterica serovar Typhi (*S. Typhi*) causes a systemic illness in humans commonly known as Typhoid fever. Approximately 2–5% of patients are unable to completely clear the bacteria primarily due to biofilm formation on cholesterol gallstones. Typhoid fever lacks a direct *in vivo* model, as *S. Typhi* is host restricted to humans. Recently, the collaborative cross project has produced immunocompetent mouse strains permissive to *S. Typhi* infection (CC003/Unc; CC053/Unc). We have investigated if these mice can serve as a novel model of chronic *S. Typhi* infection.

Mice were fed a lithogenic diet (1% cholesterol and 0.5% cholic acid) for 6 weeks to induce gallstone formation and subsequently intraperitoneally infected with 2×10^4 – 5×10^5 colony forming units (CFU) of *S. Typhi*. At 21 days post infection (dpi) the mice were euthanized, and the gallbladder, liver, and spleen were aseptically removed, homogenized, and plated for CFU enumeration. We were able to recover *Salmonella* within the gallbladder, liver, and spleen at 21 dpi, similar to the current *S. Typhimurium* model in 129X1/SvJ mice. Additionally, lithogenic diet contributed to higher bacterial burden in the hepatobiliary system. While there are no sex differences in tissue CFU, male mice demonstrate significant decreases in body weight and increased mortality.

We have determined that the CC003 and CC053 mice are permissive to chronic *S. Typhi* infection and that we can reliably detect bacteria within the gallbladder, liver, and spleen. These data support that these models can be used as more physiologically relevant murine models of Typhoid fever for future studies.

P03 Severe Cerebellar Dysplasia and Congenital Hydrocephalus Associated with Mice Lacking Hsd17b7 in Wnt1 Cell Lineages

Bayla Bessemer^{1,2}, Rolf Stottmann^{1,3}

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Abstract

Cholesterol malformation syndromes cause a range of phenotypes including CNS developmental abnormalities. Underlying mechanisms are poorly understood; treatment is limited. Studying enzymatic steps within this pathway will yield a more comprehensive understanding of causal mechanisms and could elucidate therapeutic targets. The gene Hsd17b7 encodes a post-squalene cholesterol biosynthesis enzyme. This study investigates the impact of Hsd17b7 deletion on the midbrain by genetically ablating Hsd17b7 using the Wnt1-Cre2 transgene. Sixteen litters from Wnt1Cre2/wt x Hsd17b7flox/wt (or flox/flox) were collected at embryonic day (E)18.5, postnatal day (P)3, and P17. PCR confirmed the genotypes. Brains were harvested from fourteen P3 and twelve P17 neonates for H&E histology. Eight E12.5 and eighteen E16.5 fetuses were then collected to determine the earliest timepoint of neural dysgenesis to elucidate developmental mechanisms leading to the neural phenotype. Wnt1cre2/wt; Hsd17b7flox/wt mice displayed domed heads and runting. Mutant brains featured severe hydrocephalus and small cerebella with vertically oriented vermes. Histology revealed severe dysplasia of the cerebellum and subcommissural organ as well as ventricular distension. Wnt1cre2/wt; Hsd17b7flox/wt mice could not be retrieved in Mendelian ratios after E18.5 suggesting increased perinatal lethality. The earliest timepoint of aberrant neural development is being determined, and immunohistochemical marker analysis will be utilized to explore underlying molecular mechanism(s). Thus, conditional inactivation of Hsd17b7 in the Wnt1 cell lineage results in congenital hydrocephalus associated with an abnormal subcommissural organ and severe cerebellar dysplasia. Our data reveals that Hsd17b7 and cholesterol biosynthesis are required for dorsal midline development and midbrain-hindbrain boundary patterning.

P04 Revised Clinicopathologic Reference Intervals and Urinalysis Data for Virscio's African Green Monkeys (*Chlorocebus sabaeus*)

Elizabeth Hines, Nadine R. Swierzawski, Kevinette Wattle, Amanda Murti, Rahul Dange, Matthew S. Lawrence

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Abstract

INTRODUCTION: ASVCP-compliant reference intervals (RIs) aid in characterizing animals' responses in GLP and non-GLP nonclinical studies. The African green monkey (AGM; *Chlorocebus sabaeus*) is a translational species that shares significant genetic, anatomic, physiologic, metabolic, and pharmacologic characteristics with humans and non-human primates (NHPs). Here, we expand the body of literature on clinical pathology parameters of AGMs, and add urinalysis data, taking in consideration the partitioning factors for clinical pathology RIs.

METHODS: The data to generate the RI are from pre-study or routine screening of Virscio's colony AGMs (a mix of wild-caught and captive bred). The data are from 2023–2024 for hematology, chemistry, and coagulation data; and from 2020–2024 for urinalysis. All animals were test article-naïve and clinically healthy. All samples were collected and processed according to facility SOPs. Reference intervals were generated in R, according to ASVCP recommendations.

RESULTS: The inclusion criteria were met by 491 animals for hematology, 415 animals for clinical chemistry, 179 animals for coagulation, and 56 animals for urinalysis. Males were slightly overrepresented. Most samples were from adult AGMs (5–12 years old), with fewer juveniles (<5 years) and a small number of geriatric animals (13+ years). Partitioning was not performed for UA data. The statistical and clinical reasoning behind partitioning for other clinical pathology parameters is discussed, and RIs are compared to published human and NHP RIs.

CONCLUSION/IMPACT STATEMENT: Updated RIs for hematology, clinical chemistry, coagulation, and urinalysis parameters for Virscio's AGMs will aid in interpreting pharmacology and nonclinical safety studies.

P05 Neurologic Deficits in a Three-Year-Old Australian Shepherd with Disseminated *Cryptococcosis* of the Central Nervous System

Jingyi Li, Shakirat Adetunji

Department of Veterinary Clinical Medicine, School of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA.

Abstract

Cryptococcus spp. infections are less common in dogs than in cats and often present with systemic dissemination, affecting the central nervous system (CNS), respiratory tract, skin, and eyes. However, simultaneous involvement of both the brain and the entire spinal cord has not been reported. A 3-year-old castrated male Australian Shepherd was submitted to the University of Illinois at Urbana-Champaign (UIUC) for necropsy following a history of progressive neurological deficits, including seizures, proprioceptive deficits in the hindlimbs, and altered mentation. Necropsy and histopathology examination were performed. Special stains, including Periodic Acid-Schiff (PAS) and Alcian blue (pH 2.5), were used to better visualize fungal organisms. Additionally, molecular analysis utilizing 18S rRNA gene sequencing was conducted to confirm the pathogen. Petechiae were widely distributed in the spinal cord. Histopathology demonstrated multifocal hemorrhage, Wallerian degeneration, and perivascular cuffing within the spinal cord parenchyma. Numerous yeast-like organisms were expanding the meninges of both the brain and spinal cord, accompanied by pyogranulomatous and lymphoplasmacytic inflammation. The lumbar-sacral spinal cord was most severely affected, with dense inflammation effacing the dura mater, compressing the spinal cord, and extending into the cauda equina. Special stains and 18S rRNA sequencing confirmed *Cryptococcus neoformans* Infection. The case reported extensive fungal infiltration in the brain and spinal cord of a dog, suggesting widespread dissemination via the bloodstream or cerebrospinal fluid. This case expands the understanding of cryptococcal infections in dogs, emphasizing the importance of considering widespread CNS involvement in cases of progressive neurological disease.

P06 Atopic Dermatitis Modulates AHR Ligands in the Intestine: An Explanation for the Skin-Gut Axis in the Atopic March

Katelin L. Davis^{1,2}, Estefania Claudio-Etienne¹, Derron A. Alves¹, Elisabeth Meyer¹, Benjamin Schwarz¹, Karen Laky¹, Pamela A. Frischmeyer-Guerrero¹

¹National Institutes of Health, Bethesda, MD, USA. ²Purdue University, West Lafayette, IN, USA.

Abstract

Early life atopic dermatitis (AD) predisposes children to food allergy (FA), an epidemiologic phenomenon known as the atopic march. Sensitization to food antigens through a disrupted skin barrier helps to explain this predisposition; however, we hypothesize that the atopic march is further driven by a skin-gut axis where inflammation in the skin poises the intestine for allergic disease. We used a mouse model of the atopic march where mice develop AD and FA following epicutaneous exposure to calcipotriol and ovalbumin. AD induced type 2, proinflammatory immune cell population remodeling in the small intestine (SI). Using LC-MS, we found decreased levels of aryl hydrocarbon receptor (AHR) ligands known to influence immune homeostasis in AD mice SI. Both host metabolism and the microbiome can influence AHR ligands. Shotgun metagenomic sequencing revealed minimal changes to the microbiome in AD versus control mice. No differences in AHR ligand levels were observed following transfer of stool from AD versus control mice into germfree mice, and intestinal remodeling was still observed in germfree mice with AD. We further found that AHR-dependent transcripts were downregulated in intestinal tissues of AD mice compared to controls, except for *cyp1a1*, a cytochrome P450 enzyme and negative regulator of AHR via ligand degradation. Collectively, our data point to increased host metabolism as the primary mechanism driving decreased AHR ligands in AD mice. These experiments highlight the interconnectedness of barrier tissues and suggest that inflammation in the skin can influence host metabolism in the intestine, which may contribute to the atopic march.

P07 Telomere Attrition as a Key Link Between Prenatal Heavy Metal Exposure and Developmental Neurotoxicity: A Scientometric and Mechanistic Mapping Review

Vanitha Thurairasu, Mohd Hasni Ja'afar

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Abstract

Prenatal exposure to heavy metals (HMs) such as lead, cadmium, and arsenic has been increasingly associated with disrupted telomere biology and adverse neurodevelopmental outcomes. Telomere attrition represents a critical biomarker of genomic instability and serves as a mechanistic link between environmental exposures and early-life brain vulnerability. This study aimed to elucidate the role of telomere attrition as a key pathway linking prenatal HM exposure to neurotoxicity in early brain development.

A structured literature search was conducted in Scopus and Web of Science for peer-reviewed articles published between 2000 and 2024, focusing on prenatal heavy metal exposure, telomere biology, and early neurodevelopment, using a keyword-based search strategy. A scientometric analysis was applied to map publication trends, citation metrics, and keyword co-occurrence. Thematic content analysis was used to identify recurring biological mechanisms linking telomere dysfunction to neurodevelopmental outcomes.

The initial search yielded 692 articles, of which 216 met the criteria for scientometric analysis. Publication activity increased notably after 2015. Keyword co-occurrence mapping highlighted "oxidative stress," "telomere shortening," and "neurodevelopment" as dominant terms. Thematic analysis of 45 full-text articles identified telomere attrition as a central mechanism through which prenatal HM exposure impairs neurogenesis and earlylife neurodevelopmental processes.

While telomere biology remains underrepresented in toxicologic pathology, its integration into developmental neurotoxicology is crucial. Its role as a mechanistic link between prenatal heavy metal exposure and early neurodevelopmental impairment supports its relevance in toxicologic pathology.

This review supports the integration of telomere biology for early detection and prevention of environmentally induced neurodevelopmental disorders.

P08 Development of an HTLV-1 mRNA Vaccine Using a New Zealand White Rabbit Model

Emily King¹, Joshua Tu¹, Victoria Maksimova¹, Susan Smith¹, Ramon Macias¹, Xiaogang Cheng², Tanmayee Vegesna³, Lianbo Yu¹, Lee Ratner², Patrick Green¹, Stefan Niewiesk¹, Justin Richner³, Amanda Panfil¹

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Abstract

INTRODUCTION: Human T-cell leukemia virus type 1 (HTLV-1) is the cause of several diseases including adult T-cell leukemia/lymphoma (ATL) and a neurodegenerative disease (HAM/TSP). There is no clinically approved vaccine for HTLV-1. **EXPERIMENTAL DESIGN:** We designed and synthesized a codon optimized HTLV-1 envelope (Env) mRNA encapsulated in a lipid nanoparticle (LNP) and evaluated its efficacy as a vaccine candidate in an established rabbit model of HTLV-1 infection and persistence. **METHODS:** NZW rabbits were subjected to a prime/boost protocol using Env mRNA-LNP or control GFP mRNA-LNP. Rabbits were challenged with HTLV-1 producer cells five weeks and fifteen weeks after vaccine boost. Blood was collected before each vaccine dose, before viral challenge, and at weekly time points. Rabbit PBMCs and plasma were isolated for detection of HTLV-1 antibodies (western blot), measurement of proviral load (qPCR), viral gene expression (qRT-PCR), functional T-cell responses (flow cytometry) and antibody neutralization (syncytia inhibition assay). **RESULTS:** Env mRNA-LNP immunized rabbits exhibited lower proviral load and viral gene expression with protection against viral challenge and sterilizing immunity. Elevated CD4+/IFN- γ + and CD8+/IFN- γ + T cells were detected following viral challenge. An anti-Env neutralizing antibody response after prime/boost vaccination was detected in all animals, which correlated with reduced proviral load. **CONCLUSION:** Our Env mRNA-LNP produced protective immune responses against viral challenge in a preclinical rabbit model. Env neutralizing antibodies were identified as an immune correlate of protection. **IMPACT:** These findings hold promise for future development of preventive strategies and/or therapeutic interventions against HTLV-1 infection and disease.

P09 A Multiple Instance Learning Approach for Detecting Foci of Cellular Alteration in the Liver

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Abstract

OBJECTIVE: Foci of hepatocellular alteration are common spontaneous changes in rat studies over one year of age. Diagnosing foci is a challenging task. This experiment aims to develop a qualified method assisting pathologists in evaluating this finding.

METHOD: 1,180 rat liver slides were used from multiple carcinogenicity studies with 52% of slides containing basophilic foci (positive class). Multiple Instance Learning (MIL) was used to train the model. MIL is a weakly supervised learning method where labels are assigned to bags of instances. The whole slide image is treated as a bag and divided into tiles (instances). The tiles were passed through a pretrained Resnet50 model to obtain feature vectors. The MIL model uses these features and labels to classify and identify key instances and generate a heatmap for the model's prediction.

RESULTS: Results were calculated on 129 unseen slides, of which 56% were positive having minimal, mild and moderate severities. F1 score for the positive class was 74.5% using standard ImageNet features, and 81.6% using foundational model feature embeddings. The attention mask also provided correct localization of the lesions.

CONCLUSION: Multiple Instance Learning (MIL) is an effective approach for identifying hepatocellular alterations without individual instance annotations. By leveraging bag-level labels, MIL reduces manual annotation labor. The method achieves promising accuracy in detecting lesions and provides visual evidence through attention mapping. Future work will explore MIL for non-clinical settings.

IMPACT STATEMENT: MIL holds potential for broader applications, including non-clinical domains where similar challenges in annotation and data scalability exist.

P10 Poster Withdrawn

P11 Utility of Prechronic Histologic Lung Lesions as Early Markers of Chemical-Induced Carcinogenesis in Rodent Toxicology Studies

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Abstract

INTRODUCTION: Two-year rodent bioassays are commonly used to assess lung carcinogenicity, but they are costly and time-consuming. A key gap is whether subchronic assays can efficiently predict lung carcinogenicity. Here, we tested whether histologic lung lesions in subchronic studies predict lung carcinogenicity in 2-year studies.

EXPERIMENTAL DESIGN: A retrospective analysis of 269 matched subchronic (90-day) and 269 chronic (2-year) Division of Translational Toxicology studies across 75 chemicals was conducted. We assessed the association between subchronic lung lesions including hyperplasia, inflammation, and histiocytic infiltration and subsequent lung cancer outcomes.

METHODS: Subchronic and chronic studies were analyzed using Poly-3-trend test to assess dose-related tumor trends. Fisher's exact test evaluated associations between significant subchronic lesions and chronic tumor outcomes.

RESULTS: The combination of hyperplasia, inflammation, and histiocytic infiltration increased lung cancer risk, although they rarely occurred together. Inflammation and hyperplasia co-occurred frequently and were associated with carcinogenesis. Interestingly, while inflammation occasionally occurred without hyperplasia, hyperplasia was nearly always accompanied by inflammation. Fisher's exact test identified an association between the three subchronic lesions, both individually and in combination (hyperplasia and inflammation), and lung carcinogenicity in mice. In rats, all lesions except histiocytic infiltration showed this association.

CONCLUSION: Hyperplasia and inflammation co-occurred frequently and were associated with subsequent lung carcinogenesis. Fisher's exact test further confirmed the associations between subchronic lesions and chronic tumor outcomes.

IMPACT STATEMENT: The co-occurrence of hyperplasia and inflammation shows an association with subsequent lung carcinogenicity. Future studies will investigate molecular biomarkers in subchronic studies to identify potential correlations with lung carcinogenicity.

P12 Defining Homeostatic TGF β Responses and Their Evolution in Mammary Tumorigenesis Using a Novel TGF β Pathway Reporter Mouse

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Abstract

Transforming growth factor-beta (TGF β) has a complex dual role in cancer, suppressing tumor growth early by limiting epithelial proliferation and promoting tumor progression later by driving invasion, metastasis, and tumor microenvironment (TME) remodeling. To explore these dynamics, our lab developed a novel transgenic TGF β pathway reporter mouse ("Lime" mouse) with a strong Smad3-driven GFP reporter that labels cells with active TGF β signaling, as canonical signaling occurs through Smads2/3/4. Validation efforts included siRNA knockdown of Smad proteins in Lime mouse embryonic fibroblasts, which confirmed reporter dependence on Smad3. Unexpectedly, GFP expression also required Smad1/5 but not Smad4, suggesting a role for "mixed" Smad signaling in homeostasis. Additionally, a whole-body GFP immunohistochemistry (IHC) survey suggested a role for TGF β in maintaining secretory epithelial differentiation in adult tissues. In Lime MMTV-PyMT mammary tumors, GFP IHC revealed striking spatial heterogeneity in TGF β -responding cells: well-differentiated tumor cells exhibited strong GFP expression, while poorly differentiated regions showed weak GFP expression, supporting a role for TGF β in epithelial differentiation. Furthermore, bulk RNA-seq of TGF β -treated MMTV-PyMT tumor cell cultures demonstrated enrichment of epithelial sheet morphogenesis and cellular differentiation pathways. Future studies will examine the hypothesis that TGF β -driven differentiation is a tumor-suppressive mechanism lost in advanced mammary tumorigenesis using bulk and scRNA-seq to track TGF β responses across tumor stages. The Lime mouse provides a unique tool to investigate TGF β signaling in tumors, metastases, and the TME, enabling recovery of TGF β -responsive cells for FACS, RNA-seq, spatial transcriptomics, and high-dimensional imaging, deepening our understanding of TGF β in breast cancer progression.

P13 Early-Life 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Exposure Induces Long-Term Neurotoxicity in Adult Zebrafish (*Danio rerio*)

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Abstract

INTRODUCTION: 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo), a metabolite of the industrial solvent trichloroethylene (TCE), shares structural similarity with the neurotoxicant 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and has been linked to Parkinson's disease. Previously, we demonstrated TaClo induces MPTP-like neurotoxicity in larval zebrafish, characterized by neurobehavioral impairments, dopaminergic neuronal damage, apoptosis, and altered inflammatory and antioxidant enzymatic activity. However, the long-term effects of early-life exposure to TaClo and MPTP on neurodegeneration remain largely unexplored. **EXPERIMENTAL DESIGN:** Zebrafish embryos were exposed to TaClo (0, 5, 50, 500 ppb), vehicle (0.001% DMSO), or 1.75 μ M MPTP from 0 to 5 days post-fertilization, then rinsed and maintained in chemical-free aquaria water until 6 months post-fertilization (mpf). Neurotoxicity endpoints at 6 mpf included behavioral assessments, expression of dopaminergic neurons, apoptosis, microglia, and astrocytes, oxidative stress evaluation, and gene expression analysis. **RESULTS:** Developmental exposure to MPTP led to reduced locomotor activity and increased female-biased anxiety-like behavior in 6 mpf zebrafish. While apoptosis, microglial, and astrocytic expression were not significantly altered, both TaClo (5 and 500 ppb) and MPTP exposure resulted in persistent dopaminergic neuronal loss in the posterior tuberculum. TaClo (500 ppb) increased mitochondrial reactive oxygen species levels, while MPTP elevated catalase activity. TaClo (5 and 500 ppb) exposure increased glutathione peroxidase activity. **CONCLUSION:** Our findings demonstrate that transient early-life exposure to TaClo and MPTP leads to long-lasting neurotoxicity in adult zebrafish. **IMPACT STATEMENT:** These results highlight the long-term neurotoxic risks of early-life TaClo and MPTP exposure and underscore the potential health risks of environmental TCE contamination.

P14 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo)-Induced Developmental Toxicity and Neurotoxicity in the Zebrafish Model Following the Early-Life Exposure

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Abstract

INTRODUCTION: 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) is an endogenous neurotoxicant formed in the brain after exposure to trichloroethylene (TCE) and tetrachloroethylene (PERC). TCE and PERC are industrial solvents and have the potential to induce developmental toxicity and neurotoxicity; however, it is unknown if their metabolite, TaClo, triggers similar effects in developmental and neural systems. This study tested the hypothesis that embryonic zebrafish exposure to environmentally relevant concentrations of TaClo induces developmental toxicity and neurotoxicity. **EXPERIMENTAL DESIGN:** Fertilized embryos were dosed with embryo water (control), DMSO (carrier control, TaClo at 5 ppb, 50 ppb, and 500 ppb, and MPTP (positive control) for 24 or 120 hours post fertilization (hpf). Endpoints for developmental toxicity evaluation include survival and hatching percentage, body measurement, and heart rate measurement. Photomotor and visual motor behavioral response tests, and assessment of relative dopaminergic neuronal numbers in the ventral diencephalon using whole mount in-situ hybridization and immunofluorescence assay were used to assess neurotoxicity. **RESULTS:** TaClo exposed zebrafish showed altered neurobehavior (5 ppb and 500 ppb) at 24 hpf, decreased distance moved and velocity (5 ppb) at 120 hpf, decreased pericardial area (50 ppb) at 120 hpf, and decreased relative dopaminergic neuronal cell numbers in the ventral diencephalon (5 ppb) compared to the carrier. **CONCLUSION:** Environmentally relevant concentrations of TaClo induced developmental toxicity and neurotoxicity in the zebrafish model following the early-life exposure. **IMPACT STATEMENT:** These results highlight the importance of studying TCE and PERC metabolites and demonstrate TaClo as a developmental and neurotoxicant in the zebrafish model.

P15 Combined Liver-Specific Deletions of RNA-Binding Proteins, ZFP36L1 and ZFP36L2, Attenuate Carbon Tetrachloride-Induced Hepatotoxicity

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Abstract

Zinc Finger Protein 36 Like 1 (ZFP36L1) and Zinc Finger Protein 36 Like 2 (ZFP36L2) are functionally redundant mRNA destabilizing proteins. They are enriched in the liver, however, their function in xenobiotic-induced hepatotoxicity remains poorly understood. In this study, we investigated the role of ZFP36L1 and ZFP36L2 in xenobiotic-induced hepatotoxicity. Liver-specific ZFP36L1 and ZFP36L2 double knockout (L1/L2^{dkO}; AlbCre⁺/Zfp36l1^{flox/flox}/Zfp36l2^{flox/flox}) and flox-only control (L1/L2^{FLX}; AlbCre⁺/Zfp36l1^{flox/flox}/Zfp36l2^{flox/flox}) adult mice were intraperitoneally challenged with carbon tetrachloride (CCl₄, 0.8 ml CCl₄ in corn oil/Kg body weight). Endpoints associated with CCl₄ metabolism, glutathione (GSH) synthesis, and liver injury were assessed in unchallenged and CCl₄-challenged mice at 48 h post-challenge. Liver GSH levels were significantly higher in unchallenged L1/L2^{dkO} compared to L1/L2^{FLX} mice. Interestingly, both mRNA and protein expressions of CYP2E1, an enzyme known to bioactivate CCl₄, were significantly downregulated in unchallenged L1/L2^{dkO} compared to the L1/L2^{FLX} group. Unexpectedly, the CCl₄ challenge resulted in significantly less hepatic necrosis, ~10-fold lower serum ALT and AST levels, and less perturbation in the hepatic transcriptome (359 differentially expressed genes (DEGs) versus 3,862 DEGs) in L1/L2^{dkO} mice compared to L1/L2^{FLX} mice. Notably, the mRNA levels of *Gdm* and *Gpx1*, enzymes associated with the GSH pathway, remained significantly higher in the CCl₄-challenged L1/L2^{dkO} versus the CCl₄-challenged L1/L2^{FLX} group. The combined deletion of ZFP36L1 and ZFP36L2 attenuated CCl₄-induced hepatotoxicity by preventing the bioactivation of CCl₄ and strengthening the antioxidant defense system. These findings suggest a novel pathogenic role of ZFP36L1 and ZFP36L2 in CCl₄-induced hepatotoxicity.

P16 Immunohistochemical Evaluation of Ketamine-Induced Neurotoxicity in Neonatal Sprague Dawley Rats as a Model for Pediatric Anesthesia

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Abstract

INTRODUCTION: Brain histopathology is required by regulatory agencies to assess the potential neurotoxic effects of anesthetic compounds in juvenile animal models of human pediatric exposure. This study aimed to immunohistochemically investigate the effects of Ketamine administered during brain development in a neonatal rat model of pediatric anesthesia. **EXPERIMENTAL DESIGN:** Male and female Sprague Dawley rats at postnatal day 7 [CrI:CD (SD)] were intraperitoneally injected with 20 mg/kg of Ketamine or Vehicle for 5 times every 90 minutes and sacrificed 2 and 16h after the last administration. **METHODS:** Brains were fixed in 4% paraformaldehyde, paraffin embedded, sectioned, and immunostained with Cleaved Caspase-3, γ H2AX, Iba-1, GFAP and Olig2. A total of 27 images were acquired from each brain section from the cortex (12), hippocampus (12), and corpus callosum (3), to quantify the number of apoptotic cells, DNA-damaged cells, oligodendrocytes, and the astrocytic and microglial areas using QuPath software. Statistical analyses of the data obtained were performed. **RESULTS:** No sex-related differences were observed. The number of Cleaved Caspase-3, γ H2AX, and Olig2 positive cells showed a significant increase at 2h post-treatment, while no effects on astrocytes and microglia were observed. **CONCLUSION:** Ketamine-induced apoptosis and DNA damage were associated with an increase of oligodendrocytes at 2h post-treatment, while at 16h, a return to basal levels was observed. The increase in Olig2 can be interpreted as a response to the toxic insult caused by Ketamine. **IMPACT STATEMENT:** Ketamine-induced neonatal neurotoxicity after repeated administrations was demonstrated in this rat model of pediatric anesthesia.

P17 Spontaneous Accumulation of Basophilic Granular Material in Neurons of Vagal/Nodose Ganglion and Superior Cervical Sympathetic Ganglion of Cynomolgus Monkeys

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Abstract

The supply of non-human primates (NHPs) has lately been put under pressure due to several factors including the development of new therapeutics modalities with the NHP as the non-rodent species or the only on-target nonclinical species. With these supply challenges, it is important to characterize background changes in NHPs to avoid their misinterpretation in toxicity studies.

STUDY MATERIAL/METHOD: Two non-GLP studies- Animals in both studies were from the same supplier, but they were from different origins (Cambodian vs Mauritius) and had different age/body weight ranges at study initiation.

STUDY RESULTS: Accumulation of basophilic granular material within the neurons in the nodose/vagal ganglion and superior cervical sympathetic ganglion that was interpreted as of uncertain relationship to test article due to the increased incidence of the finding in treated NHPs in the first study. In the follow up study, the same finding occurred with similar incidence/severity in treated and concurrent control NHPs. This allowed determination that the finding was a spontaneous change in these non-routinely examined ganglia in NHPs. A detailed microscopic description of the finding as well as results of additional investigations (special stains for mineral and transmission electron microscopy of affected ganglia) will be provided.

CONCLUSION/CLOSING STATEMENT: We report a background change in non-routinely examined ganglia of NHPs likely representing a various stage of mineral deposition. It is important that pathologists be aware of this change that may erroneously be interpreted as treatment-related when it occurs only or with higher incidence/severity in treated NHPs.

P18 Hemangioma in the Urinary Bladder of a Cynomolgus Monkey Used as a Control Animal in a Toxicity Study

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Abstract

Reports of spontaneous proliferative and neoplastic lesions in cynomolgus monkeys used in toxicity studies are scarce primarily due to the relatively young age of animals used in these toxicity studies.

STUDY MATERIAL/METHOD: A dark focus in the serosa of the urinary bladder of a control female of Mauritius origin and 24-36 months of age used in a 3-Month Repeat Dose Toxicity Study by Once-Weekly Subcutaneous or Intravenous Injection in Cynomolgus Monkeys

RESULTS: Microscopic examination of the urinary bladder from the affected animal revealed a lobulated proliferative lesion expanding the submucosa and extending into to the muscularis. The lesion is characterized by multiple and variably sized vascular spaces lined by flattened or occasionally plump cells and supported by a scant to moderate amount of fibrous connective tissue. The lesion was diagnosed as a hemangioma of the urinary bladder. A detailed microscopic description of the finding as well as additional investigations done (immunohistochemistry with marker for endothelial cells and for cell proliferation) to better characterize the proliferative change will be provided.

CONCLUSION/CLOSING STATEMENT: It is very important to report this proliferative spontaneous finding in the urinary bladder of a control cynomolgus monkey used in a toxicity study as it is of rare occurrence in NHPs, and it can serve as a reference if a such finding occurs in a test article-treated NHP in toxicity studies.

P19 Total Western Diet Exacerbates Colonic Proliferative Lesions in Pirc Rats

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Abstract

INTRODUCTION: Risks and mechanisms linking dietary choices to the development of colorectal cancers (CRC) remain poorly understood.

EXPERIMENTAL DESIGN: Evaluation of CRC risk associated with common dietary compositions (17%, or 35% fat, or high-fructose corn syrup bolus (HFCS)) in a susceptible rat model.

METHODS AND MATERIALS: Male and female *Apc* heterozygous Polyposis-in-Rat-Colon (Pirc, (F344/NTac-*Apc*^{am1137})) rats and their wildtype (WT) littermates on Fischer 344 background were placed on one of three different diets, AIN93G with 17% fat calories (as a control), AIN93G+HFCS bolus, and total western diet (TWD) with 35% fat calories. Weekly body weights were monitored, and monthly colonoscopies were conducted. After study termination, gross and microscopic evaluation of the tumors were evaluated.

RESULTS: With the exception of Pirc females on HFCS, all other treatment groups had significantly increased body weights (all $P < 0.05$) compared to the corresponding control groups. No colorectal lesions were observed in the WT rats. As expected, Pirc males harbored a greater tumor burden, and tumor size compared to Pirc females. TWD Pirc females had significantly higher incidences of atypical hyperplasia ($P= 0.030$) compared to the control Pirc female group. TWD Pirc males had a significantly higher proportion of carcinomas ($P=0.024$) compared to the control Pirc males.

CONCLUSIONS: TWD but not HFCS increased the proliferative colonic lesions in Pirc rats.

IMPACT STATEMENT: An increase in tumor grade in TWD is likely a significant public health concern especially in susceptible populations. Additional mechanistic studies will help to understand the translational relevance of these rodent tumors.

P20 Spontaneous Findings in the Reproductive System of Male Mice

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Abstract

Mice are widely utilized in basic research and nonclinical toxicity studies; however, the prevalence of spontaneous findings in the reproductive tract of male mice is not well documented. Recognition of such findings is crucial for the differentiation of lesions that are directly caused by xenobiotics. The aim of this review was to identify spontaneous findings in the reproductive tract of sexually mature male mice assessing whole slide images of testes, epididymides, prostate, and seminal vesicles. Male mice (n=151) from vehicle control groups from nonclinical toxicology studies were selected, and included CD1, NIH:III, CByB6F1-Tg(HRAS)2Jic, and CByB6F1-Tg(HRAS)2Jic (hemizygous) RasH2 strains. Animal age at necropsy ranged from 9 to 46 weeks old. The most prevalent microscopic findings in the testes were tubular degeneration/atrophy, followed by multinucleated giant cells, and atypical residual bodies. In the epididymides, the most prevalent finding was epithelial vacuolation, whereas in the prostate were mononuclear inflammatory infiltrates.

P21 Poster Withdrawn

P22 Impact of *Plasmodium* spp. Infection on Spleen Weights and Absolute White Blood Cell, Lymphocyte, and Basophil Counts in Captive-Bred Laboratory Cynomolgus Macaques

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Abstract

INTRODUCTION: Although infection with *Plasmodium* spp. is variable among laboratory primates, literature describing the impact on pre-clinical research data is limited. In this study, we examined the impact of *Plasmodium* infection on spleen and liver weights, as well as hemogram parameters, in cynomolgus macaques. **METHODS:** At terminal sacrifice, organ and body weights were collected from 8 macaques which were *Plasmodium* positive upon testing facility arrival (5 males/3 females) and 24 which were negative (11 males/13 females). Animals were age 2–4 years, purchased from a single vendor, and used in the same toxicology study, and positive animals received chloroquine before sacrifice. Venous blood samples in K₂EDTA were analyzed with an ADVIA 2120i. **RESULTS:** Mean spleen weights and spleen to bodyweight and brain weight ratios were significantly higher in *Plasmodium* positive compared to negative macaques (t-test, p=0.0010, 0.0001, 0.0006). There was no significant difference in spleen weights when comparing sex, treatment group, or collection timepoint (t-test, p=0.7182; ANOVA, p=0.6262, 0.3918), nor in liver weights between positive and negative animals (t-test, p=0.085). There was a weak negative correlation between spleen weight and age (R²=0.0558). In the pre-study hematology data, absolute white blood cell, lymphocyte and basophil counts were higher among *Plasmodium* positive animals that had not received chloroquine compared to negative animals (t-test, p=0.025, 0.0011, 0.0027). **CONCLUSION/IMPACT:** Because *Plasmodium* spp. infection can result in higher spleen weights and white blood cell counts in macaques, it is crucial to screen for infection and exercise caution when interpreting data from studies including positive animals.

P23 The Trigeminal Pathway in Drug-Induced Peripheral Neuropathy

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Abstract

BACKGROUND AND OBJECTIVES: With the exception of the three special senses, histopathological evaluation of potential sensory neuropathy remains generally neglected in drug development safety assessments. Given the recent challenges in translating histopathology findings in the sensory ganglia with adeno-associated virus (AAV)-based therapeutics, this review aims to provide an integrated perspective to address some of the translational gaps for sensory neuropathy (with a focus on the trigeminal sensory pathway) in drug safety risk assessments.

METHODS: Morphological assessment of somatosensory (touch, pain, temperature, and proprioception) neuropathy is evaluated through histopathological examination of specific compartments along the three trigeminal pathways: the trigeminal lemniscal pathway (coding mechanoreceptors), the trigeminal thalamic pathway (coding nociceptors and thermoceptors), and the trigeminal proprioceptive pathway (coding input from the muscles of mastication, the temporomandibular joint, and the teeth). Routine histopathology evaluations are portrayed with the specific components of the trigeminal sensory pathways to allow comparative assessments for meaningful interpretation of histopathology findings.

RESULTS: Histopathology findings in the trigeminal ganglion and its associated pathways along the neuroanatomical trajectory of DIPN, and sensory neuropathy in particular, are depicted. Specifically, the absence of clinical observations in a routine nonclinical study as a false negative interpretation of a lack of toxicological findings is emphasized.

CONCLUSIONS: Strategic tissue sampling in contextual evaluation of neural components along their respective topographical pathways and integrated toxicology evaluation is found to be key to meaningful safety assessments.

P24 Cerebrospinal Fluid Collection-Induced Nerve Fiber Changes in the Monkey

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Abstract

OBJECTIVES: To investigate the background changes in the nerve fibers induced by multiple cerebrospinal fluid (CSF) collections in monkeys.

MATERIAL & METHOD: Two groups of 8 male monkeys (4/group) were administrated with saline by intrathecal injection at the lumbar level (L3-L5) once every 2 weeks (for a total of two doses). In the dosing period, CSF was collected from lumbar vertebrae and foramen magnum, totaling six collections/each dose (pre-dose, post-dose 0.25, 24, 48, 72, and 168h) in monkeys from Group 1. Monkeys from Group 2 had no CSF collection during the dosing period. All animals in both groups underwent a final CSF collection before necropsy. Clinical observation and neurological examinations were performed. Gross observation and histopathologic evaluation were conducted on the brain, spinal cords, and sciatic nerves.

RESULTS: The clinical observation, neurological examination, and gross observation were all unremarkable. Histopathologically, multifocal, minimal axonal degeneration of nerve fibers was observed in the brain, spinal cords, sciatic nerves, and injection sites of animals from Group 1, characterized by dilated myelin sheaths containing pale, eosinophilic fibrillar debris, and few macrophages (digestion chambers/Wallerian degeneration). The nerve fibers in animals from Group 2 appear normal.

CONCLUSION: Repeat CSF collection may induce axonal degeneration in nerve fibers in multiple neural tissues.

IMPACT STATEMENT: The procedure-related nerve changes have been reported in the intrathecal delivery approach. Here we first report repeating CSF collection-induced nerve changes in monkeys.

P25 Foresight V2: Development and Performance of an AI Decision Support Tool for the Detection of Inflammatory Cell Infiltration in Rodent Brain for Nonclinical Toxicological Studies

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Abstract

INTRODUCTION/OBJECTIVES: Brain histopathological assessment is part of the standard evaluation performed in nonclinical safety studies. A commonly found microscopic finding in rat short-term studies is inflammatory cell infiltration. A convolutional neural network (CNN)-based deep learning model was developed to assist in efficient and consistent microscopic identification of inflammatory cell infiltration in the brain.

METHODS AND MATERIALS: The model was trained using 244 hematoxylin and eosin stained slides with pathologist-made pixel annotations. The slides originated from 20 studies, from 8 laboratories, were scanned using 3 different scanners and contained samples from 2 rodent strains.

EXPERIMENTAL DESIGN: The classifier was trained using a U-Net architecture with an EfficNetb0 backbone at 20x magnification (0.5 microns per pixel), with geometry and color augmentation.

Precision, recall and F1 score were calculated at 3 levels of validation (tile, slide level and study levels).

RESULTS: Infiltration and hemorrhage reached F1 scores on 0.96 and 0.99, respectively (tile level), our target for tile level F1 is 0.9. The model is currently under pathologist qualification, where 3 different pathologists will evaluate the performance across a diverse set of brain slides and determine if it is fit for use.

CONCLUSION: By providing a consistent, efficient, and accurate method for detecting inflammatory cell infiltration in the brain, this model promises to streamline the histopathological assessment process and reduce variability in microscopic evaluations across diverse study conditions. With ongoing pathologist qualification further evaluating its robustness, the classifier represents a critical advancement towards integrating deep learning technologies into routine safety evaluations, supporting faster and more reliable decision-making in non-clinical research.

P26 Effects of Excessive Fructose on Kidney in SDT Fatty Rats

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Abstract

INTRODUCTION/OBJECTIVES: Excessive fructose intake has been reported to increase the risks of obesity, diabetes and kidney diseases, including diabetic nephropathy. We investigated the effects of a high-fructose diet on nephropathy in Spontaneously Diabetic Torii (SDT) fatty rats, a model for obese type 2 diabetes.

METHODS AND MATERIALS: Male and female Sprague-Dawley (SD) and SDT fatty rats were divided into 2 groups, receiving either a high-fructose diet (60% fructose) or a standard diet. Each group consisted of 5 animals. The diets were fed *ad libitum* for 7 weeks. Body weights, food consumption, clinical chemistry, urinalysis, kidney weights and histopathology were evaluated.

RESULTS: In the SDT fatty rats, fructose intake increased the urinary excretion of protein, albumin, uric acid, calcium and inorganic phosphate, and decreased urinary pH. Histopathological examination revealed that fructose intake worsened nephropathy (mineralization, inflammatory cell infiltration, increased mesangial matrix in the glomeruli and inflammation in the renal pelvis) in female SDT fatty rats.

CONCLUSION: A 7-week high fructose diet induced changes in urinary parameters and accelerated kidney damage in SDT fatty rats. These results were considered to be mainly caused by fructose inducing an imbalance in the urinary electrolytes, leading to increased urinary stones.

IMPACT STATEMENT: The SDT fatty rat model is useful for studying type 2 diabetes and diabetic nephropathy, and this study suggests that excessive fructose intake is a risk factor for urinary stone formation and the progression of kidney injury in diabetes.

P27 Outcome Assessment of Non-Matrix-Assisted Trimming Utilizing Anatomic Landmarks for Reliable Canine Brain Sampling in Nonclinical General Toxicity Studies

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Abstract

INTRODUCTION: Microscopic evaluation of brain in nonclinical toxicity studies is supported by consistent review of recommended neuroanatomic structures including caudate/putamen, cerebellum, cerebral cortex, choroid plexus, hippocampus, hypothalamus, medulla oblongata, midbrain, pons, and thalamus. Following published matrix-assisted sampling guides, a procedure was developed for canine brain trimming using free-hand targeting of external anatomic landmarks (olfactory tracts, piriform lobes, optic chiasm, infundibulum, cerebral peduncles, pons, cerebellum, occipital lobe, cerebellar vermis, and parafolliculi) to capture recommended neuroanatomic structures.

EXPERIMENTAL DESIGN: The free-hand procedure was evaluated for capture of recommended neuroanatomic structures.

METHODS: Coronal sections from 40 Beagle dog brains were processed routinely according to the targeted free-hand canine brain trimming procedure, and evaluated independently by two pathologists for the presence of 11 core and 22 supplemental targeted neuroanatomic structures.

RESULTS: Utilizing major external landmarks, the majority of core structures (caudate, putamen, cerebellum, cerebral cortex, hippocampus, hypothalamus, medulla oblongata, midbrain, and thalamus) were captured in 100% of examined brains. The pons and choroid plexus were identified in 95% of examined brains. The 22 supplemental structures had a $\geq 85\%$ capture rate.

CONCLUSION: Consistent capture of recommended neuroanatomic structures was achieved utilizing free-hand targeting of major external landmarks. This trimming procedure required no special equipment and was easily implemented with standard training processes.

IMPACT STATEMENT: A canine brain trimming scheme utilizing free-hand targeting of external anatomic landmarks can be successfully deployed by trained laboratory staff and yield reliable capture of recommended neuroanatomic structures for evaluation in canine nonclinical general toxicity studies.

P28 Procedure-Related Findings Associated with Intravenous Injection in Non-Human Primates Utilized in Preclinical Toxicity Studies

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Abstract

Recognition of test-article effects at the injection site and differentiation from procedure-related findings are imperative for accurate identification and reporting of potential toxicity in preclinical intravenous (IV) injection studies. Description of background injection site findings in non-human primates (NHPs) is limited in recent literature. A retrospective review of historical control data was conducted to identify and describe procedural findings commonly observed with IV injections in NHPs. Microscopic data from IV injection sites were compiled from 40 control *Cynomolgus macaques* (*Macaca fascicularis*) in seven (4 IV bolus, 3 IV infusion) toxicology studies over two years (2022–2024). Study duration ranged from 3 to 183 days, with time since final dosing ranging from 1 to 7 days. Only the most recent dose site was surveyed. Terminology was aligned among studies. Common regional (cutaneous and/or subcutaneous) findings at intravenous injection sites included hemorrhage (42.5%), neutrophilic infiltrate (25.0%), and epidermal hyperplasia (22.5%); infrequent findings included mononuclear cell infiltrate (17.5%), mixed cell inflammation (17.5%), granulation tissue (12.5%), and mixed cell infiltrate (10.0%); and rare findings (2.5%) included crust, erosion/ulcer, edema, fibroplasia, and necrosis. There were no specific vascular findings. IV injection site findings in control NHPs reflect trauma and initial inflammatory/healing response including findings that may be confounded with test material-related effects. Toxicologic pathologists evaluating IV injection studies should be familiar with typical microscopic findings associated with IV dosing to ensure the accurate identification and differentiation of findings indicative of potential toxicity at the injection site.

P29 An Evaluation of scAAV-Mediated Combinatorial Gene Therapy in an Equine Model of Post-Traumatic Osteoarthritis

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Abstract

INTRODUCTION: Gene therapy approaches using self-complementary adeno-associated viruses (scAAVs) have been successfully tested in the equine post-traumatic osteoarthritis (PTOA) model. This study aimed to develop a scAAV-based combinatorial approach to diminish the progression of PTOA in a highly translational equine preclinical model. **EXPERIMENTAL DESIGN AND METHODS:** A 10-week dosing trial identified 5×10^{11} vg as a safe therapeutic dose of scAAVIL-1ra. This dose was further used in this 4-month preclinical trial evaluating the efficacy of intra-articular injections of scAAVIL-1ra or scAAVIGF-I alone or in combination. Thirty-one horses were divided into four groups and treated with scAAVIL-1ra, scAAVIGF-I, a combination of both, or saline (control). Horses were evaluated throughout and at the end of the study. **RESULTS:** Synovial IL-1ra levels were significantly higher in the scAAVIL-1ra and the combination groups compared to the control. The peak synovial fluid IL-1ra levels in the scAAVIL-1ra and the combination groups were observed at 14 days and 7 days post-treatment, respectively. Correlating with IL-1ra expression levels, the cartilage summation scores were lower (improvement) in the scAAVIL-1ra and the combination group compared to the scAAVIGF-I group. No differences in synovial membrane summation scores were observed across the different treatment groups. **CONCLUSION:** These preliminary results suggest that suppression of IL-1 β via IL-1ra alone or in combination has a positive treatment effect for PTOA. IGF-I expression alone did not improve cartilage healing. **IMPACT STATEMENT:** A combinatorial gene therapy approach aimed at reducing inflammation while enhancing the joint anabolic environment may offer a promising gene therapy strategy for PTOA.

P30 Organ Weight Data Interpretation in Toxicology Studies

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Abstract

Organ weight analysis is an important endpoint for identification of potential effects of test articles in toxicology studies. In repeat-dose studies, body weights are often affected, which may accompany organ weight changes, thus making organ weight interpretation between control and test article-administered animals more difficult. Therefore, it is important to understand the relationship between organ weight, body weight, and brain weight to properly interpret organ weight changes in relationship to test article administration. Based on published literature and the authors' experiences, organs in laboratory animal species often affected by a decrement in body weight include the heart, kidneys, spleen, pituitary gland, prostate gland, seminal vesicles, liver, and thymus. In contrast, the absolute weight of brain, adrenal glands, testes, and thyroid gland are generally not affected by a decrement in body weight. In addition to considering organs' relationships to body weight, other factors to consider when interpreting organ weight changes include, but are not limited to, stress-related effects, enzyme induction, estrous cycle stage (females), variability of sexual maturity, and collection and trimming difficulties. Examples of interpretation of organ weight data that are complicated by body weight decrement are presented.

P31 Metastatic Malignant Granular Cell Tumor in the Distal Reproductive Tract of a Control Wistar-Han Rat

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Abstract

INTRODUCTION: Granular cell tumors in rodents were described in various tissues and considered to be mostly benign including in the distal reproductive tract. Occasional reports of malignant granular cell tumors in the literature were classified based on the morphologic features. For the first time, we describe a case of malignant granular cell tumor with potential mesenchymal cell origin in a control Wistar-Han rat uterine cervix with extensive regional metastasis. **METHODS:** A female rat from a vehicle control group was sacrificed in a poor clinical condition during Week 97 of a 104-week carcinogenicity study. Several H&E-stained tissue samples and uterus and cervix from the euthanized animal were stained with Periodic acid-Schiff (PAS; with/without diastase) and immunohistochemically with antibodies against Vimentin, S-100, Cytokeratin (CK) and CD 68 were examined. **RESULTS:** Microscopic examination of H&E sections of cervix revealed an infiltrative non capsulated neoplasm with a homogeneous population of round polygonal cells with abundant pale eosinophilic to amphophilic finely granular, variably vacuolated cytoplasm and oval, small, dense nuclei with abundant chromatin. The neoplastic cells infiltrated serosal surfaces of multiple tissues in the abdominal cavity. The neoplastic cells in the cervix were stained positive for Vimentin and PAS, retained eosinophilic PAS positivity following diastase digestion, and were stained negative for S-100, CK or CD 68. **CONCLUSION:** A malignant granular cell tumor in the uterine cervix of an aged rat with extensive local metastasis that was diastase resistant PAS positive and stained immunohistochemically positive for vimentin, indicating a potential mesenchymal nature.

P32 Anti-Drug Antibody (ADA)-Associated Hepatotoxicity in Rats Administered an Anti-SARS-CoV-2 Antibody

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Abstract

INTRODUCTION: SARS-CoV-2 spike protein mediates cell entry by binding to the host cell receptor ACE2. A human monoclonal neutralizing antibody, REGN17092, targeting SARS-CoV-2 spike protein was developed and the non-clinical safety profile was evaluated in Sprague-Dawley rats. **EXPERIMENTAL DESIGN:** Rats were administered REGN17092 via intravenous (30, 100 or 300 mg/kg) or subcutaneous injections (100 and 300 mg/kg) once weekly for 4 weeks and euthanized on Day 29 (dosing) and Day 113 (recovery). **RESULTS:** No REGN17092-related changes in clinical signs and in-life endpoints were observed. There were no test article-related adverse findings except for one unscheduled animal death 3 days following the second SC dose of 300 mg/kg/dose; this animal showed bridging hepatocellular degeneration/necrosis and immune complex deposition on IHC assays (consistent with ADA formation) and mixed cell inflammation at SC injection site. The test article related non-adverse microscopic findings in other animals during dosing period (SC route) included hepatocellular vacuolation, extramedullary hematopoiesis in spleen, increased myeloid cellularity in bone marrow and mixed cell inflammation at injection sites, which were predominantly recoverable. **CONCLUSION:** The hepatotoxicity observed in the rat administered REGN17092 via SC route (300 mg/kg/dose) was test-article related and associated with ADA formation. The NOAEL was the highest dose tested via the IV route.

P33 Historical Control Incidence of Göttingen Minipig Microscopic Background Lesions in Drug Safety Assessment: 2014–2024

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Abstract

INTRODUCTION: The Göttingen minipig is widely used in toxicological safety evaluation studies. Generation of updated historical control data allows accurate interpretation and evaluation of potential test article-related effects.

EXPERIMENTAL DESIGN: Microscopic data was collected from 1199 control Göttingen minipigs from three Charles River Laboratories sites in North America, including 597 males and 602 females, from 144 studies performed between 2014 and 2024. All the animals were obtained from Marshall Farms, Inc. Microscopic data was collected in Provantis®. Terminologies were unified and harmonized by the authors, guided by previous publications, International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) publications, and the global open Registry Nomenclature Information System (goRENI).

RESULTS: Histopathological findings are presented across 13 organ systems. The three most common findings noted in male and female Göttingen minipigs are intrasinusoidal erythrocytes in lymph nodes (47.8% and 53.4%), inflammatory cell infiltration in kidneys (22.7% and 22.9%) and pigment/pigmented macrophages in lymph nodes (20.9% and 22.7%), respectively. Other findings with incidence over 2% are also presented.

CONCLUSION: A historical control publication for Göttingen minipigs has not been released in almost a decade. Given that Göttingen minipigs remain the most common swine breed in nonclinical toxicity studies, current historical control data is crucial for providing a reliable basis of comparison. Therefore, we report the most common microscopic background findings from a large cohort of animals, from the past decade. This data will provide useful guidance for research investigators in toxicological safety assessment.

P34 Safety Assessment of a 1940 nm Tm:YAP Laser for Fractional Skin Ablation in a Swine Model

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Abstract

INTRODUCTION: Laser skin resurfacing is widely used in dermatology for treating photodamage, scars, and dyschromia. Fractional ablative lasers create controlled injury, promoting collagen remodeling while minimizing complications. The Epicare 1940 nm Thulium-doped Yttrium Aluminum Perovskite (Tm:YAP) laser represents a novel approach for fractional skin ablation. This study evaluates its safety and preliminary efficacy in a swine model, with histopathological analysis of tissue responses.

METHODS: Two female domestic swine underwent fractional skin ablation at 52 marked abdominal sites using varying energy settings of the Epicare laser. Macroscopic and histopathological analyses assessed epidermal regeneration, dermal remodeling, inflammation, and collagen deposition at 1, 7, and 29 days post-treatment.

RESULTS: Immediate macroscopic observations revealed well-demarcated ablation zones with erythema and edema, resolving by day 14. Histopathology showed early necrosis with complete epidermal regeneration by day 7. By day 29, enhanced collagen deposition and minimal residual inflammation confirmed effective dermal remodeling. No infections, ulcerations, or scarring were observed. Differences between treatment settings demonstrated a correlation between energy levels and lesion width, supporting the laser's capacity for precise ablation depth control.

CONCLUSIONS: The Epicare 1940 nm Tm:YAP laser demonstrated a favorable safety profile and effective tissue ablation in this preclinical model. Findings suggest its potential for dermatologic applications, warranting further clinical studies to optimize treatment parameters and confirm efficacy in human skin resurfacing.

IMPACT STATEMENT: This study provides essential preclinical data supporting the safety and efficacy of the 1940 nm Tm:YAP laser for fractional skin resurfacing, with implications for advancing laser-based dermatologic treatments.

P35 Development and Evaluation of a Deep Learning-Based Tool for Interpretable Abnormality Detection in Preclinical Toxicology Whole Slide Images

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Abstract

Before human testing, drugs must undergo testing in mammalian models to identify potential toxic effects. However, manual evaluation of preclinical toxicology samples is often time-consuming and variable. AI-based models have the potential to improve the efficiency, speed, and accuracy of this process. We developed an AI model to identify histological abnormalities in whole slide images (WSIs) of rat specimens. The model was developed using a subset of histopathology slides from the TG-GATEs database, including liver and kidney samples obtained from the testing of 70 compounds, divided into training (N=3287) and testing (N=877) sets. The testing subset contained 16 compounds in liver samples, which were held out during model development. An additive multiple instance learning model using embeddings from PLUTO, a pathology foundation model, was trained using 4-fold cross-validation to predict the probability of a slide containing significant histological abnormalities. Folds were tested as an ensemble model against the test set, with an overall area under the receiver operating curve (AUROC) of 0.87, with an AUROC of 0.81 on the set containing the held-out compounds. Exploratory analysis using a previously trained liver histology model revealed positive associations between model-predicted histological remarkability and lobular inflammation ($p < 0.001$), microvesicular steatosis ($p < 0.001$), and steatosis ($p = 0.02$), consistent with acute injury to the liver. Thus, AI models can provide quantitative and interpretable insights and have the potential to increase the speed and accuracy of toxicological pathology evaluations.

P36 Using Microinjection to Test Developmental Toxicity of Pharmaceuticals in Zebrafish

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Abstract

INTRODUCTION/OBJECTIVES: Thalidomide is an established teratogen and causes severe developmental malformations in humans and other species. Replicating these effects in zebrafish is challenging because of its low solubility and stability in water. This study addresses these limitations by employing microinjection techniques to directly deliver thalidomide into zebrafish embryos. **EXPERIMENTAL DESIGN/METHODS AND MATERIALS:** Two methods were used to dose zebrafish embryos with thalidomide, bath application and microinjection. During bath application, adjustments were made to the chorion status and DMSO concentration to evaluate their impact on thalidomide absorption and the development of associated phenotypes (abnormal fin development). The second method involved microinjecting thalidomide into zebrafish embryos at various doses and developmental stages. **RESULTS:** Bioanalysis results showed that the chorion status did not affect the levels of thalidomide in larvae 72 hours after treatment. Increasing DMSO levels to 1.5% during bath application allowed higher thalidomide dosing (600 μ M) and improved aqueous solubility. Despite these modifications, the most significant developmental effect observed was a decrease in the length of the larvae. Abnormal fin development was present in less than 2% of animals following various bath application methods. However, by microinjecting thalidomide before 8 hours post-fertilization, we significantly increased the occurrence of altered fin development to over 30% of the animals. **CONCLUSION:** Microinjection effectively increases thalidomide exposure and the penetrance of altered fin development. **IMPACT STATEMENT:** Microinjection opens new avenues for applying zebrafish models to study the developmental effects of compounds with low aqueous solubility.

P37 Utilizing Spectral Entropy to Enhance Neurotoxicity Prediction in iPSC-Derived 3D Neural Cultures

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Abstract

INTRODUCTION: Accurately predicting neurotoxicity is critical during preclinical drug development, as drug-induced neurotoxicity contributes to safety-related failures in clinical trials. Ideally, reliable *in vitro* assays capable of screening large sets of molecules for neurotoxic potential could be used to cull neurotoxicants in early development. Human iPSC-derived 3D neural cultures, which display spontaneous calcium oscillations indicative of neural activity, are a promising model for predicting neurotoxicity. Conventionally, high-throughput characterization of activity in these models is predicated upon assessing calcium oscillation waveforms using parameters such as peak count and amplitude. These analyses fail to capture synchrony and irregularity in the patterns of neural activity, thereby limiting predictive accuracy. To address this issue, we introduce spectral entropy, a novel feature designed to enhance the identification of synchronization irregularities, thereby improving the prediction of neurotoxicity.

METHODS AND MATERIALS: Commercially available 3D human iPSC-derived neural co-cultures, composed of neurons and glial cells, were treated with various neuromodulatory compounds. Neural activity was measured by capturing calcium oscillation using a high-throughput fluorometric imaging plate reader. Conventional features and the spectral entropy were evaluated in oscillation traces from 3D neural spheroids and simulated seizurogenic waveforms.

RESULTS: Spectral entropy provides neurotoxicity predictability comparable to conventional features. Additionally, spectral entropy exhibited superior performance in detecting seizure activity from simulated data.

CONCLUSION: Spectral entropy provides comparable neurotoxicity predictability and improved detection of seizurogenic liability over traditional features.

IMPACT STATEMENT: Spectral entropy enhances the prediction of neurotoxicity and seizurogenic toxicity in high-throughput screening assays, aiding early detection of potential neurotoxic compounds.

P38 Utilizing Cynomolgus Macaque Induced Pluripotent Stem Cells (iPSCs)-Derived Neurons as a Viable Tool for Regulatory and Investigative Neurotoxicology in NHP Models

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Abstract

Nonhuman primates (NHP), specifically cynomolgus macaques (*Macaca fascicularis*) are used as an appropriate animal model for efficacy and toxicology of human drugs. There have been many cases of human drug candidates that failed to pass preclinical safety evaluations in NHP *in vivo* studies (e.g., neurotoxicity). Despite the large number of studies failed in NHPs, post *in vivo* evaluation of the drugs in *in vitro* models of NHPs has not been possible so far because there has not been a reliable NHP *in vitro* models (e.g., neurons).

In this study we present development of reproducible cynomolgus macaque induced pluripotent stem cells (iPSCs) as a viable tool for the development of NHP *in vitro* cell models for investigative pathobiology after NHP *in vivo* studies. Additionally, in collaboration with major pharmaceutical companies, we have validated differentiation of the cynomolgus macaque iPSCs into NGN2 neurons, motor neurons (MNs), endothelial cells, and hepatocytes. These cells exhibit markers and functional properties of primary cells that were measure by surface marker expressions, albumin production in iPSCs-derived hepatocytes, and neuronal activities measured through multielectrode arrays (MEA) in NGN2 and motor neurons. In this study we propose a new set of iPSC-derived *in vitro* tools and platform for investigative pathobiology in nonhuman primate models. This *in vitro* model can be used as a complementary tool to investigate mechanism of failure of drugs in NHPs *in vivo* by testing pathway analysis upon exposure of cells *in vitro* to drug candidates.

P39 A Technique for Half Brain Fixation-Perfusion in Macaques

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Abstract

INTRODUCTION: Neurological investigations of nonhuman primates often require collection of both fresh and perfusion-fixed tissues. We developed a technique to flush the entire brain with saline, followed by collection of the right hemisphere and fixation-perfusion of the right hemisphere. This allows collection of both fresh and fixed brain from the same animal.

METHODS: Immediately after euthanasia, the thoracic cavity is opened, the heart is removed, and the right carotid artery is cannulated. The axillary arteries are clamped off, and a peristaltic pump is used to perfuse the brain with saline. During perfusion, an incision is made in the brow to assess flow. If flow is adequate, a hacksaw is used to remove the calvarium and dura to expose the brain. Once the cerebral vasculature has cleared, an incision is made between the cerebral hemispheres to bisect the brain, and the left side is removed for assays that require fresh tissue. The perfusate is changed to fixative, and the right hemisphere is perfused with 10% neutral buffered formalin.

RESULTS: With trained personnel, the time from euthanasia to removal of fresh tissue is less than 10 minutes. Histological quality of brains perfused using our method is comparable to standard perfusion methods and superior to immersion fixation. The method is effective in fetal, juvenile, and adult macaques.

CONCLUSION: Our method provides a rapid, simple, and reliable method for collecting both fresh and fixed neurological tissue from the same animal without compromising quality of either collection.

P40 Poster Withdrawn

P41 Interpretable Predictions of Toxicological Liver Abnormalities Through Multiple Instance Learning Algorithms Applied to Digital Whole Slide Images

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Abstract

Accurate detection of abnormal histology in nonclinical toxicology studies of experimental therapeutics can be hindered by inter-pathologist variability and the potential subtlety of meaningful changes in tissue architecture. To explore the utility of incorporating artificial intelligence into toxicologic pathology evaluations, we developed an algorithm to predict the presence of abnormalities in hematoxylin and eosin (H&E)-stained specimens. H&E-stained rat liver whole-slide-images (WSIs n=406) were divided into training-validation (n=304) and held-out test sets (n=102). An additional 160 WSIs comprised an independent test-set. An artifact detection model identified regions of usable tissue for analysis. An additive multiple instance learning (aMIL) model was trained using 3-fold cross-validation to predict the probability of a slide containing histologically abnormal ("remarkable") regions; folds were tested as an ensemble model against the held-out and independent test sets. Slide-level remarkable-probability scores and heatmaps identifying areas with predicted remarkability were compared to ground-truth histology labels. These attention heatmaps allow users to view regions contributing to slide-level predictions. On the held-out and independent test-sets, model accuracy was 97% and 81%, sensitivity was 94% and 78%, and specificity was 99% and 85%, respectively, for binary classification of cases as remarkable/non-remarkable, and remarkable-probability scores were often correlated with drug dosage. Most false-positive predictions (77%) occurred in control animals administered vehicles known to alter liver morphology (e.g., corn oil). Thus, this model accurately classifies specimens as remarkable if histological abnormalities are present. Digital pathology approaches have the potential to improve accuracy and speed, while allowing quantitative sample evaluation in toxicology.

P42 Atypical Grade II Canine Meningioma: A Comparative Approach to Understanding Human Brain Tumors

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Abstract

An 11-year-old, castrated male, mixed breed dog presented with a history of stumbling and appearing off balance. On neurologic examination, the dog had a right-sided head turn, intermittent right head tilt, and a right sided vestibular ataxia with severe atrophy of the right temporalis and masseter muscles. A mass was identified by MRI imaging in the right ventral caudal fossa in the region of the trigeminal ganglion. On gross examination, the right tympanic bulla was filled with abundant yellow, transparent, mucoid fluid. An oval, tan to dark red, slightly irregular, firm mass overlaid the ventral and slight lateral aspect of the right pons and medulla of the brainstem. On histopathology, the brain mass was an atypical grade II meningioma based on the 2021 World Health Organization classification of meningioma. The most common primary intracranial tumor in both humans and dogs are meningiomas. Immunohistochemistry (vascular endothelial growth factor [VEGF] and E-cadherin) will be used in this case to identify characteristics that may provide insight into using dogs as models for meningiomas in humans. VEGF expression has been reported as possible prognostic markers in both canines and humans. E-cadherin expression in human meningiomas has been reported as a negative prognostic factor but has not been explored as a prognostic factor in canine meningiomas. Mouse models of meningiomas often involve xenotransplant models performed in immunocompromised mice and thereby cannot evaluate the natural response by the adaptive immune system. Dogs could be used as an alternative model for meningiomas, especially for preclinical pharmacologic testing.

P43 Glucose-Starvation-Adapted Cancer Cells Obtained Resistance to Alpelisib by an Increase of YAP Through Fatty Acid Oxidation

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Abstract

Through the adoption of alternative metabolic processes, cancer cells can thrive in nutrient-deprived tumor microenvironment. Where access to key nutrients such as glucose is limited, cancer cells rely on metabolic flexibility to maintain their survival and proliferative capacity. While many studies have focused on the immediate effects of glucose starvation on cancer cells, the long-term consequences of adaptation to such stress remain largely unexplored. This study aims to bridge this gap by investigating how cancer cells adapt to glucose starvation and how this adaptation affects lipid metabolism and drug resistance. To delve deeper into the relationship between lipid metabolism and chemoresistance following glucose starvation, we generated glucose-starvation-adapted cells from NSCLC cells with activated PIK3CA. Upon adaptation to glucose starvation, the cells exhibited an elevation of stemness markers and resistance to Alpelisib, a PI3K inhibitor commonly used in targeted cancer therapy. In the glucose-starvation-adapted cells, FAO was significantly upregulated, which was linked to the regulation of stemness and chemoresistance. This process was found to be mediated by the elevation of YAP expression. The findings of this study highlight the complex interplay between glucose and lipid metabolism in cancer cells, particularly under metabolic stress conditions.

P44 Upper Urinary Tract Urothelial Cell Carcinoma in Two Macaques

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Abstract

Urothelial cell carcinomas (UCCs) have been rarely reported in non-human primates in the urinary bladder or in the upper urinary tract. Here, we describe two cases of spontaneous urothelial cell carcinoma in macaques arising within the renal pelvis. One 22-year-old rhesus macaque and one 24-year-old cynomolgus macaque were humanely euthanized for hindlimb osteoarthritis and diarrhea, respectively. Macroscopically, the cynomolgus macaque's kidneys were described as spongy, though no mass effect was described in either animal. Sections of kidney were routinely processed and stained with H&E. In both cases, the renal parenchyma was focally expanded by small cystic spaces lined by neoplastic polygonal urothelial cells arranged in nests and papillary projections supported by fine fibrovascular stroma that extended from the renal pelvis to medulla. Neoplastic cells displayed distinct cell borders, and had moderate to abundant amounts of eosinophilic, variably vacuolated cytoplasm, with rare mitotic figures. The neoplastic cells were focally infiltrative into the underlying parenchyma. Immunohistochemistry for Uroplakin III showed positive membranous immunoreactivity within a proportion of the neoplastic cells, consistent with urothelial cell origin. No evidence of distant metastasis was observed. These two cases expand the current understanding of spontaneous neoplasia in rhesus and cynomolgus macaques as important comparative oncology models for age-related neoplasia.

P45 Tumor-Derived VEGFA Limits Prostate Cancer Growth and Osteolysis in a Mouse Model of Bone Metastasis

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Abstract

INTRODUCTION: Prostate cancer (PCa) in men is frequently complicated by incurable bone metastasis. Blood vessel growth (angiogenesis), governed by vascular endothelial growth factor A (VEGFA), supports both bone homeostasis and metastatic cancers. Little is known about VEGFA in bone-metastatic PCa and its effects on the bone microenvironment. **METHODS:** Human PCa gene expression databases were explored for genes involved in angiogenesis. The canine PCa cell line Ace-1 was retrovirally transduced with human prostate-specific membrane antigen (PSMA) and canine VEGFA for experimental studies. The proximal tibiae of male, 5-week-old, nude mice (n = 20) were inoculated with Ace-1-hPSMA or Ace-1-hPSMA-cVEGFA and monitored by bioluminescent imaging for two weeks. Following, mice were euthanized for qRT-PCR, microCT, histology, and immunohistochemistry. Effects of VEGFA on cell proliferation and function were investigated in macrophage, osteoblast, bone marrow mesenchymal stem cell (BMSC), and osteoclast cell lines and primary cultures from male, C57BL/6 mice (n = 6). Flow cytometry and gene expression were performed for VEGFA receptors, VEGFR1 (*Flt1*) and VEGFR2 (*KDR*), on primary and established cell cultures of bone-derived cells. **RESULTS:** VEGFA was consistently upregulated in human PCa metastatic samples compared to prostate gland and primary tumors. All mice formed tibial intramedullary tumors. Ace-1-hPSMA-cVEGFA tumors had reduced *in vivo* growth compared to control tumors (Ace-1-hPSMA). Genes for bone lysis were downregulated in VEGFA-expressing tumors. VEGFA receptors were only detected in BMSCs. MicroCT and histology results are pending. **CONCLUSION/IMPACT:** High VEGFA levels in the tumor-bone microenvironment paradoxically inhibited PCa growth, possibly by maintaining bone volume.

P46 Ovarian Teratoma in a rasH2 (Wild Type) Mouse

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Abstract

OBJECTIVE: Report a case of spontaneous benign (mature) ovarian teratoma in a rasH2 (Human HRAS transgene) wild type mouse.

EXPERIMENTAL DESIGN/METHODS/RESULTS: At the terminal necropsy of a 4-week toxicity study, a 12- to 14-week-old female mouse was noted with a single, clear fluid-filled cyst in the left ovary, measuring 10 mm³. Microscopically, this cyst contained well-differentiated multipotential embryonic tissue derived from all three primary embryonic germ layers: ectoderm (nervous tissue), mesoderm (skeletal muscle), and endoderm (gastrointestinal epithelia) and was diagnosed and interpreted as a benign spontaneous teratoma.

CONCLUSION/IMPACT STATEMENT: Teratomas are rare in domestic animals. As far as the authors know, an ovarian teratoma has not been previously reported in rasH2 (wild type) mice.

P47 Two-Year Carcinogenicity Study of SJP-0132, a TRPV1 Antagonist, in Rats

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Abstract

INTRODUCTION: SJP-0132 (Motugivatrep) is a transient receptor potential cation channel subfamily V member 1 (TRPV1) antagonist for treatment of dry eye disease. Some reports suggest that TRPV1 agonists/antagonists may induce tumors in rodents, and role of TRPV1 in carcinogenesis remains controversial. To assess the carcinogenicity, SJP-0132 was administered to rats and the potential was evaluated.

EXPERIMENTAL DESIGN: SJP-0132 was given by oral gavage to 60/group male and female Crl:CD(SD) rats at doses of 0 (control, vehicle: corn oil), 0.5, 5, and 50 mg/kg once daily for 104 weeks. Additionally, toxicokinetics satellite groups were set for evaluation of systemic SJP-0132 exposure. Scheduled necropsy amended to Week 99 in females since the cumulative mortality reached 75% in the control group. After necropsy, histopathological examination was performed.

RESULTS: Treatment with SJP-0132 did not increase mortality. Histopathological examination revealed no increases in tumors in any organs/tissues in the SJP-0132-treated groups. Increased severity/frequency of age-related quadriceps femoris muscle atrophy was shown in males in the 5 and 50 mg/kg/day groups. Systemic exposure to SJP-0132 increased in a dose-dependent manner, with systemic exposure in females being approximately 2-fold higher than those in males.

CONCLUSION: SJP-0132 did not show carcinogenic potential in rats under the condition of this study. Age-related skeletal muscle atrophy progressed in males only at doses much higher than those used clinically.

IMPACT STATEMENT: This investigation will be an evidence supporting the safety of SJP-0132 as a treatment for dry eye disease.

P48 Whole Brain *In Vivo* Assessment of Brain Development in a Rat Model of BPA Exposure

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Abstract

Years of epidemiological research have associated anxious behavior in children to exposure of the endocrine disrupting chemical (EDC) Bisphenol-A (BPA). However, other EDCs have also been implicated, thus confounding the role of EDCs on mental health. Because BPA has been associated with influencing oligodendrocyte differentiation and maturation, we have developed a translational neuroimaging rat model to investigate the role BPA plays on myelination and anxiety. 115 Sprague Dawley rats were used in this study (53% Female). Beginning at P1, rats were dosed with BPA (2.5, 25, 250 ug/kg-bw, oral gavage). 2D high-resolution T2-weighted images and Diffusion Tensor Imaging (DTI, 6 directions) were acquired on rats on a Bruker 7T MRI scanner prior to euthanasia at P60 and P90. Using software developed to register T2w and DTI images to an anatomical atlas (SIGMA), we extracted regions of interest (ROI) and created ROI x ROI correlation matrices representative of whole-brain myelination relationships in each Dose x Sex x Timepoint group. Spearman correlation was calculated to assess similarity between each matrix. We found greater similarity between dosed BPA groups but were all dissimilar to P30 control rats. However, control P30 rats appear to have brains more similar in brain development to BPA dosed rats at P90. This suggests a differential time effect on brain development possibly due to BPA exposure. However, it is premature to conclude much beyond this. This is a work-in-progress; however, this method of analysis will be an essential for translating our neuroimaging rat model results to human studies.

P49 Elevated Serum Neurofilament Light Chain (NfL) Levels Associated with Drug-Induced Phospholipidosis in Dorsal Root Ganglion (DRG) Neurons

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Abstract

INTRODUCTION: This investigation aimed to determine the diagnostic value of serum neurofilament light chain (NfL) levels, a recognized biomarker for neuroaxonal injury, in rats following a 4-week repeat-dose toxicity study that showed widespread phospholipidosis in several tissues including dorsal root ganglion (DRG) neurons.

EXPERIMENTAL DESIGN: Vehicle or Compound A at low, mid and high dose was administered to Sprague-Dawley rats (10/sex/group) daily by oral gavage for 4 weeks followed by a 4-week dose-free recovery period.

METHODS: Transmission electron microscopy (TEM) was performed on DRGs from selected animals to further characterize the microscopic findings. Serum at the end of dosing and recovery phases was collected from each rat and was analyzed using the single molecule array (Quanterix SR-X) to determine NfL concentration.

RESULTS: Cytoplasmic foamy vacuolation was observed in several tissues including DRG neurons from rats dosed with Compound A. There were no associated degenerative and/or inflammatory changes in DRGs. Phospholipidosis within DRG neurons was confirmed by TEM which revealed characteristic lamellar bodies. Serum NfL levels were significantly higher in the high dose group compared to the controls, low or mid dose groups but reverted to the control levels after 4-week recovery period.

CONCLUSION: Elevated NfL levels were associated with increased severity of neuronal phospholipidosis present in the high dose group.

IMPACT STATEMENT: Our results demonstrate that serum NfL is a sensitive biomarker for drug-induced neuronal phospholipidosis and can be utilized in preclinical safety studies to monitor the progression of subtle neuronal findings in the absence of overt pathological changes.

P50 Neurofilament Light Chain as a Good Indicator for Monitoring the Neurodegenerative Changes in SOD^{1G93A} Rats

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Abstract

INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by a progressive loss of motor neurons leading to paralysis and death typically within 3–5 years of onset. Neurofilament light chain (NfL) has recently been reported as a promising blood biomarker for monitoring various neurological diseases in both clinical and nonclinical studies. Thus, we evaluated the correlation between pathological findings and NfL levels to monitor the disease progression in the SOD^{1G93A} rat, a well-known rodent model of ALS. **EXPERIMENTAL DESIGN AND METHODS:** Twenty male wild-type and of transgenic (Tg) rats were used in this study. Body weight, electrical impedance myography (EIM), anatomic pathology, and blood and CSF NfL levels were evaluated at 10, 17, and 23 weeks of age and the endpoint (25–27 weeks of age). **RESULTS:** Degeneration of neuropile in the brain and spinal cord, and nerve fiber degeneration in the sciatic nerve were observed at 17 weeks of age. The incidence and severity of these central and peripheral nerve changes, and muscular findings increased with age. The EIM and NfL levels were well correlated to muscular atrophy and nerve degenerative changes, respectively. **CONCLUSION:** Considering the pathological findings in nervous tissues, NfL was a good indicator of disease onset and progression in Tg rats. **IMPACT STATEMENT:** Based upon our study, NfL will be a powerful biomarker to detect neurodegenerative changes in non-clinical studies.

P51 Serum N^G, N^G-Dimethyl-L-arginine (SDMA) Concentrations in Rhesus Macaque (*Macaca mulatta*) Models for Renal Transplantation

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Abstract

INTRODUCTION: The utility of SDMA as a biomarker is not well understood in non-human primates (NHPs). This study aimed to evaluate serum SDMA concentration as a marker for kidney injury in rhesus macaque models for renal transplantation. **EXPERIMENTAL DESIGN:** Serum samples were collected from rhesus macaques (n=69) in one of three kidney health states: (1) two healthy kidneys (clinically healthy, pre-renal transplant; n=19); (2) one healthy kidney (bilateral nephrectomy followed by transplantation of one healthy kidney; n=21); and (3) no healthy kidney (bilateral nephrectomy followed by transplantation and rejection of one kidney; n=30). Routine serum chemistry (including SDMA) (all animals) and histopathology (n=29) were performed. Statistical analysis was performed to assess the relationship between serum creatinine (sCr), urea, and SDMA concentrations; the association of sCr, urea, and SDMA concentrations with the three kidney health states; SDMA values pre- and post-transplantation; and the associations between serum SDMA concentration and histology scores. **RESULTS:** Serum SDMA concentrations strongly correlated with both sCr and urea concentrations ($P < 0.001$). Serum SDMA, urea, and sCr concentrations inversely correlated with the number of healthy kidneys ($P < 0.001$). Serum SDMA concentrations increased significantly post-transplantation ($P < 0.0001$). Significant differences ($P < 0.001$) in serum SDMA concentrations were observed between the three health states. No association between serum SDMA concentration and histopathology scores was detected. **CONCLUSION:** In rhesus macaque models for renal transplantation, serum SDMA concentration increased with decreasing kidney function and rejection. **IMPACT STATEMENT:** Serum SDMA concentration predicts kidney function and overall health status in NHPs.

P52 Clinical Pathology Standard Parameters in Nude Rats

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Abstract

INTRODUCTION: Long term pharmacology and safety evaluation of innovative human-derived cell therapy products require the use of immunocompromised animals to avoid immune-mediated destruction of injected human-derived cells. Athymic Nude (RH-FoxN1^{rnu}) rats represent an interesting alternative to broadly used Nude mice, particularly when implantation surgery is required and animals of larger size are preferred. Clinical pathology data obtained from this rat strain remain sparse in the literature.

MATERIALS AND METHODS: We sampled 34 untreated Nude male (N=16) and female (N=18) rats and analyzed a standard panel of hematology (Sysmex XN-1000V), biochemistry (Pentra C400) and coagulation (STA-Satellite Max) parameters routinely used in toxicology studies. Blood smears were prepared and examined. Data were qualitatively reviewed and compared with data obtained from conventional rat strains.

RESULTS: Whilst most biochemistry and coagulation parameters were overall unchanged, in hematology, white blood cell differential counts strongly differed from those recorded in other rat strains. Alterations in blood smear examination data were present and matched with hematology analytical findings.

CONCLUSIONS: Full clinical pathology profiles of nude rats including blood smear examination enabled to characterize the Nude rat strain and to produce first reference intervals. Specific hematology changes were observed and consistent with the T-cell deficient phenotype of this rat strain. Those results should be taken into consideration when interpreting study results.

P53 Cytological Bone Marrow Cell Differential Counts and Morphological Findings in the Common Marmoset (*Callithrix jacchus*)

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Abstract

INTRODUCTION: Cytological bone marrow evaluation is performed in nonclinical toxicological studies to identify potential effects of tested compounds on hematopoiesis. The common marmoset (*Callithrix jacchus*) is a nonhuman primate (NHP) species used in biomedical research. Due to its small size and the current limited supply of other NHP species such as the Cynomolgus monkey, the number of marmosets used in toxicological studies is expected to increase. The purpose of this study is to describe bone marrow cell differential counts and morphological findings from healthy marmosets.

MATERIALS AND METHODS: Bone marrow smears were prepared from 26 control marmosets (13 males, 13 females). Two bone marrow smears from one proximal femur were prepared and stained with MGG stain. An approximative 400-cell complete differential cell count and morphological assessment were performed. For each sex, myeloid to erythroid (M:E) ratios and the percentage of each individual cell types, total granulocytic cells, total erythroid cells, total lymphocytes related to total bone marrow cells were calculated.

RESULTS: All smears were of adequate quality. M:E ratios ranged from 0.61 to 1.77. Percentages of total granulocytic cells, total erythroid cells and lymphocytes ranged from 30.7% to 58.3%, 32.1% to 58.3% and 4.8 to 22.4%, respectively. Morphological findings were similar to those observed in the Cynomolgus monkey and consisted of occasional giant metamyelocytes and band neutrophils and metarubricyte nuclear blebbing and binucleation.

CONCLUSIONS: These results demonstrate technical and scientific appropriateness of cytological bone marrow evaluation in the common marmoset and provide preliminary data regarding background morphological findings.

P54 Placental Pathology in Per- and Polyfluoroalkyl Substance (PFAS) Gestational Exposure

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Abstract

INTRODUCTION: The class per- and polyfluoroalkyl substances (PFAS), commonly used in industrial products, include traditional perfluorooctanoic acid (PFOA) and structurally similar replacement analogs, hexafluoropropylene oxide dimer acid (GenX). PFAS exposure has been associated with adverse developmental and reproductive toxicity for which the placenta is a suspected target tissue. The objective of this study was to evaluate the placenta to help identify the pathogenesis of PFAS toxicity and any differences between PFOA and GenX toxicity. **EXPERIMENTAL DESIGN:** Pregnant CD-1 mouse dams were administered either 1 or 5 mg/kg/day of PFOA or 2 or 10 mg/kg/day of GenX via oral gavage from embryonic day 1.5 to 17.5 to assess embryo-placental development. **METHODS:** Endpoints assessed included embryo and placenta weights and histopathology of the placenta ($n=11-13$ litters/group). **RESULTS:** PFOA and GenX resulted in higher placenta weights and lower placental efficiency ($p \leq 0.001$ in 5 mg/kg/day PFOA). Microscopic findings in the placenta for PFOA and GenX were similar. When compared to untreated controls, these findings included increased incidence and/or severity of spiral artery fibrinoid necrosis, decidual necrosis, increased cellularity of glycogen cells and size/density of their lacunae, increased junctional zone thickness, and congestion, atrophy and necrosis of the labyrinth. Sporadic nodular dysplasia was present in PFOA females and GenX males and females. **CONCLUSIONS/IMPACT:** *In utero* exposure to PFOA or Gen-X resulted in increased placental weights/decreased placental efficiency and microscopic findings compatible with maternal vascular malperfusion as the pathogenesis of PFAS placental toxicity.

P55 Thyrotoxicity in the Developing Male Reproductive Tract

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Abstract

INTRODUCTION: Chemical exposure inducing thyroid hormone (TH) disruption in the perinatal period is associated with severe adverse outcomes. The effects of thyrotoxicants on the developing male reproductive tract have not been evaluated in safety assessment. **EXPERIMENTAL DESIGN:** A GLP Comparative Thyroid Assay (CTA; USEPA, 2005) was performed to assess pre and postnatal developmental effects. To induce perinatal hypothyroidism, timed-mated pregnant adult female Sprague Dawley rats were administered 1, 3, or 10 ppm 6-propyl-2-thiouracil (6-PTU), an antithyroid agent, in feed from gestational implantation through weaning of juvenile pups compared to two groups of vehicle controls ($n=55$ /group). **METHODS:** In addition to CTA endpoints, the testes and epididymides from male pups at postnatal day (PND) 4 and PND 21 were evaluated ($n=25$ /group). Testis and epididymis weights and histopathology and immunohistochemistry (IHC) for GATA Binding Protein 4 (GATA-4) were performed. Quantitative image analysis for mean diameter of seminiferous tubules was performed on testes (Visiopharm). **RESULTS:** At PND 21, 6-PTU-related findings in the testes included lower mean weights at the ≥ 3 ppm dose level ($p \leq 0.001$) and lower mean diameter of seminiferous tubules at the 3 ppm ($p \leq 0.05$) and 10 ppm ($p \leq 0.001$) dose levels ($n=25$ /group). Microscopic findings at all dose levels were decreased cellularity and delayed maturation of germ cells in the testes. GATA-4 evaluation in 6-PTU administered animals demonstrated loss of spermatogonia and spermatocytes, indicating loss of maturation and steroidogenesis. **CONCLUSIONS/IMPACT:** The testes in rats are a sensitive end-point for male reproductive toxicity in perinatal thyrotoxicant chemical assessment.

P56 Intra-neuronal Mutant Protein Accumulation in an Alpha-1-Antitrypsin Deficiency Mouse Model

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Abstract

INTRODUCTION: Alpha-1-antitrypsin (A1AT) deficiency is a genetic disorder in which mutant A1AT accumulates in hepatocytes, inducing hepatic damage. The most common cause of severe disease is the PiZ mutation of *SERPINA1*, which encodes A1AT. The multicopy transgenic PiZ mouse model recapitulates the hepatic disease of A1AT deficiency, but mutation-associated lesions in other tissues in this model are not well-characterized.

EXPERIMENTAL DESIGN: To identify mutant A1AT protein accumulation and associated pathology, we histologically evaluated all major organs in one PiZ mouse cohort (12-month-old heterozygous and homozygous mice) and additional neuromuscular tissues in another cohort (5-month-old C57BL/6J WT and homozygous PiZ mice), using H&E, PAS with diastase, and IHC and ISH for human *SERPINA1* (*hSERPINA1*). To evaluate neuromuscular function, wire hang and grip strength tests were performed on 5-month-old WT and homozygous PiZ mice.

RESULTS: Within neurons of dorsal root and trigeminal ganglia, we observed PAS-positive, diastase-resistant eosinophilic cytoplasmic globules, which had hSERPINA1 signal by IHC. *hSERPINA1* ISH signal was present in the cytoplasm of globule-laden neurons, as well as in neurons lacking globules in the spinal cord. Finally, a wire hang test identified faster latency to fall in PiZ mice compared to matched WT mice.

CONCLUSION: These microscopic findings identified expression of mutant A1AT in neurons in PiZ mice, and a wire hang test identified a behavioral phenotype, suggesting a functional effect of this expression.

IMPACT STATEMENT: Transgenic gene and protein expression in non-hepatic tissues, including widespread neuronal expression, is an important consideration in use of this multicopy PiZ model.

P57 Drug-Induced Bone Marrow Effects Resembling Hypereosinophilic Syndrome (HES) in Dogs

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Abstract

NNRTI-1 is a novel nonnucleoside reverse transcriptase inhibitor that was previously explored as a potential oral treatment for human immunodeficiency virus type 1 (HIV-1) infection. Nonclinical safety profile of NNRTI-1 was characterized through the conduct of repeat dose toxicity studies in mice and dogs. NNRTI-1 was administered once-daily by oral gavage to beagle dogs at dose levels of 30, 100, or 450 mg/kg/day for up to 4- or 39-weeks. In the 4-week study, NNRTI-1 related changes consisted of minimally increased neutrophil, monocyte, and white blood cell counts in females at 450 mg/kg/day and minimally to moderately eosinophilia in males and females at 100 and 450 mg/kg/day. These hematology changes correlated with increased myeloid hematopoiesis in the bone marrow histology and bone marrow smear findings of increased myeloid to erythroid ratio and increased numbers of eosinophilic precursors with no changes in maturation or dysplastic changes. Secondary causes of eosinophilia, such as underlying parasitic, neoplastic, vascular, or allergic disease, were absent. In the 39-week study, NNRTI-1 related hematology and bone marrow changes were generally similar to the 4-week study findings with additional pathology findings of mixed inflammatory cells in the liver, spleen, small intestine and granulomatous inflammation of mesenteric lymphnodes. NNRTI-1 related marked peripheral eosinophilia with infiltration of eosinophils in multiple tissues in the dogs resemble the hypereosinophilic syndrome (HES). Data will be presented on the pathological findings associated with drug induced sustained peripheral eosinophilia resembling HES which has not been previously described in beagle dogs.

P58 Drug-Related Brain Vacuolation as an Unusual Presentation of Neurotoxicity in Sprague-Dawley Rats

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Abstract

In a 4-week rat study, a nucleic acid antiviral agent containing a non-natural amino acid (C-331) induced a dose-related vacuolation in the brain's white matter tracts without concurrent neuronal loss, gliosis, or neurological deficits. Routine safety pharmacology studies showed no effects. Penetration of the drug through the blood-brain-barrier was negligible. Notably, vacuolation was absent in cynomolgus monkeys after 4 weeks at a human equivalent dose (HED) 19 times greater than the expected efficacious dose in the clinic. Rat-specific metabolites were not evident. A follow-up 13-week rat study with a 4-week interim assessment was performed using oral doses of 0, 100, 300, or 1000 mg/kg/day for 4 or 13 weeks. No test article-related clinical signs, neurobehavioral or open-field changes, or ophthalmic abnormalities were noted. At all dose levels, widespread vacuolation predominantly affecting white matter was observed in the cerebellum, thalamus, and striatum. The severity of vacuolation increased over time and persisted after the 4-week recovery period. Based on ultrastructural evaluation, the vacuolation resulted from myelin sheath splitting (intramyelinic edema) without axonal degeneration. The vacuolation was considered to be an adverse effect based on the severity and extent of the changes and the potential for causing oligodendrocyte dysfunction. Because of multiple unknowns, including the lack of neurobehavioral signs, species specificity, low exposure multiples, and the potential human significance, further development of C-331 was terminated. The study describes the unusual presentation of species-specific, drug induced splitting of myelin sheaths/intramyelinic edema without clear correlative neurofunctional effects.

P59 Epigenetic Alterations Associated with Trichloroethylene (TCE): Developmental and Transgenerational Effects in Zebrafish (*Danio rerio*)

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Abstract

INTRODUCTION/OBJECTIVES: Trichloroethylene (TCE), a volatile organic solvent and legacy environmental toxicant, may induce epigenetic toxicity in developmental stages by altering DNA methylation. This study examines whether TCE exposure during zebrafish development affects DNA methyltransferase expression and transgenerational DNA methylation. **EXPERIMENTAL DESIGN/METHODS:** Zebrafish embryos were exposed to 0, 5, 50, or 500 ppb TCE from 1–120 hours post fertilization. Larvae were then rinsed, pooled, and homogenized for RNA extraction using TRIzol and Qiagen RNeasy Mini Kits. cDNA was synthesized with the Invitrogen SuperScript First-Strand System, and the expression of seven DNA methyltransferase genes was analyzed via QuantStudio 3, using β -actin as a reference. Six biological replicates were tested in triplicate per MIQE guidelines. The Quest 5-hmC™ DNA ELISA Kit quantified 5-hmC DNA in F0, F1, and F2 generations using a Tecan M200 Infinite® Microplate Reader. **RESULTS:** Significant changes in gene expression were demonstrated with increased relative expression of *dnmt1* in the 5 and 50 ppb exposure groups and decreased relative expression of *dnmt3bb.2* in the 500 ppb exposure group. 5-hmC DNA quantification indicated significantly higher levels of global DNA methylation in the F0 generation, while F2 larvae consistently had the lowest. **CONCLUSION:** Results indicate that developmental exposure to TCE does change gene expression involved in DNA methylation, with a presumed cumulative impact over generations. **IMPACT STATEMENT:** Altered gene expression and generational decline in DNA methylation suggest potential short and long-term risks associated with TCE and the need for active surveillance in contaminated sites.

P60 Variation in Canine Neurohypophyseal Histology Associated with Sectioning Plane

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Abstract

INTRODUCTION: As a master endocrine organ and relay between nervous and hormonal systems, pituitary gland assessment is standard in nonclinical toxicology studies. However, factors including small size, anatomic location, and structural complexity can complicate assessment across animals. **EXPERIMENTAL DESIGN:** Due to suspected section orientation-related observations including increased cellularity and/or vacuolation in the neurohypophysis, a review of canine pituitary gland slides was performed to document sectioning planes and associated histologic appearance. Historical control data (Nov 2022–Dec 2024) was also screened. **METHODS:** Pituitary sections were classified by approximate plane orientation (straight/distal transverse, angled/proximal transverse, angled/parasagittal longitudinal, straight/sagittal longitudinal, or fragmented/other), cysts were recorded, and findings within the neurohypophysis graded. **RESULTS:** Pituitary sections (79) were 39% angled/proximal transverse, 22% angled/parasagittal longitudinal, 18% straight/distal transverse, 13% fragmented/other, and 9% straight/sagittal longitudinal. An appearance of vacuolation (minimal-mild) and increased cellularity (minimal-moderate) occurred at increasing incidence and severity with proximity to the third ventricle (infundibular) recess, from 31% in straight/distal transverse to 100% in straight/sagittal longitudinal sections. Cysts occurred in ~2/3 of most section planes (59–68%) but were less frequent in straight/sagittal longitudinal sections (29%) and were not correlated to appearance of cellularity and/or vacuolation. The only historical control observations were cysts (21%). **CONCLUSION:** There is wide variability of canine pituitary gland sectioning and accompanying variation in histologic appearance reflecting normal anatomy. **IMPACT STATEMENT:** Standardization of canine pituitary gland sectioning may assist with interindividual consistency and avoid potential confusion with 'non-lesions'.

P61 Urinary Bladder Background Findings in Beagle Dogs: Detrusor Myopathy Remains an Infrequent but Important Finding

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Abstract

INTRODUCTION: Urinary bladder lesions are uncommon background findings in pre-clinical studies. Detrusor myopathy has been reported at 13% incidence in beagle dogs with no sex predilection and possible association to catheterization and/or arteritis (Cain et al. 2000). **EXPERIMENTAL DESIGN:** Following observation of lesions consistent with detrusor myopathy in beagle dogs on study, a review of historical control data and contemporaneous studies was performed. **METHODS:** Urinary bladder control data was compiled for recent finalized studies (Nov 2022–Dec 2024). For contemporaneous non-finalized studies, urinary bladder slides and urinalysis data from all animals were reviewed. **RESULTS:** Historical control data comprised 87 dogs (12 studies, 2–39 weeks) with single occurrences of minimal hemorrhage, fibroplasia, and arterial mineralization and no muscle findings. Contemporaneous studies encompassed 198 single-source and naïve dogs across 6 studies (3 to 301 days) and two test facilities. Multifocal degeneration/regeneration of bladder smooth muscle occurred in 10 (5.1%) dogs, all males. Lesions were minimal (6), mild (3), and moderate (1) severity, with no relationship to test article administration. Most (9/10) were associated with a test site practicing catheterization for urine collection. There were no associated vascular or consistent urinalysis findings. Other bladder findings (minor hemorrhage, inflammation, and/or ulcer) were rare and lacked direct correlation to myopathy. **CONCLUSION:** Urinary bladder findings were rare in historical background data. Detrusor myopathy occurred at low incidence in male dogs primarily with standardized catheterization procedures. **IMPACT STATEMENT:** In dogs, detrusor myopathy remains an infrequent but important background finding of which pathologists should be aware.

P62 Overview and Interspecies Comparison of the Neuroanatomy and Neurophysiology of the Caudate Nucleus

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Abstract

INTRODUCTION/OBJECTIVES: Degeneration (characterized by neuronal loss and gliosis) of the caudate nucleus in laboratory beagle dogs has recently been reported as a potential spontaneous lesion. Investigation into the incidence and pathogenesis of this lesion in nonclinical toxicity studies is ongoing by an international pharmaceutical and contract research organization working group. Understanding the neuroanatomy and neurophysiology of the caudate nucleus is essential to design and interpret results of investigative studies.

METHODS AND MATERIALS: Literature on neurotransmitter systems and functional neuroanatomical connections of the caudate nucleus in dogs and other species was reviewed, using PubMed, EMBASE, and veterinary neuropathology texts.

EXPERIMENTAL DESIGN: Literature review considering a novel observation of degeneration of caudate nucleus of laboratory beagle dogs.

RESULTS: The caudate nucleus is part of the basal ganglia of the cerebrum with connections throughout the forebrain and functions in movement, learning and memory. All neurotransmitter systems (dopamine, GABA, glutamate, acetylcholine and serotonin) are present in the caudate nucleus.

CONCLUSION: The neuroanatomy and neurophysiology of the caudate nucleus is complex and contributes to the clinical and neuropathological features of spontaneous and acquired diseases of the basal ganglia.

IMPACT: Comparative information about the neuroanatomy and neurophysiology of the caudate nucleus is limited. Enhanced understanding from this review will directly inform the design and interpretation of investigative studies in the laboratory beagle dog, there by strengthening drug safety assessments.

P63 Toxicological Assessment of Combined Exposure to PFOA and Ethanol Using Human Renal Proximal Tubule Organoids

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Abstract

Per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanoic acid (PFOA), are persistent environmental pollutants that bioaccumulate and pose long-term health risks, particularly to the kidney. Ethanol also impairs organ function under chronic and combined exposure. However, their combined toxicity remains poorly understood, especially in human-relevant 3D organoid models. This study aimed to evaluate the physiological and pathological effects of combined exposure to PFOA and ethanol using human renal proximal tubule organoids. Initially, RPTEC/TERT1 cells were exposed to PFOA (10 μ M), ethanol (2.5%), and their combination for 24 and 72 hours in 2D. WST-1 assay revealed significantly reduced viability in the co-exposure group ($p < 0.05$), indicating a synergistic cytotoxic effect. These results guided dosing for 3D organoid experiments. In the organoid model, immunofluorescence staining of physiological markers Na⁺/K⁺-ATPase and AQP1 showed significant decreases in membrane localization after 21 days of co-exposure ($p < 0.01$), suggesting disrupted ion and water transport. Pathological analysis revealed altered expression of epithelial-mesenchymal transition (EMT) markers: E-cadherin shifted from the membrane to the cytoplasm, and N-cadherin was upregulated, indicating EMT induction and potential fibrosis ($p < 0.01$). Additionally, long-term co-exposure significantly increased carcinogenic markers S100A4 and MTS-1 ($p < 0.01$), suggesting that low-dose chronic exposure may trigger tumorigenic processes. This study highlights the utility of human renal organoids in evaluating chronic, low-dose combined toxicity. EMT analysis provides early indicators of renal injury and fibrogenesis. These findings contribute valuable insights into the health risks of environmental toxicants and support organoid-based screening for complex exposure.

P64 Organophosphate Toxicity in a Four-Year-Old Dog

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Abstract

A four-year-old, castrated male, Australian Shepherd dog was presented post euthanasia to the University of Illinois Veterinary Diagnostic Lab for necropsy with a history of "presented comatose." On gross examination, the lungs were red to dark red and meaty and a section of the jejunum was reddened and contained red mucoid material. The gross morphological diagnoses were pneumopathy and a suspected enteropathy.

Histopathologically, the cerebral gray matter was mildly hypercellular with increased numbers of oligodendrocytes and astrocytes. The oligodendrocytes often clustered near neurons and the astrocytes were scattered. The lungs were edematous and the heart had mild myocardial fibrosis with associated myofiber atrophy and degeneration. Suspected cause of morbidity was heart disease leading to pulmonary edema.

Additional, more extensive, history was provided that indicated a 1-day history of acute seizures, muscle fasciculations, hypersalivation, and diarrhea. Liver tissue sent to the Michigan State University Veterinary Diagnostic Laboratory confirmed the presence of ethyl-parathion and chlorpyrifos, both organophosphate pesticides.

Organophosphate pesticides inhibit acetylcholinesterase leading to increased acetylcholine levels. Clinical signs are broad and can include muscarinic (salivation, lacrimation, urination, defecation), nicotinic (tremors, ataxia, weakness), and central nervous system (depression, hyperactivity, seizures) effects. Microscopic findings are largely vague and can include pulmonary edema, neuronal degeneration, chromatolysis, satellitosis, and necrosis of axons with axonal swelling, microcavitation, and myelin loss.

This case is an example of the importance of clinical history in investigating toxicity cases and how miscommunication between diagnostician and clinician can lead to incomplete or inaccurate diagnoses.

P65 Poster Withdrawn

P66 Retrospective Review of Histopathology Findings in Cynomolgus Monkeys in Powder Inhalation Toxicology Studies

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Abstract

INTRODUCTION: Safety testing of powder blends used in inhaled drugs is commonly performed in laboratory species in inhalation toxicology studies. Nonhuman primates are often used for assessing potential risk for human health, but information on pathological findings related to powder carriers is very limited. **EXPERIMENTAL DESIGN:** 74 cynomolgus monkeys used as control animals in powder inhalation studies conducted at ITR Laboratories Canada in last decade were retrospectively reviewed for histopathological findings at all levels of respiratory tract. **METHODS:** Provantis software was used to acquire incidence and severity of microscopic findings from control animals exposed to air and control/vehicle powders such as lactose and leucine. **RESULTS:** Histopathological findings were observed in respiratory tissues of 27% of monkeys receiving control/vehicle items as compared to 10% in air control monkeys. Most common finding in powder control monkeys was minimal hyperplasia of mucous cells in respiratory epithelium of trachea, carina (22%) and lung bronchi and bronchioles (35%). Minimal inflammatory cell infiltrates were observed in nasal cavity of 18% of powder controls compared to 6% of air controls. **CONCLUSION:** Differences in incidence of histopathological findings in lungs were observed between powder and air control monkeys. Although incidence of findings was higher in powder control animals, severity was comparable between powder and air control monkeys, suggesting that these control/vehicle items can safely be used as carriers of inhalation drugs. **IMPACT STATEMENT:** These results can serve as historical background data for future inhalation studies in Cynomolgus monkeys exposed to most commonly used powder carriers.

P67 Histopathologic Characterization of Eyes from a Laser-Induced Primate Glaucoma Model

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Abstract

INTRODUCTION: This study characterizes laser-induced glaucomatous lesions in African green monkeys (*Chlorocebus sabaues*).

METHODS: Fifteen monkeys underwent laser scarification of the trabecular meshwork to obstruct aqueous outflow induced through laser spots (50–100 µm) applied circumferentially with a four-mirror gonioscope (0.5–1 second pulse durations, 0.3–0.75 W). Weekly assessments included slit lamp examinations, fundus imaging, and optical coherence tomography (OCT) to measure retinal ganglion cell (RGC) thickness, retinal nerve fiber layer (RNFL), and Bruch's membrane opening minimum rim width (BMO-MRW). The eyes and optic nerves were microscopically evaluated using hematoxylin and eosin.

RESULTS: Histopathology findings varied in magnitude and duration across eyes, primarily influenced by sustained intraocular pressure (IOP). High laser energy with prolonged IOP elevation (>30 mm Hg for >2 weeks) caused slight to marked optic nerve head (ONH) cupping, lamina cribrosa bowing, RGC and RNFL atrophy (consistent with OCT findings), and minimal to moderate optic nerve degeneration/necrosis. In contrast, transient IOP elevation (<2 weeks) was associated with minimal or no optic nerve degeneration/necrosis. RGC and RNFL atrophy, along with BMO-MRW decline, correlated strongly with IOP elevation. Change in ONH cupping was weakly correlated with IOP elevation.

CONCLUSION: This nonhuman primate trabecular laser model replicates key features of human glaucoma, including retinal atrophy, ONH cupping, decreased BMO-MRW, and optic nerve degeneration. Sustained increased IOP was strongly associated with BMO-MRW and RGC and RNFL atrophy.

IMPACT STATEMENT: This model provides translational clinical and histopathologic endpoints that can robustly support the preclinical evaluation of novel therapeutic interventions targeting glaucoma.

P68 Histological Evaluation of Neuronal Necrosis and Microvasculopathy in a Neonatal Rat Model of Hypoxic-Ischemic Encephalopathy

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Abstract

INTRODUCTION: Hypoxic-ischemic encephalopathy (HIE) is a leading cause of neonatal brain injury, contributing to infant mortality and chronic neurological disabilities in term newborns. Currently, the standard of care for HIE (therapeutic hypothermia) is only partially protective. Microvessels play a crucial role in forming the blood-brain barrier (BBB), and alterations in cellular and non-cellular components of the BBB may contribute to HIE. Despite their importance, the response of the developing microvasculature to HIE remains underexplored. Here, we perform a histopathological assessment of the microvasculature and surrounding regions in a rodent model of neonatal hypoxic-ischemic (HI) brain injury. Histopathology scoring for these models has been reported, although the assessment of vascular lesions is not generally included in published scoring systems.

METHODS: The Vannucci model of unilateral hypoxic-ischemic brain injury was performed in postnatal day (P) 10 rats. Brains were harvested at P13 and P17 for histopathology and immunohistochemistry. A modified version of a nine-step scoring system for HIE was used to grade the neuronal necrosis and microvessels. **RESULTS:** Histopathology revealed extensive unilateral neuronal necrosis, loss, and neuropil rarefaction within the cortex, striatum, hippocampus, and thalamus. The affected regions displayed a significant proliferation of small-caliber blood vessels, featuring notable tortuosity, endothelial hypertrophy, and increased perivascular gitter cells. Vascular lesions were more severe in animals with higher histopathology scores, and at P13 compared to P17. **CONCLUSIONS/IMPACT STATEMENT:** This study presents a modified grading system for HI lesions in rats and underscores the responses of developing microvasculature to HIE injury, representing potential therapeutic avenues.

P69 Immunohistochemical Characterization of Bilateral Basal Nuclei Lesions in Beagle Dogs Using Novel Neurotransmitter Marker Panels

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Abstract

INTRODUCTION/OBJECTIVES: Bilateral basal nuclei lesions (BNL) in Beagle dogs have been identified as a potential spontaneous neuropathology with implications for regulatory toxicology studies. To support differentiation between spontaneous and test article-related effects, we are developing an expanded immunohistochemical (IHC) panel to characterize these lesions. This includes the novel application of neurotransmitter markers—such as Tyrosine Hydroxylase, VGLUT1/2, and GAD67—not previously used in basal nuclei evaluation in dogs.

METHODS AND MATERIALS: Formalin-fixed, paraffin-embedded brain tissues from an unaffected control Beagle and two positive control dogs with encephalopathies were sectioned at 4 μm and stained on a Leica Bond RX automated platform. A panel of 30 IHC markers targeting neuronal, glial, vascular, neurotransmitter, degenerative, and metabolic pathways was optimized. Digital slide scanning was performed at 40x magnification.

EXPERIMENTAL DESIGN: Following pathology review, the IHC panel, verified by available evidence, will be applied to archived brain tissues from four Beagle BNL cases.

RESULTS: Pending analysis, we plan to assess neuronal integrity, glial responses, and neurotransmitter expression using digital image analysis and semi-quantitative scoring to compare BNL cases with controls.

CONCLUSION: This study aims to define the cellular composition and chronicity of BNL, providing insights into their pathogenesis and supporting their evaluation as a potential background lesion.

IMPACT STATEMENT: By applying an expanded IHC panel—including first-time use of key neurotransmitter markers in canine basal nuclei—this work will support differentiation of spontaneous neuropathology from test article-related effects and inform future nonclinical neurotoxicity studies.

P70 Bioavailable Iron Coal Dust-Induced Oxidative Damage and Histopathological Changes in Rat Lung

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Abstract

INTRODUCTION: Hazard related health issues in coal mine workers and bioavailable iron (BAI) levels are still not defined in regards to prevalence of Coal workers' pneumoconiosis (CWP). This study was designed to investigate oxidative damage and histopathological changes in lung tissue of rats exposed to BAI coal. **EXPERIMENTAL DESIGN:** Twenty-four male Wistar rats were randomly divided into four groups (6/group) which included an air controlled group (N), and three BAI coal dust groups which were exposed at 40 (A), 20 (B), 10 mg/m^3 (C). Groups were exposed for five hours per day for 30 and 60 days. **METHODS:** Oxidative damage was evaluated using malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) parameters in lung homogenate. Histopathological alteration was studied by evaluation of hematoxylin and eosin and Masson's trichrome stained slides. **RESULTS:** BAI coal dust exposure caused significantly increased MDA levels while decreasing GSH, SOD and CAT activity compared to control rats. Pathological assessment of lung tissue showed intact ciliated tracheal epithelium and normal lamina propria and submucosal structures in the N group. Collagen deposition was highly dense in (A) as compared to (C) and (N) groups. BAI coal dust exposure at 20 (B) and 40 (A) mg/m^3 following 30 and 60 days exposure caused mild to moderate bronchiolar epithelial necrosis, collagen deposition, and decreased goblet cells with increased exposure. **CONCLUSION:** Findings suggested that coal dust, even with modest levels of BAI, may be accountable for inflammatory responses and oxidative damage to pulmonary cells, leading to CWP development. **IMPACT STATEMENT:** Study suggested that BAI can be an important characteristic to assist with establishing safety standards for coal dust exposure prior to active mining.

P71 INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions - An Update – 2025

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Abstract

The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) coordinates objectives of the project. Development of terminology for rodent organ systems and non-rodent species is the responsibility of Working Groups, with experts from North America, Europe, and Japan. All rodent organ systems have been published – Respiratory, Hepatobiliary, Urinary, Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands and Hematolymphoid System in *Toxicologic Pathology* and the Integument and Soft Tissue, Female Reproductive System, Digestive System, Cardiovascular System, Skeletal System, Special Senses and Endocrine System in the *Journal of Toxicologic Pathology* as supplements and on a web site – www.goReni.org. Mini-pig and Dog were published in *Toxicologic Pathology* in 2021 and Non-human primate and Rabbit were published in the *Journal of Toxicologic Pathology* in 2021. Non-rodent ocular toxicity manuscript was published by *Toxicologic Pathology* in late 2024 and the Fish INHAND manuscript is targeted for publication in *Journal of Toxicologic Pathology* in 2025. INHAND guides offer terminology, diagnostic criteria, differential diagnoses, images, and guidelines for recording lesions in toxicity and carcinogenicity studies. INHAND GESC representatives work with Clinical Data Interchange Standards Consortium (CDISC) to incorporate INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. Interest in INHAND nomenclature, based on input from industry and government scientists, is encouraging wide acceptance of this nomenclature.