



Poster Setup and Presentation Times

Poster Presentation Times

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

Sunday, June 25 (Welcome Reception) 6:00 PM–6:30 PM
 Monday, June 26.....10:15 AM–10:45 AM and 3:00 PM–3:30 PM
 Tuesday, June 27.....10:15 AM–10:45 AM and 2:50 PM–3:20 PM
 Wednesday, June 28.....9:45 AM–10:15 AM

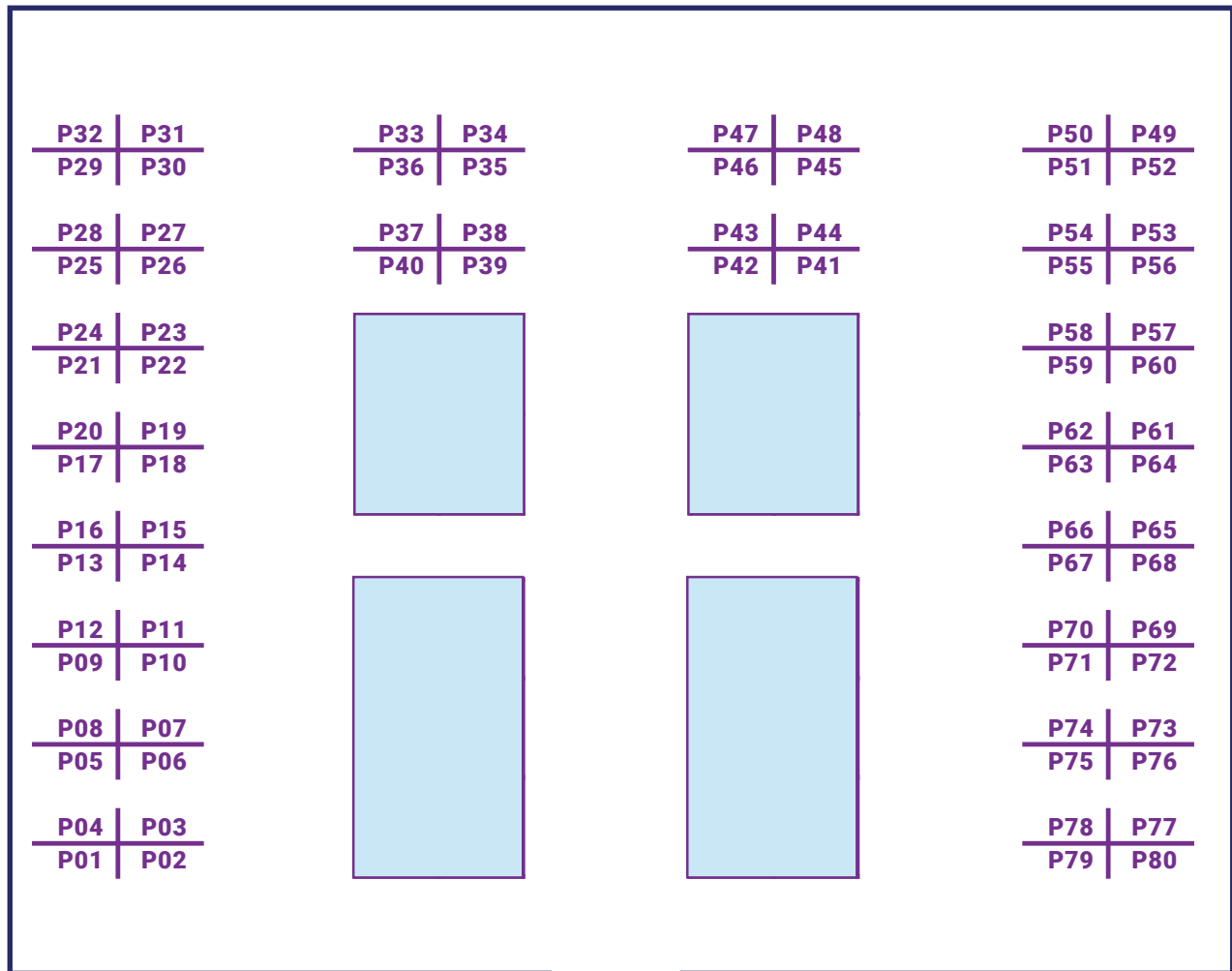
Poster Setup

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Poster Teardown

Wednesday, June 28..... 1:00 PM–2:00 PM

If your poster is not removed before 2:00 PM, it will be removed and placed near the Registration Desk for pickup.



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Bradley C. Wright^{1,2}, *Joel Levoy*², *Beth Fugate*², *Diana Lindquist*², *Alex Edmondson*^{1,2}

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Abstract

Exposure to endocrine disrupting chemicals (EDC) potentially dysregulates myelination during brain development. Magnetic resonance imaging (MRI) can be used to measure myelination *in vivo*, but this method must be validated histologically. Therefore, we compared *in vivo* MRI and *ex vivo* histological measures of myelination in rats. For this pilot study, seven control rats were scanned in a Bruker 7T MRI scanner at PND 90. Upon completion, rats were euthanized and perfused with 4% paraformaldehyde. Brains were harvested, fixed, cryoprotected (20% and 30% sucrose), and cryosectioned at 8µm. Sections were stained with fluoromyelin (488nm), phalloidin (695nm), and DAPI (460nm), then imaged using fluorescence microscopy at 4x. From four regions of interest (corpus collosum, hippocampus, hypothalamus, cingulate tract), we extracted mean myelin water fraction (MWF) and fractional anisotropy (FA) from MRI images and mean luminescence from the 488nm fluorescent channel, in histological sections. MWF and FA were positively correlated (Pearson's correlation, $r = 0.79$, $p = 6.37e-7$). There was no correlation between fluoromyelin and MWF or FA within brain regions of interest. However, pooled data demonstrated positive correlations between fluoromyelin and MWF ($r = 0.69$, $p = 4.48e-5$) and FA ($r = 0.41$, $p = 0.02$). Our results suggest MRI measures of myelination (MWF, FA) are representative of biological features found in rat brain histology. These strong relationships between *in vivo* and *ex vivo* outcomes support the validity of using MRI for assessing the effects of EDCs on myelination *in vivo*.

P02 Comparison of Two Histomorphologic Methods to Quantify Vascular Profiles in Topical Antimicrobial Therapy: Is It Worth the Hassle?

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Abstract

Introduction: Mitigating bias in quantifying routine hematoxylin and eosin (HE) stained sections remains a challenge without *preplanned tissue* preparation for more comprehensive methods of evaluation, such as isotropic sectioning for stereological techniques. This study aims to utilize modified stereological techniques to compare the number of dermal vascular profiles in HE stained skin sections challenged with different topical treatments.

Methods: Six full-thickness, cutaneous biopsies are sampled from one pig treated with a control and five different treatments. Tissues are formalin-fixed, paraffin-embedded, and stained with HE. Three tissue sections of each treatment area are examined histologically. Two methods of evaluation are employed: 1) A superimposed arbitrary line across the superficial dermis or; 2) a cycloid grid over a randomly selected area. Intersections of the line or cycloid arc with vascular profiles are counted. For the cycloid method, three, 4µm, serial sections are utilized to quantify intersections in a volume of space. Statistical analyses drawing comparisons between control and treatment groups are performed with 1-factor ANOVA for each 2D and 3D sampling method.

Results & conclusion: There are no statistically significant differences between the control and five treatment groups using the cycloid probe and linear probe methods. In this pilot study, there is no benefit to utilizing a cycloid quantification method versus a simple linear probe in standard HE sections of skin.

Impact statement: Consideration of the advantages/disadvantages of sophisticated quantification methods compared with more simple estimations is warranted in studies with limited resources, including limited pathologist time.



P03 The Ikaros Zinc Finger Transcription Factor Aiolos as a Novel Regulator of T Cell Migration

Melissa R. Leonard^{1,2}, Kaitlin A. Read^{1,3}, Devin M. Jones^{1,3}, Srijana Pokhrel¹, Robert T. Warren¹, Kenneth J. Oestreich^{1,4}

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Abstract

Introduction: Chemokine signaling is an integral component of lymphocyte migration, activation, and survival. The chemokine receptor CXCR3 is highly expressed on CD4+ T helper 1 (TH1) cells, which play a critical role in the adaptive immune response against intracellular pathogens. While CXCR3 helps direct TH1 cells to sites of inflammation or infection, it has also been implicated in a multitude of autoimmune diseases and cancers. Thus, understanding the molecular mechanisms that regulate CD4+ T cell migration is imperative for preventing immunopathology. **Methods & Results:** Here, we identify the Ikaros zinc finger (IkZF) transcription factor Aiolos (IkZF3) as a positive regulator of CXCR3 expression in CD4+ T cells in a murine influenza model. Initially, we show decreased numbers of Aiolos-deficient antigen-specific CD4+ T cells in the lung, which correlates with decreased CXCR3 expression. RNA-seq and Assay for Transposase Accessible Chromatin (ATAC)-seq analyses of *in vitro*-generated wild-type and *Ikzf3*^{-/-} TH1 cells revealed decreased *Cxcr3* transcript and reduced chromatin accessibility at regulatory regions of *Cxcr3* in the absence of Aiolos, respectively. **Conclusion:** These data imply a novel role for Aiolos in positively regulating CXCR3 expression and migration in TH1 cells. Given these findings, we seek to define the functional effects of Aiolos deficiency on T cell migration and the molecular mechanism(s) by which Aiolos directly and/or indirectly regulates migration. **Impact statement:** This work will provide functional and mechanistic insight regarding the role of Aiolos in T cell migration and inform the design of novel therapeutics to treat various immunologic disorders.

P04 A Preclinical Common Marmoset Model of Gammaherpesvirus Infection and Associated Lymphoma

Stacey L. Piotrowski^{1,2}, Allison Tucker¹, Amanda Lee¹, Jennifer E. Dwyer³, R. Mark Simpson³, Matthew F. Starost⁴, Heather Narver¹, Steven Jacobson¹

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Abstract

Introduction: Callitrichine herpesvirus 3 (CalHV-3), a gammaherpesvirus, was first identified in the common marmoset (*Callithrix jacchus*) in the early 2000s. Viral prevalence, viral loads, and associated pathology, including lymphoma, were investigated to further characterize CalHV-3 in the marmoset as a model of human Epstein-Barr virus (EBV). **Experimental Design/Methods and Materials:** Peripheral blood mononuclear cells (PBMCs) from over 140 marmosets in a research colony were screened for CalHV-3 via droplet digital PCR (ddPCR). Longitudinal saliva and PBMC samples were similarly screened in a subset of animals. Archived formalin-fixed paraffin-embedded (FFPE) marmoset tissues were searched for neoplasms, and DNA was extracted from blocks for CalHV-3 ddPCR. Immunohistochemistry (IHC) was performed on FFPE tissue to confirm neoplastic origin. **Results:** 19% of the colony was positive for CalHV-3, with fluctuations in PBMC and saliva viral loads over time. Lymphoma was diagnosed postmortem in an animal with a high PBMC viral load of over a million copies of CalHV-3 per million cells. All archived B-cell lymphomas (8/8, 100%) were positive for CalHV-3, with millions of copies of virus per million cells in neoplastic tissue. **Conclusion:** Characteristics of CalHV-3 infection in the common marmoset, including persistent infection and variations in viral loads, are comparable to human EBV infection. All analyzed cases of B-cell lymphoma are associated with CalHV-3 infection, similar to the relationship of EBV infection with subsets of lymphoma in humans. **Impact Statement:** CalHV-3 infection in the common marmoset is a potentially useful translational model of EBV-like gammaherpesvirus infection and virally induced hematopoietic neoplasia.

P05 Comparative Study on the Effects of Human CD34+ Hematopoietic Stem Cell Engraftment in NSG-SGM3 and NOG-EXL Mice

Elinor Willis¹, Jillian Verrelle¹, Esha Banerjee¹, Charles-Antoine Assenmacher¹, James C. Tarrant², Nicolas Skuli³, Moriah Jacobson⁴, Zev A. Binder⁵, Enrico Radaelli¹

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Abstract

Engraftment efficiency of human immune cell lineages in severely immunodeficient mice varies, and numerous strains have been developed to improve reconstitution. NSG-SGM3 and NOG-EXL mice combine severe immunodeficiency with transgenic expression of human myeloid stimulatory cytokines, resulting in improved expansion of myeloid populations. However, humanized NSG-SGM3 (huNSG-SGM3) mice develop a lethal macrophage activation syndrome (MAS) and mast cell hyperplasia that limit their use in long-term studies, especially those requiring humanization followed by tumor xenotransplantation. It is currently unclear to what extent humanized NOG-EXL (huNOG-EXL) mice suffer from the same conditions observed in huNSG-SGM3 mice. We compared the effects of human CD34+ hematopoietic stem cell engraftment in these two strains in an orthotopic glioblastoma patient-derived organoid xenograft model. NSG-SGM3 mice humanized in-house were compared to commercially available huNOG-EXL mice. Mice were euthanized at humane or study endpoints, and a complete pathological assessment was performed. A semiquantitative multiparametric clinicopathological scoring system was developed to characterize the myeloid proliferative disorder. HuNOG-EXL mice survived longer (22 vs 17 weeks), with less severe MAS and lack of mastocytic proliferation. Major findings included mast cell infiltration of the pancreas and liver (huNSG-SGM3 only), increased eosinophilopoiesis, anemia, and histiocytic infiltration of the spleen (both strains). Engraftment of human lymphocytes, assessed by immunohistochemistry, was similar in the two strains. The longer survival and decreased MAS severity in huNOG-EXL mice enabled their use in a patient-derived xenograft transplantation study. The NOG-EXL model may be better suited than the NSG-SGM3 model for immuno-oncology studies requiring long-term survival post humanization.

P06 Identification of a Novel Actinomyces Species Causing Mandibular Abscesses in a Southern Giant Pouched Rat (*Cricetomys ansorgei*) Research Colony

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Abstract

African pouched rats are muroid sub-Saharan African rodents that are so named for their large cheek pouches, which are used for storing food. While African pouched rats have been utilized in the detection of land mines, tuberculosis, and *Salmonella* spp., the research colony housed at Cornell University is utilized primarily in the study of reproductive behavior. In the last 8 years, there have been 17 cases presented for postmortem evaluation, and of these cases, 5 presented for mandibular abscesses. In the particular case discussed, the presenting complaint was a right sided facial swelling, with suppurative material exuding from the ear canal, and evidence of bone lysis on imaging. Gross evaluation revealed a 1 cm diameter nodule in the caudal mandibular region containing viscous, opaque, yellow-white material. Histologically, there was effacement of the bone by neutrophils with club-shaped foci of eosinophilic material (Splendore-Hoeppli material), surrounding Gram-positive filamentous bacteria. Culture results revealed Gram-positive branching rods resembling *Actinomyces* species. Further workup with whole genome sequencing revealed a previously unidentified species. Based on the high number of cases with jaw abscesses, it was discussed that there may be trauma to the oral cavity in this colony, related to wooden chewing block given as toys. A survey of all necropsied cases also revealed renal disease, chronic myocarditis, and individual cases of mesothelioma and fibrosarcoma in this colony. This presentation provides an overview of potential lesions in this less well-studied laboratory animal, and highlights a novel bacterial species affecting these animals.



P07 A Retrospective Study of Veterinary Diagnostic Toxicology in Auburn University and Thompson Bishop Sparks State Diagnostic Lab from 2005 to 2023

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Abstract

Introduction: Veterinary diagnostic toxicology is a special field in veterinary medicine with the vast majority of the cases reported as accidental exposures. More attention is paid on the relationship between chemical or poison-related cases in animals and humans. However, it remains challenging to identify the toxicants as it requires a complete clinical history, pathologic findings, and chemical analyses. The purpose of this study is to perform a retrospective review of the diagnostic toxicology cases on the animals submitted to Auburn University (2005-2023) and Thompson Bishop Sparks State Diagnostic Lab (Alabama Veterinary Diagnostic Laboratory System) (2019-2023). **Methods:** Archived cases with known toxicants were selected, and the medical records, gross and histopathologic findings, and toxicology reports were reviewed. **Result:** A total of 52 cases were retrieved, in which overexposure to rodenticide (14/52) and heavy metals (14/52) were determined as the most commonly diagnosed, followed by ethylene glycol (8/52), toxic plants (8/52), insecticides (4/52), minerals (3/52), and drug (1/52). Second-generation anticoagulant rodenticides were mostly detected. Copper toxicity in ovine accounted for half of the heavy metal cases. Toxic plants such as senna plant, sago palm, oak tree, maple tree, and azalea tree were recorded. Aldicarb was uniformly detected in cases with insecticide poisoning. **Conclusion/Impact statement:** This is the first retrospective survey of diagnostic toxicology in Alabama. Rodenticides, insecticides, heavy metals, minerals, toxic plants, and drugs were documented in the archives. Data obtained from this study represent an ongoing environmental pollution alert to the ecosystem we shared.

P08 Investigating the Interactions of Dopamine Receptor D1 in Lung Cancer

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Abstract

Background: In lung cancer, although mutation-targeting therapies including EGFR inhibitors have improved patient outcomes, resistance is common, so novel and adjunct treatments are still needed. Our lab recently identified dopamine receptor D1 (DRD1) as a novel tumor suppressor in lung cancer and discovered DRD1 signaling modulates EGFR signaling, cell proliferation, and cell death. However, these mechanisms remain poorly understood.

Objective: Our lab seeks to better understand how DRD1 regulates cancer cell growth and if those mechanisms could be co-opted as adjunct lung cancer treatments. In this study we investigate whether DRD1 interacts directly with EGFR and through a protein-protein interaction (PPI) screen identify other proteins that interact with DRD1 and through which DRD1 could regulate cell proliferation.

Methods: We investigated DRD1's PPIs *in vitro* using proximity ligation assay (PLA), immunoprecipitation (IP), and proximity-dependent biotinylation (PDB).

Results: While PLA suggested DRD1 and EGFR exist in close proximity on the cell membrane, IP did not support direct binding of DRD1 and EGFR. The PDB screen for DRD1's PPIs also supported a lack of direct DRD1-EGFR binding but identified many potential mediators of DRD1's regulation of EGFR signaling and cell proliferation, including GSK3 β , an important Wnt/ β -catenin regulator.

Conclusions: These results suggest a close but not direct-binding interaction of DRD1 and EGFR. As we continue to evaluate our PPI candidates, we hope elucidation of how DRD1 regulates cancer cell proliferation will lead to novel therapies that alone or paired with EGFR inhibitors result in better outcomes for lung cancer patients.

P09 Class I Glucose Transporters as Therapeutic Targets in Advanced Prostate Cancer

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Abstract

Introduction: Progression of prostate cancer (PCa) to bone metastasis relies on glucose. Glucose transporter (GLUT) inhibitors are potential therapeutics for advanced PCa. Our hypothesis is inhibition of class I GLUTs by a second-generation small molecule (DRB18) developed by our laboratory for GLUTs 1-4 will safely reduce PCa growth measured by *in vivo* bioluminescence imaging, histopathology, and biochemistry in a preclinical mouse model. **Experimental Design/Methods:** Ace-1, a canine, androgen-independent PCa cell line representing advanced PCa in men was used. RNA sequencing measured *in vitro* expression of carbohydrate transporters. Glucose uptake and cytotoxicity were determined in Ace-1 cells following 24 or 72h DRB18 treatment. Canine kidney cell line (MDCK) was used for cytotoxicity comparison. Male, 8-week-old, nude mice were injected with Ace-1 cells in proximal tibias and receive DRB18 (20mg/kg intraperitoneal q48h) or vehicle (DMSO/PBS). Intratibial tumor growth will be monitored for three weeks using bioluminescent imaging. Tibias will be collected postmortem for histopathology and bone histomorphometry. DRB18 safety will be assessed by postmortem examination, body weights, and serum chemistry, including insulin, glucose, and triglycerides. **Results:** Ace-1 has high expression of GLUT1 (2.5-fold greater than related transporters). DRB18 ($\geq 10\mu\text{M}$) significantly decreased glucose uptake and induced dose- and time-dependent cytotoxicity in Ace-1 with IC50 of 15-26 μM (compared to 37 μM for MDCK). *In vivo* and *ex vivo* analyses are in progress. **Conclusion:** Glucose transport antagonism with DRB18 has promising anti-proliferative effects in PCa. **Impact Statement:** Inhibitors of glucose metabolism are untested for the treatment of advanced PCa, a currently fatal disease stage.

P10 Progression and Persistence of Lung Injury in Diabetic Mice Repeatedly Exposed to Ozone

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Abstract

Epidemiological studies suggest that people with metabolic diseases are particularly prone to adverse health effects of air pollution. We recently reported that diabetic KKAY mice have more severe lung injury than nondiabetic C57BL/6 mice after repeated exposures to ozone, a common gaseous air pollutant. In the present study we further elucidated the progression of ozone-induced lung injury and resolution in KKAY mice as compared to C57BL/6 mice. Male mice of both strains were exposed to 0 or 1 ppm ozone for 4, 8 or 12 consecutive weekdays, 4 h/day, and euthanized 1-day postexposure (PE). Another group of these mouse strains were exposed for 12 weekdays but sacrificed 26 d PE (recovery group). Lung tissue was prepared for light microscopic, immunohistochemical and morphometric analysis. Minimal lung lesions were present in ozone exposed C57BL/6 mice. Lesions were restricted to centriacinar regions and resolved by 26 d PE. In contrast, ozone exposed KKAY mice had multifocal, necrotizing alveolitis that expanded beyond centriacini to more distal alveolar parenchyma. There was a time dependent increase in severity of alveolar histopathology that included necrosis and loss of alveolar type I epithelial cells, influx of eosinophils, fibrin accumulation, hemorrhage, proliferation of alveolar type II epithelial cells and alveolar transitional epithelial cells, and interstitial fibrosis. KKAY recovery mice had resolving yet persistent epithelial, inflammatory, and interstitial lung lesions. These findings give biological plausibility to the epidemiologic suggestion that people with diabetes are particularly susceptible to health effects of air pollution.



P11 Background Lesions in the Harderian Glands of Naïve or Control Göttingen Minipigs

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Abstract

Introduction: Göttingen minipigs (*Sus scrofa domestica*) are often used as a large animal model in pre-clinical safety assessment studies, particularly in ocular toxicity studies due to similar anatomy and physiology to human eyes. Associated glands, including the Harderian gland, are also examined histologically for test article-related effects in these studies. However, minimal historical control data is present regarding background findings in minipig Harderian gland. **Experimental Design:** In this study, 30 total minipig Harderian glands were examined (18 males and 12 females). Harderian glands were either harvested from naïve minipigs from an internal stock colony or from control animals on safety assessment studies. Harderian glands were trimmed per standard operating procedure, stained with hematoxylin and eosin, and assessed for various background lesions. **Results:** Minimal mineralization was observed in 8/18 males and 3/12 females. Minimal to mild cellular debris within ductular lumen was noted in 4/18 males and 3/12 females. Minimal mononuclear infiltrate was present in 3/18 males and 1/12 females. Minimal to mild atrophy was observed in 3/12 females. Minimal duct dilation was observed in 2/18 males and 1/12 females. Mild gland dilation was noted in 1/18 males. **Conclusion and Impact Statement:** This assessment highlights that a minimal to mild severity of multiple background lesions are noted in both male and female Harderian glands from Göttingen minipigs. Careful observation of the severity of lesions should be performed to determine if observed lesions are background or potentially test article-related on various safety assessment studies.

P12 Liver-Specific Deletion of RNA-Binding Proteins ZFP36L1 and ZFP36L2 Protects Against Carbon Tetrachloride-Induced Acute Liver Injury

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Abstract

Combined deletion of mRNA destabilizing proteins, Zinc Finger Protein 36 like 1 (ZFP36L1) and Zinc Finger Protein 36 like 2 (ZFP36L2), has been shown to induce inflammatory changes in the liver in our previous experiments. Accordingly, we hypothesized that the given phenotype would exaggerate chemical-induced acute liver injury. Liver-specific ZFP36L1 and ZFP36L2 double knockout (L1L2LKO; AlbCre+/Zfp36l1flox/flox/Zfp36l2flox/flox) and flox-only control (L1L2FLX; AlbCre-/Zfp36l1flox/flox/Zfp36l2flox/flox) adult mice were intraperitoneally injected with carbon tetrachloride (CCl₄) dissolved in corn oil (0.8 ml CCl₄/Kg body weight). The basal levels of liver glutathione (GSH) and cytochrome P450 2E1 (CYP2E1) were assessed in untreated mice. Gene expression, histopathology, serum ALT, and AST levels were assessed in untreated and CCl₄-challenged mice at 48 h post-administration. The basal liver GSH was significantly elevated in L1L2LKO than in L1L2FLX control mice. Interestingly, both mRNA and protein expressions of CYP2E1, a pathogenic factor, were significantly downregulated in L1L2LKO than in the L1L2FLX group. CCl₄ administration resulted in milder hepatic necrosis in L1L2LKO mice compared to L1L2FLX mice. Furthermore, the serum levels of liver injury biomarkers, ALT and AST were also significantly lower in L1L2LKO than in the L1L2FLX group. Genes involved in GSH synthesis, Gclm, and Gpx1 were significantly upregulated in L1L2LKO than in the L1L2FLX group following CCl₄ administration. In conclusion, the combined deletion of RNA-binding proteins, ZFP36L1 and ZFP36L2, protects against CCl₄-induced acute liver injury by preventing the bioactivation of CCl₄ and promoting the antioxidant defense mechanism. These data suggest a pathogenic role of ZFP36L1/ ZFP36L2 in CCl₄-induced acute liver injury.

P13 Morphologic Changes and Expression of Epithelial to Mesenchymal Transition (EMT)-Related Markers in Human HPV-Immortalized Ectocervical Cells Exposed to Cigarette Smoke Condensate

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Abstract

Introduction: High-risk human papillomavirus (HR-HPV) such as HPV16 is a major known risk factor for cervical cancer (CC). Studies revealed that cigarette smoking is also associated with CC; however, the underlying molecular mechanism(s) remains unclear. Tobacco components have been found in the cervical mucus of women smokers. Our **objective** was to determine the potential effects of cigarette smoke condensate (CSC; 3R4F) on human ectocervical cells (HPV16 Ect/E6E7). **Experimental Design:** HPV16 Ect/E6E7 cells were exposed to CSC at concentrations of 1×10^{-6} $\mu\text{g}/\text{mL}$ -100 $\mu\text{g}/\text{mL}$. **Methods:** Cell proliferation was measured by MTS assays. Cell morphology was determined by light and transmission electron microscopy, and expression of EMT markers E-cadherin (CDH1) and vimentin (VIM) were evaluated by immunofluorescence (IF), confocal microscopy, and western blotting. **Results:** CSC induced increased proliferation in ectocervical cells at 1×10^{-6} $\mu\text{g}/\text{mL}$ -10 $\mu\text{g}/\text{mL}$ for 24h, 48h, and 72h. Cells exposed to CSC (10 $\mu\text{g}/\text{mL}$) had morphologic changes that consisted of a loss of their "cobblestone" appearance with a shift toward a "spindle-like" morphology. Ultrastructural changes showed CSC-treated cells were enlarged nearly 1.5 times that of controls. Additionally, these cells had decreased surface filopodia, and cytoplasmic swelling. We found a significant reduction in E-Cadherin expression in CSC-treated cells, compared to controls at 24h, which remained reduced at 72h and 168h. **Conclusion:** Our data suggest that CSC induces EMT in human cervical epithelial cells. **Impact Statement:** CSC can induce EMT in ectocervical cells which may be a molecular mechanism important in the progression of CC.

P14 Evaluating Brain MicroRNA-29 Reduction as a Key Event in Alzheimer's Disease

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Abstract

Introduction: MicroRNA-29 (miR-29) is vital to mature brain homeostasis, and reduced levels of miR-29 in cerebrospinal fluid and blood have been associated with Alzheimer's disease (AD). While miR-29 offers a potential avenue of therapy for AD, the effects of miR-29 reduction in the brain are not entirely understood.

Objectives: We aim to: 1) Define the changes in miR-29 levels in multiple murine models of AD; 2) Evaluate the effects of reduced miR-29 in the neurons of adult wild type mice.

Methods: 1) We measured brain miR-29 levels (qRT-PCR) in three murine AD models: APP/PS1, PS19, N-L-GF; 2) We created a cre/lox mouse model to induce complete (miR-29 F/F) or partial (miR-29 F/wt) deletion of miR-29 in Thy1-positive neurons at 4 months of age. These mice were evaluated for survivability, gene expression, behavioral abnormalities, and AD-associated pathology.

Results: 1) miR-29 was reduced by approximately 10-40% in the brains of all three murine models of AD evaluated; 2) While partial deletion of miR-29 did not affect survivability, complete deletion resulted in lethality by 9 months. Deletion of miR-29 also resulted in significantly altered expression of several genes (e.g. COX7A2) that have been linked to AD.

Conclusions: 1) miR-29 levels appear to be reduced in several established AD mouse models; 2) miR-29 expression in neurons is vital to brain homeostasis, and reduced miR-29 likely plays a role in AD pathogenesis.

Impact Statement: The links between miR-29 and AD shown here further support miR-29 overexpression as a promising AD treatment.



P15 SARS-CoV-2 Infection Persists in the Gastrointestinal Tract Beyond Respiratory Clearance in Experimentally Infected Domestic Cats

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Abstract

Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causes multisystemic clinical syndromes in humans. Infection is reported in felines, but little is known about non-respiratory lesions, specifically in the gastrointestinal tract. Our objective was to investigate viral persistence in extrapulmonary tissues in domestic cats experimentally infected with SARS-CoV-2. **Experimental design:** Tissues from eleven cats were evaluated at 3, 6, 10, or 28 days post-inoculation (DPI). Quantitative reverse transcriptase-polymerase chain reaction and immunofluorescence was performed on formalin-fixed paraffin-embedded tissues. **Results:** SARS-CoV-2 RNA was detected in the ileum, colon, heart, and all respiratory tissues. By 10 DPI, viral RNA was below the limit of detection in all respiratory tissues and heart, except the trachea of one cat at 28 DPI. In the colon and ileum, viral RNA was detected at all time points in nearly all cats, including 28 DPI for both tissues. Surface enterocytes and colonocytes, both individual and clusters, had positive cytoplasmic immunoreactivity to SARS-CoV-2 nucleoprotein at all time points. Positively staining intraluminal material, within or overlaying the brush border, was most abundant at 28 DPI. **Conclusion:** SARS-CoV-2 replication occurs in the gastrointestinal tract, and viral RNA persists beyond respiratory clearance. **Impact statement:** Cats are susceptible to SARS-CoV-2, spend considerable time interacting with humans, and develop disease similarly; this non-conventional but translational model should be used to study viral pathogenesis and investigative pathology. Furthermore, intestinal tissues may serve as a useful target tissue in chronic SARS-CoV-2 studies where viral shedding from the respiratory tract has been cleared.

P16 Histologic and Immunohistochemical Characterization of Graft-versus-Host Disease with Chimeric Antigen Receptor T Cell Therapy in a Murine Model of Mantle Cell Lymphoma

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Abstract

Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a treatment strategy for patients with mantle cell lymphoma (MCL), an incurable B-cell lymphoma. The long-term efficacy of CAR-T therapy is unknown, however, and relapses are reported. In addition to the FDA-approved CD19-targeting CAR-T product, other targets have been explored but ultimately have performed poorly in clinical trials. We thus developed a novel CAR-T, and our data show that these CAR-T effectively kill MCL cells *in vitro* and *in vivo* while showing minimal cytotoxicity against normal immune cells. NOD-SCID γ c^{-/-} mice were engrafted with a human MCL cell line and randomly allocated to control group (untreated) or treatment groups receiving untransduced T-cells, CD19-targeting CAR-T, or our novel CAR-T. The primary study endpoint was overall survival. Despite significant reduction of disease burden in the group receiving CAR-T therapy, survival benefit was attenuated due to development of xenograft graft-versus-host disease (GVHD)-like disease. The present study is aimed at histologic and immunohistochemical characterization of this CAR-T therapy-induced GVHD-like disease. We found that primary tissues targeted included skin, nasal sinuses, liver, and stomach. To characterize cellular infiltrates, immunostaining for human T-cells, murine macrophages, and MCL cells was performed. Understanding of the risks and toxicities with CAR-T as a therapeutic modality is imperative to advance preclinical development of a safe and efficacious agent.

P17 Novel Murine Models to Study the Pathogenesis and Possible Therapies for the Liver Disease in Niemann-Pick Disease Type C1

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Abstract

Introduction/Objectives: Niemann-Pick disease type C (NPCD) is a lysosomal storage disorder characterized by disrupted cholesterol transport and other lipids, leading to its accumulation within the lysosomes. It is caused by mutations in NPC1 or NPC2 genes. Current mouse strains used for NPC1 studies are useful for the evaluation of the neurodegenerative phenotype; however, they rarely develop lesions in other organs, including the liver. Our goal was to create a liver-specific knockout to characterize the liver disease in NPC1 and evaluate possible therapies. **Experimental design:** We developed a liver specific knockout model using the Cre-lox tissue specific knockout system by breeding a liver-specific or Kupffer cell-specific with a homozygous NPC1 "floxed" mouse. **Methods and Materials:** Genotype was confirmed by PCR. Mice were euthanized, and the liver was removed, post-fixed in 4% paraformaldehyde, paraffin embedded, and 4 µm sections were prepared for H&E staining. **Results:** We have successfully developed a hepatocyte-specific and Kupffer cell-specific NPC1 mouse knockout models. Our preliminary results show that both knockout models have enlarged liver. **Conclusions:** We expect our mouse model provides a valuable tool for studying liver disease in NPC1. **Impact statement:** The creation of this liver-specific knockout model provides a significant contribution to the field of NPC1 research and could lead to improved understanding of the disease, which may ultimately lead to the development of better therapies for patients.

P18 Characterization of Serum Biomarkers of Bone Turnover in Sprague-Dawley Rats

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Abstract

Introduction: Skeletal findings have been observed in nonclinical toxicity studies; however, routine histopathology is often insensitive for their detection and tools such as serum biomarkers and imaging enhance assessment. Bone turnover biomarker concentrations were measured in naïve rats and rats treated with a compound with known bone effects to support bone biomarker application in toxicity studies. **Experimental Design:** Serum, femorotibial joint, and lumbar vertebra were collected from naïve male and female Sprague-Dawley rats (1- to 8-months-old) and from male rats treated with prednisolone for 14 days. **Methods:** Serum N-terminal propeptide of procollagen type I (P1NP), osteocalcin, and C-terminal telopeptides of type I collagen (CTX-1) were measured. Femorotibial joint with distal femur and proximal tibia was decalcified, processed, and stained with hematoxylin and eosin for routine microscopic examination and lumbar vertebra or femur were imaged with micro-computed tomography (CT). **Results:** Biomarker concentrations in naïve rats decreased over time with lowest levels at 8-months-old without evidence of bone mass decreases with routine histopathology; however, imaging showed decreased lumbar vertebral trabecular number in older animals. Decreases in all biomarkers were observed in prednisolone-treated rats; however, there was no change in femoral bone mass with routine histopathology or CT. **Conclusion:** Serum biomarkers provide a sensitive tool for monitoring changes in bone mass even after short steroid treatment without changes detected with routine histopathology or imaging. **Impact Statement:** These data enhance understanding of bone biomarker changes over the normal course of aging in rats and support their use in routine nonclinical toxicity studies.



P19 Preclinical Assessment of a MUC12-Targeted BiTE® (Bispecific T-cell Engager) Molecule

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Abstract

MUC12 is a transmembrane mucin that is highly expressed in >50% of primary and metastatic colorectal tumors. MUC12 is also expressed by normal epithelial cells of the colon and small intestine. Although MUC12 localization in normal epithelial cells is restricted to the apical membrane, expression in tumors is depolarized and shows broad membrane localization. The differential localization of MUC12 in tumor cells as compared with normal cells makes it a potential therapeutic target. Here, we evaluated targeting of MUC12 with a BiTE® (bispecific T-cell engager) molecule. We generated a panel of proof-of-concept half-life extended (HLE) BiTE molecules that bind MUC12 on tumor cells and CD3 on T cells. We prioritized one molecule based on *in vitro* activity for further characterization *in vivo*. *In vitro*, the MUC12 HLE BiTE molecule mediated T-cell-redirected lysis of MUC12-expressing cells with half-maximal lysis of 4.4 ± 0.9 to 117 ± 78 pmol/L. In an exploratory cynomolgus monkey toxicology study, the MUC12 HLE BiTE molecule administered at 200 µg/kg with a step dose to 1,000 µg/kg was tolerated with minimal clinical observations. However, higher doses were not tolerated, and there was evidence of damage in the gastrointestinal tract, suggesting dose levels projected to be required for antitumor activity may be associated with on-target toxicity. Together, these data demonstrate that the apically restricted expression of MUC12 in normal tissues is accessible to BiTE molecule target engagement and highlight the difficult challenge of identifying tumor-selective antigens for solid tumor T-cell engagers.

P20 Lymphohistiocytic Steatitis in Research Cynomolgus Macaques with Chagas Disease—A Novel Chagas Finding?

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Abstract

Chagas disease, caused by the hemoflagellate protozoan *Trypanosoma cruzi*, causes a fatal myocarditis in humans, non-human primates, and other mammals. The disease is endemic to the Southern United States and Latin America, where the arthropod vectors of the protozoa are naturally found. Non-human primates serve as an important experimental model for studying the disease in humans, and inadvertent exposure to the protozoa can have significant negative consequences for both primate health and biomedical/toxicology research. This case series highlights a group of Cynomolgus research monkeys that were intentionally orally infected with Chagas disease between February and June 2019 at the Keeling Center for Comparative Medicine and Research at The University of Texas MD Anderson Cancer Center. After starting this study, most of the animals developed an active co-infection with Simian Varicella Virus, an alphaherpesvirus, during a small outbreak of the virus on campus. On postmortem examination, in addition to the expected Chagas-related findings of pancarditis and skeletal myositis, all animals also developed a lymphohistiocytic perivascular and interstitial steatitis (not previously reported in Chagas cases in primates) affecting various fat deposits throughout the body, most commonly the abdominal, subcutaneous, and peri-aortic, and pericardial/peri-thymic fat. Immunohistochemistry confirmed this inflammatory cell population consisted predominantly of CD3+ T cells, with fewer CD68+ macrophages, and small numbers of CD20+ B lymphocytes. As the animals in this study group who did not have SVV also developed this steatitis, we propose this is a novel Chagas related finding in Cynomolgus monkeys, unrelated to the SVV co-infection.

P21 Characterization of Phase Formation in Uterine Fibroid Spheroids Using Three-Dimensional (3D) Co-Cultures of Human Uterine Leiomyoma Cells and Myofibroblasts

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Abstract

Introduction: Uterine leiomyomas (fibroids) are the most common benign tumors of the uterus in reproductive-aged women. Leiomyomas consist of smooth muscle cells, myofibroblasts, and extracellular matrix (ECM). Four phases (1-4) have been described in fibroids in women based on collagen content (0, <10%, 10-50%, >50%). **Experimental Design:** Ht-UtLM cells and myofibroblasts were seeded at varying ratios to create 3D spheroids to recapitulate each fibroid phase using ultra-low attachment 96-well plates. **Methods:** Spheroids were collected at day 7, fixed with 10% formalin, and stained with hematoxylin and eosin (H&E). Caspase 3, collagen I and III, and F-actin expression was evaluated by immunofluorescence and confocal microscopy. A receptor tyrosine kinase (RTK) array was used to assess growth factor receptor activation. Positive-stained cells were analyzed using a Multiwavelength Cell Scoring application (MetaMorph Imaging software). **Results:** H&E and F-actin staining showed 3D spheroids with cells that had spindled morphology. Caspase 3 and collagen III were increased, and collagen I expression was decreased in phases 2-4 compared to phase 1 spheroids. EGFR, FGFR3, EphB3 and RYK were differently expressed between phases. **Conclusions:** The 3D spheroid phases (1-4) were morphologically similar to fibroid tumor phases observed in women. FGFR3 activation, collagen III content, and fibrosis were all increased with phase progression. **Impact Statement:** Our 3D co-culture uterine fibroid phase model is a novel *in vitro* system for studying the molecular mechanisms of the life cycle of the fibroid myocyte, and for identifying environmental hazards or translational intervention and prevention strategies for fibroids in women.

P22 KNTP Technical Report on the Toxicology Studies of LAC Color in Sprague-Dawley Rats (Gavage Studies)

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Abstract

Food additives are essential in the food industry and can be classified as natural or synthetic. Natural food additives have a higher customer preference than synthetic ones, and as consumer preference for natural dyes increases, the use of natural colorants also increases. Lac color can be used in processed livestock products to reduce sodium nitrite, and its use is continuously increasing. Herein, the general and genetic toxicity studies were performed to produce safety data on lac color via oral exposure. The approximate lethal dose 50 of lac color to SD rats was > 5,000 mg/15 mL/kg bw. A 90-day repeated oral toxicity was performed using lac color. The test animals (SD rats) were administered 0, 50, 100, 200, and 500 mg/10 mL/kg bw for 90 days. Significant changes were observed in several endpoints, but these were considered sporadic or natural because the incidences were within the historical control range. Lac color was negative in the bacterial reverse mutation assay at 5,000 µg/plate or less concentration. Lac color was negative for the *in vitro* chromosomal aberration test (CHL cells) under the following conditions: 1,500 µg/mL 6 h treatment without S-9 mix, 500 µg/mL 24 h treatment without S-9 mix, and 2,500 µg/mL 6 h treatment with S-9 mix. Lac color was negative for the *in vivo* micronucleus test at doses of 2,000 mg/kg bw/day or less. In conclusion, the NOAEL of lac color in a 90-day repeated oral toxicity study was 500 mg/10mL/kg bw/day.



P23 KNTF Technical Report on the Toxicology Studies of Hexadecylpyridinium Chloride Monohydrate in Sprague-Dawley Rats (Gavage Studies)

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Abstract

With the development of technology, living in a chemical-free environment is now considered an insurmountable challenge. Considering several chemicals, exposure to household chemicals such as cetylpyridinium chloride (CPC), used in wet wipes and mouthwashes, is continuously increasing, but safety data for these remain scarce. Therefore, the present study examined the toxicity of CPC in rodents following oral exposure route and provided information regarding the risk assessment of CPC for household application. For hazard evaluation of CPC, studies, including acute oral toxicity, dose-range finding (DRF), 90-day repeated oral toxicity study, genetic toxicology study, bacterial reverse mutation test, *in vitro* mammalian chromosomal aberration test, *in vivo* micronucleus test using mice, pharmacokinetics study, and toxicokinetics, were performed. An acute oral toxicity study showed treatment-related deaths, which can be classified as GHS category 4. The subchronic repeated oral toxicity study showed that females treated with CPC at 72 mg/kg/day displayed histological lesions in the forestomach. CPC was negative for the bacterial reverse mutation tests, *in vitro* mammalian chromosomal aberrations, and mouse *in vivo* micronucleus test. In conclusion, the no observed adverse effect levels (NOAELs) in males and females were 15 and 24 mg/kg/day, respectively.

P24 Histopathological and Immunohistochemical Characterization of Renal Pathology in a Mouse Model of APOL1-Mediated Kidney Disease

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Abstract

Introduction: Apolipoprotein L1 (APOL1)-mediated kidney disease (AMKD) is a severe, debilitating, chronic kidney disease, caused by gain of function variants in the APOL1 gene and is a primary cause of end-stage kidney disease (ESKD) in individuals of sub-Saharan African descent. In the United States, approximately 13% of African Americans (~5 million individuals) are homozygous for APOL1 risk alleles; and a significant fraction of them will develop AMKD. Currently, there are no approved therapeutics treating the underlying cause of AMKD. APOL1 expression is limited to humans, baboons, and gorillas and there is no universally accepted animal model to study AMKD. Here we present a detailed histopathologic and immunohistochemical characterization of renal findings from a 14-day study using a transgenic homozygous mouse model expressing the human APOL1 G2 risk variant gene. This mouse model recapitulates key histopathological findings consistent with AMKD in humans and provides insight into the progression of the disease.

Materials and Methods: Transgenic mice on FVB background harboring a single copy of the G2 allele of human APOL1 gene were generated and bred to homozygosity. A CpG-free plasmid encoding for murine interferon-gamma (IFN γ) was introduced into these mice via hydrodynamic tail vein injection. Body weight, proteinuria (UACR), and serum IFN γ levels were measured at routine intervals. Mice were euthanized on each day starting from 6 through 14. Formalin-fixed kidney sections were analyzed for morphological changes using H&E, special stains (PAS), and immunohistochemistry to assess nephrin protein expression, an integral component of glomerular filtration barrier.

Results: The non-treated transgenic mice (control group, no IFN γ plasmid) did not develop proteinuria and had no histopathological changes in the kidneys. Transgenic mice treated with IFN γ plasmids developed severe and progressive proteinuria starting from day 5. Minimal to mild-grade tubular dilatation and protein casts were present in animals starting from day 8. Minimal to moderate grade glomerular pathology characterized by up to 60 % of glomeruli with collapsed/indistinct capillary lumina, mesangial hyalinization with increased mesangial matrix (PAS+ve), vacuolation of parietal epithelial cells (PEC), and vacuolated cells in Bowman's space were noted from day 10. The histopathology findings were consistent with a primary glomerular injury leading to tubular findings (dilatation and protein casts). Nephrin immunohistochemical labeling showed a decrease of nephrin protein expression in the IFN γ treated animals compared to the control animals. Decreased nephrin expression is consistent with the known pathogenesis of APOL1-mediated glomerulopathies in which there is a direct APOL1-mediated damage to the podocytes resulting in effacement of podocyte foot processes and loss of proteins involved in integrity of glomerular filtration barrier.

Conclusion: Results from this study indicate that transgenic mice expressing a single copy of human APOL1 risk variant (G2) treated with IFN γ exhibited progressive proteinuria (starting from day 5 post-treatment) and developed glomerulopathy starting from day 10 post-treatment. Together, the genotype and the phenotype (proteinuria, glomerulopathy, and a decrease in nephrin expression) of this animal model recapitulate key clinical and morphologic features associated with different manifestations of human AMKD.

Impact statement: Here we present the characterization and morphologic findings using a transgenic homozygous mouse model expressing a risk variant of the human APOL1 gene. Our findings recapitulate key in-life and pathological aspects of human AMKD and provide a detailed insight into the progression of glomerulopathy in this disease.

P25 Hyaline Arteriosclerosis in a Research Colony of Hemophilic Mixed Breed Dogs (*Canis familiaris*) Treated with Conventional and AAV Gene Therapies

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Abstract

Hyaline arteriosclerosis is associated with disease states in humans such as diabetes and hypertension and is a degenerative and/or proliferative lesions which results in decreased arterial elasticity. It is characterized by the accumulation of mucopolysaccharides and serum proteins, including immunoglobulins and complement within the tunica media and intima of arteries. Chronic inflammation likely contributes to this lesion. This retrospective study assessed the reported nature and tissue distribution of hyaline arteriosclerosis diagnosed or described in tissues collected between 2004 and 2022 from 32 mixed breed dogs (27 with coagulopathy, 5 without coagulopathy) housed at the University of North Carolina, Chapel Hill. The dogs with coagulation disorders have unique clinical histories and received different conventional therapies including blood transfusions and/or AAV gene therapies. Tissues were evaluated using routine H&E and histochemical stains (PAS, anti-C3) to both characterize the lesions and their distribution. The heart was the most frequently affected tissue in each group, however more tissue types were identified in dogs with coagulation disorders (5 tissue types, heart, liver, lungs, kidney, subcutis, in dogs with coagulopathy versus 2 tissue types, heart and liver, in dogs without coagulopathy) and 6/27 dogs with coagulopathy had arterial changes in multiple tissues. Hyaline arteriosclerosis was identified in a wider age range in dogs with coagulation disorders (4.5-14 years versus 11-14 years).

P26 Effect of NADPH Oxidase Inhibition in Amebic Liver Abscess Development in a Susceptible Model

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Abstract

Amebiasis is an intestinal infection caused by *Entamoeba histolytica* (*E.h*) that affects millions of people in developing countries. Amoebic liver abscess (ALA) is a frequent complication of amebiasis. Neutrophils (NPs) are the first cells to come into contact with *E.h* in the liver parenchyma. In addition, NPs produce and release reactive oxygen species (ROS) with NADPH oxidase (NOX2) being an ROS-producing oxidase enzyme. There is no information on the presence and role of NOX2 in the evolution of ALA. Objective: The aim of this work was to analyze *in vivo* the role of NOX2 in a susceptible ALA model. Methodology: Male hamsters were distributed into two groups: 1) inoculated with amebas 2) inoculated with amebas and treated with apocynin (AP) a NOX2 inhibitor. Animals were sacrificed at 3, 6 and 12 h post-infection. In samples of ALA we determined: 1) the percentage of lesion, the morphologic changes during ALA evolution and quantification of number of *E.h*. in ALA. Results. A significant difference between the two groups in the percentage of lesions were determined, also morphologic differences were observed between both groups during the evolution of ALA. Finally, hamsters treated with AP showed significant reduction in the number of *E.h* compared with the untreated hamsters. Conclusion: Our results showed that during the pathogenesis of ALA, the absence of NOX2 favors the resolution of ALA probably due to the decrease in ROS production. This work was supported by SAPP-IPN, Proyecto Multidisciplinario Módulo 20230986, 20230979, 20230994 and 20230927



P27 **INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions—An Update—2023**

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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 or 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 have been published in Toxicologic Pathology in 2021 and Non-human primate and Rabbit have been published in the Journal of Toxicologic Pathology in 2021. Fish and Non-rodent ocular toxicity group are targeted to have a manuscript for review in 2023. INHAND guides offer terminology, diagnostic criteria, differential diagnoses, images, and guidelines for recording lesions in toxicity and carcinogenicity studies. INHAND GESC representatives work with representatives of FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) 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.

P28 **Impacts of Pre-Existing Immunity on Histopathology Findings Following Single Intrathecal Injection of scAAV9-CBA-mCherry in Cynomolgus Monkeys**

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Abstract

Adeno-associated virus (AAV) is used as a vector for gene therapy and delivering target DNA to patients' targeted cells. One of the challenges for the AAV gene therapy field is pre-existing immunity to AAV in patients, which can neutralize AAV viral particles before target cell transduction can occur. Although AAVs are not pathogenic, there are no or little reports describing the impact of pre-existing immunity on pathology findings, some possible examples including cellular/humoral immune responses or reduced pathology findings due to altered AAV bio-distribution and/or transductions. The purpose of this presentation is to describe how different levels of pre-existing immunity affect histopathology findings after a single intrathecal (IT) administration, in the lumbar region of the spinal cord, of scAAV9-CBA-mCherry in female cynomolgus monkeys followed by 4-week observation period.

ScAAV9-CBA-mCherry-related microscopic findings were observed in the brain, IT injection site, spinal cord, cauda equina/spinal nerve roots, dorsal root ganglion and heart. While observed test article-related findings were mostly minimal to mild in severity with high individual variabilities, there was trends toward increased severity of mononuclear cell infiltration in the meninge and/or perivascular region in the neuropil of the brain in animals with pre-study AAV9 titer >1:800 when compared with animals with AAV9 titer <1:50. These changes were not accompanied by any detrimental effects in the vascular or peri-vascular cells, including inflammatory, degenerative, or necrotic changes.

P29 Use of Recovery Animals in Nonclinical Monoclonal Antibody Development: Continuing Opportunities for Optimization

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Abstract

Introduction: Assessment of reversibility from nonclinical toxicity findings with potential adverse clinical impact is required during pharmaceutical development, but there is flexibility around how (e.g., are recovery animals necessary?) and when this is performed. For monoclonal antibodies (mAbs) and in accordance with ICH S6(R1) if inclusion of recovery animals is warranted, this need only occur in one study. **Experimental Design:** Data on study designs for first-in-human and later-development toxicity studies were shared from a recent collaboration between the NC3Rs, EPAA, Netherlands Medicines Evaluation Board (MEB) and 14 pharmaceutical companies where 11 companies submitted anonymized data by survey. MAbs with at least one study started in 2015 or later were used in this analysis; there were 52 mAbs with 84 nonrodent (NR, 99% nonhuman primate, 1% minipig) and 11 rodent studies in total. **Results:** Recovery animals were included in 68% of FIH-enabling and 69% of later-development studies, and 52% included recovery groups in studies from both. Only 8 mAbs used two species within packages, and 4 included recovery groups in studies for both species and both FIH-enabling and later-development studies. Recovery groups were commonly in control plus one test article-dosed group or in all dose groups (45% of studies, each design). **Conclusion:** These results suggest more recovery animals may have been used than were scientifically necessary. **Impact:** Use of existing knowledge, limiting inclusion of recovery to a single nonclinical toxicity study and species when appropriate, and study design optimization provide opportunities to further reduce animal use within mAb development programs.

P30 Re-Evaluating the Need for Chronic Toxicity Studies with Therapeutic Monoclonal Antibodies, Using a Weight of Evidence Approach

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Abstract

To support registration of monoclonal antibodies (mAbs) for chronic indications, 6-month toxicity studies have historically been conducted per ICH S6(R1) guidance. Experience with nonclinical mAb development has shown a relatively benign and well-understood safety profile for this class, with many toxicity findings anticipated based on pharmacology. Under an EPAA supported project, a consortium of 14 pharmaceutical companies, the Medicines Evaluation Board (Netherlands), and the NC3Rs conducted a study to evaluate whether a 6-month toxicity study duration is still necessary to assess the long-term safety of mAbs. Data on shorter-term, subchronic, and chronic nonclinical toxicity studies were shared for 142 mAb programs submitted by 11 companies; 111 met criteria for evaluation. For 71.2%, either no toxicities or no new toxicities were noted in chronic studies compared with shorter-term studies. New toxicities not considered of concern for human safety (e.g., related to exaggerated pharmacology or immunogenicity) were identified in 15.3%. New toxicities of potential concern for human safety, or that changed clinical trial design, were identified in 13.5% with 7% being considered critical and 2% leading to program termination. An iterative, weight-of-evidence model which considers factors that influence the overall risk for a mAb to cause toxicity was developed to drive selection of the optimal duration of the longer-term toxicity study(ies) without necessarily defaulting to a duration of 6 months. This model enables an evidence-based justification of study duration, suggesting when 3-month toxicity studies are likely sufficient to support late-stage clinical development and registration for some mAbs.



P31 Challenges and Opportunities in Use of Minipigs for Nonclinical Pharmaceutical Development: Results of the Second IQ DruSafe Minipig Survey

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Abstract

Minipigs represent nonrodent species/strains infrequently utilized for nonclinical pharmaceutical development relative to dogs or monkeys. A 2014 IQ DruSafe survey indicated minipig use was largely limited to studies evaluating small molecule dermal products. In 2022, IQ DruSafe conducted another survey of member companies. An incremental increase in minipig use, primarily in protein and oral small molecule development, was noted. Despite this increased use, minipigs still represent a relatively small percentage (generally $\leq 5\%$) of all nonrodents used in pharmaceutical development. Based on survey responses, key challenges to wider use are: (i) a limited number of efficacy models compared with other species; (ii) a lack of/limited historical control data; (iii) a lack of relevant reagents to assess crossreactivity, pharmacology, or toxicity which leads to inconsistent use of minipig for assessing pharmacologic activity and immunogenicity risk of biologics; and (iv) a larger test article requirement for *in vivo* studies. Companies also expressed concerns regarding inhouse capabilities, training, and handling of minipigs as well as uncertainties regarding capabilities and experience at CROs. The EU and UKbased member companies noted an increased consideration of minipigs due to monkey shortages as well as ethical concerns related to monkey and dog use, but this has not translated to more routine minipig use. The results of this 2022 IQ DruSafe survey indicate that many of the concerns previously identified in 2014 persist. Focused, ongoing, industry-wide efforts to address these challenges may assist in more frequent consideration of minipigs for nonclinical pharmaceutical development.

P32 Subacute and Subchronic Repeated Inhalation Toxicity of Acetyl Acetone in F344 Rats

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Abstract

Introduction/Objectives: Acetylacetone is used in several industries as coating agent and solvent/stabilizer. This is also used in the laboratory to measure formaldehyde levels in water and air. However, despite its versatile uses, reported toxicity data is limited and insufficient to make toxicity profile. Here, we performed 4- and 13-week repeated toxicity studies using F344 rats to examine its toxicity. **Experimental design/Materials and Methods:** The male and female F344 rats were exposed to 0, 200, 400, and 800 ppm for 4-week study and 0, 100, 200, 400 ppm for 13-week study via whole-body chamber. Clinical sign, food consumption, body weight, bronchoalveolar lavage, hematology, serum chemistry, necropsy, organ weight, histopathology were examined. **Results:** All male and female rats exposed to 800 ppm in 4-week study found dead or moribund during exposure period. These were considered to be acetylacetone-related. Food consumption and body weights were decreased in both of two studies. Histopathologically, transitional/ mucous cell hyperplasia and squamous/transitional cell metaplasia in the nasal cavity, parakeratosis in the larynx were noted in 4-week and/or 13-week studies. Additionally, changes of HGB, MCV, MCH, reticulocytes, and WBC were observed in 13-week study. **Conclusion:** Acetylacetone-related changes were noted in food consumption, body weight, and histopathology of 4-week and 13-week studies and in hematology of 13-week study. **Impact Statement:** This is the first case to reveal subchronic toxicity of acetylacetone. Based upon our result, acetylacetone induced damage in upper respiratory tract but not in others including the lung.

P33 NADPH Oxidase Inhibition in Amebic Liver Abscess in a Resistant Model

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Abstract

Entamoeba histolytica (E.h.) is the causal agent of amebiasis, which cause amebic liver abscess (ALA). The BALB/c mouse (model of resistance). Neutrophils interact with E.h. The enzymes in neutrophils are myeloperoxidase (MPO), NADPH oxidase (NOX2) and superoxide dismutase (SOD). There is no information about the enzyme NOX2 in ALA. Objective. To analyze the role of the NOX2 in the resolution of ALA in BALB/c mice using a NOX2 inhibitor. Methodology. Male BALB/c were divided in ameba-inoculated animals (CT), ameba-inoculated animals treated with apocynin (E.h.A) and NOX2 inhibitor (0.41 mg/25 g). The animals were euthanized at 3, 6 and 12 h post-infection. In ALA samples it was determined the percentage of lesion, histological changes in ALA and the number of amebas. Results. In the E.h.A group a significant increase in the percentage of lesion compared with the CT mice at 3 and 6 h of ALA development was observed. Also, in the group E.h. A with apocynin, the presence of amebas with absence of inflammatory cells at 3 h of ALA evolution was observed, at 6 h small inflammatory foci, hepatocytes damaged and some amebas in vessels was shown. Amebas, inflammatory foci and ischemia was observed at 12 h in the ALA. Moreover, the number of amebas without apparent damage increased in E.h.A. Conclusions. The results show that the NOX2 is important during the immune response of the BALB/c at early times to eliminates E. h. Thanks to Proyecto Multidisciplinario Módulos: 20221473, 20220507, 20221514 y 20221426.

P34 Utilization of Virtual Necropsy Supervision in the Age of Remote Work

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Abstract

The COVID-19 pandemic has led to a rapid and widespread adoption of remote work for toxicologic pathology (digital peer review), and this trend is expected to continue in the post-pandemic era. Necropsy supervision historically has required a pathologist to be physically present in the necropsy room with highly trained technical staff. It is uncommon for the technical staff to have questions during necropsy and the utilization of virtual necropsy supervision could provide flexibility and efficiency for the toxicologic pathologist. At the Spencerville site, we evaluated the feasibility and effectiveness of virtual necropsy supervision. Virtual supervision was conducted using a Microsoft Surface tablet equipped with a high-definition camera affixed to a mobile tripod and Microsoft Teams software. Still images, recording of any kind, and display of faces and names were not allowed. Technical staff were trained, and the procedure was piloted with on-site and remote pathologists. We found that virtual necropsy supervision was effective in ensuring accurate diagnoses, with no significant differences observed between the in-person and virtual supervision. Moreover, the use of virtual supervision reduced the need for in-person presence and unnecessary utilization of personal protective equipment (PPE). Some limitations included occasional connectivity issues, suboptimal lighting, and difficulty in controlling the motion of the camera. Overall, virtual necropsy supervision utilizing a tablet and Microsoft Teams has the potential to be a valuable tool in the field of toxicologic pathology, particularly in the context of remote work post COVID-19 pandemic, allowing for increased geographic flexibility for pathologists and reduction in PPE use.



P35 Lower Histological Severity of Nerve Fiber Degeneration (NFD) in Three-Month Nonclinical Studies of Intrathecally (IT) Administered Antisense Oligonucleotides (ASOs) Is Interpreted as Non-Adverse Within the Study Context

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Abstract

Introduction: Like other drugs, assessment of ASO adversity and the estimation of the no observed adverse effect level (NOAEL) are critical steps in product development. Nerve fiber degeneration (NFD) is an emerging ASO class effect that has been observed in pharmaceutical industry following intrathecal (IT) administration. However, standardized histopathology grading scheme and adversity assessment criteria are lacking.

Objective and Experimental Design: Two pathology working groups for 2 separate ASOs devised a grading scheme and performed a semi-quantitative blinded evaluation of selected spinal cord segments, spinal nerve roots, dorsal root ganglia (DRG), and nerves in non-human primates (NHPs) and rats following repeat-dose IT administration of both ASOs (one for each species), each administered monthly for up to 3 months.

Results: Neurological signs attributable to NFD did not develop as assessed by standardized neurological examinations. Histologically, NFD was observed in treated and some control animals and was characterized by myelin sheath dilation with axonal degeneration, increased Schwann cells, and reactive microgliosis in spinal nerve roots (predominantly near the injection site) and the spinal cord dorsal funiculus with sparing of DRG neuronal cell bodies. These changes were of minimal or mild severity and unrelated to dose. Infiltrates of mononuclear cells, unrelated to dose, were also observed mostly at the injection site.

Conclusion and Impact Statement: NFD was mostly centered at or near the injection site supporting injection-related microtrauma as a contributory factor. It was interpreted as non-adverse in the context of these studies since it did not cause substantial disruption of organ integrity.

P36 *Entamoeba histolytica*: Effect of the Riluzole in the Resolution of the Amoebic Liver Abscess

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Abstract

Introduction: Amoebiasis is produced by the parasite *Entamoeba histolytica*; this disease affects millions of people throughout the world who may suffer from amoebic colitis or amoebic liver abscess (ALA). Metronidazole is currently used to treat this protozoan. However, metronidazole can reportedly have different adverse effects, such as mutagenic and carcinogenic effects in animals. Therefore, it is necessary to test new anti-amoebic drugs to prevent these effects. There are some studies that show that riluzole has activity against some protozoan parasites. Thus, the aim of the present study was to evaluate the efficacy of the riluzole in the resolution of ALA. **Methodology:** Hamsters (*Mesocricetus auratus*) were inoculated intrahepatically with 1×10^6 trophozoites of *E. histolytica* to produce ALA. After 3 days post-infection, the riluzole (5 mg/100 g) was administered intraperitoneally every 12 h for 15 days. Control group did not receive any treatment. After finishing the treatments, the animals were sacrificed; the livers were removed and weighed to determine the percentage of liver damage and histopathological changes were analyzed by hematoxylin and eosin staining. **Results:** The hamsters untreated presented 70% of liver lesion and showed granulomas with extensive necrosis and numerous amoebae. Animals treated with riluzole resolved the ALA, revealed the retraction of granulomas, presence of different types of cells that participate in the regeneration of the liver and the absence of amoebae. **Conclusion:** Our results suggest that riluzole could be an alternative treatment in the resolution of ALA. *This work was supported by SAPP-IPN, Proyecto Multidisciplinario Módulos 20230986, 20230979, 20230994 and 20230927.*

P37 Comparison of Fasted and Nonfasted Hematologic, Coagulation, and Biochemical Parameters in Rats and Nonhuman Primates

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Abstract

Background: The effect of nonfasting on clinical pathology parameters in toxicology studies has been incompletely characterized.

Objectives: To determine and characterize differences in hematology, coagulation, and biochemical parameters between healthy fasted and nonfasted rats and macaques.

Methods: Adult Sprague-Dawley rats (40 per sex) and cynomolgus macaques (10 per sex) were sampled for hematologic, coagulation, and biochemical parameter assessment after approximately 8 hours of food restriction and after unrestricted food access. Nonfasted results were compared to fasted results.

Results: Rats: noteworthy changes consisted of minimally lower reticulocyte (0.89x) and neutrophil (0.61x) counts in males only. Minimally to mildly higher alanine aminotransferase (1.24x-1.80x), aspartate aminotransferase (1.14x-1.26x), and alkaline phosphatase (1.56x-2.46x) activities, glucose (1.09x-1.19x), triglyceride (2.42x-2.55x), cholesterol (1.14x-1.17x), and globulin (1.10x-1.17x) concentrations were observed with minimally lower urea nitrogen (0.88x-0.91x), albumin (0.95x-0.96x), and phosphorus (0.90x-0.93x) concentrations in nonfasted animals. The incidences of serum lipemia (5% versus 0%) and hemolysis (7.5% versus 1.25%) were highest in nonfasted rats. Nonfasting of rats did not affect coagulation parameters. Macaques: noteworthy changes were minimally higher triglyceride (1.27x-1.70x) and minimally lower phosphorus (0.82x-0.95x) and total bilirubin (0.71x-0.84x) concentrations. The incidences of serum lipemia (0% versus 5%) and hemolysis (0% versus 15%) were highest in fasted macaques. Nonfasting of macaques did not affect hematologic or coagulation parameters.

Conclusions/Impact Statement: Nonfasting has a minimal effect on select hematologic and biochemical parameters in rats and biochemical parameters in macaques. Interpretation of standard clinical pathology data should not be affected in nonfasted studies that include appropriate control and/or baseline data.

P38 Recommendations for GLP-Conform Archiving of Whole Slide Images

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Abstract

In recent years, working groups of toxicologic pathology societies (ESTP, STP), consortium-driven interactions with regulatory authorities and efforts made by individual institutions have helped to develop a general understanding of elements required for the GLP-conform use of digital pathology. To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by GLP authorities, generating little feedback to build upon.

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation.

Archiving of whole slide images (WSI) must be considered in the context of raw data generation. If archival of WSI is required (primary evaluation and retrospective peer review), in addition to the archival of the glass slides, the processes to retain original electronic data generated by the computerized system as well as procedures to assure data integrity need to be defined according to the respective OECD documents. The group recommends using DICOM format for back compatibility reasons. In addition, processes such as archival log, duration of archiving as well as recovery and disaster recovery need to be defined. Finally, the decision of using either cloud solution or physical address of the data center for archiving purposes needs to be based on a suitable risk assessment.



P39 **In Vitro Application for Accessing Oxidative Stress to the Respiratory Tract by Occupational Chemicals**

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Abstract

Introduction: Respiratory damage caused by industrial chemicals is well known to cause occupational diseases. Among them, occupational asthma is difficult to judge, and various tools for assisting diagnosis are being studied. Recently, studies have been conducted to explore the mechanism for disease by applying Adverse Outcome Pathway (AOP). Through this study, we identify oxidative factors related to respiratory damage in alternative methods.

Methods and Materials: The scientific literatures on occupational asthma has been reviewed. Furthermore, AOPs, including Key events (KEs) and associated chemicals related to respiratory disorders were investigated. In our methods, we used 3D-cell culture models that were well-constructed with human respiratory cells (Mucilair™ or Smallair™, Epithelix). The factors of oxidative damage and the changes in respiratory epithelium induced by industrial chemicals were studied.

Results: Chemicals that cause occupational asthma, including isocyanates, have been identified, and AOPs associated with asthma were collected through AOP wiki. The level of LDH in each 3D-cell model was increased with the dose of chemicals. Also we identified the KEs related with oxidative stress by evaluating the increase in ROS/RNS level and morphological changes with mucus secretion of each 3D-cell model following exposure to chemicals.

Conclusion: We investigated the respiratory cell damage related with oxidative stress induced by industrial chemicals based on an AOP. However, the next phase of KEs such as cytokines or chemokines need to be considered with an additional experimental evidence.

Impact Statement: Key events related to occupational asthma were experimentally suggested using human respiratory 3D-cell model.

P40 **Recommendations for the Qualification of Instruments in Digital Pathology**

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Abstract

In recent years, working groups of toxicologic pathology societies (ESTP, STP), consortium-driven direct interactions with regulatory authorities and efforts made by individual institutions have helped to develop a general understanding of elements required for the GLP-conform use of digital pathology for peer reviews and primary reads. To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation in common use cases.

The group recommends that instrument qualification of scanners and pathologist workstations be based on a set of predetermined specifications by both the vendor (installation and operational qualification) and end-user requirement specifications (user acceptance testing).

In addition, spatial and color calibration of scanners (calibrating displayed lengths against known values) are discussed. The group felt that the monitor and the viewing environment are a point of concern. This can be addressed via a QC slide, "point of use QA" approaches, color calibration, and/or via equivalency studies. Options and open questions are discussed.

P41 Immunohistochemistry-Free Enhanced Histopathology of the Rat Spleen Using Deep Learning

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Abstract

Enhanced histopathologic evaluation of lymphoid organs involves microscopic examination with semiquantitative descriptions of changes in subanatomic compartments within the tissues. Challenges in both accurate lymphoid compartment recognition and cellularity estimation render the process inherently prone to interobserver variability. Immunohistochemistry is required for definitively differentiating T and B cell compartments; however, routine toxicologic assessments are based solely on H&E slides. We hypothesized that a deep learning (DL) model would accurately segment and quantify lymphoid compartments in the spleen from H&E slides, enabling rapid quantitative morphological assessment. A DL model was developed using naïve and vehicle control Sprague-Dawley rats to quantify area and cellularity of PALS, follicles, germinal centers, marginal zone, and red pulp from H&E slides. The ground truth for training and validation was obtained from pathologist-guided annotations of H&E slides with co-registered, dually CD3/CD79a immunostained slides. The model input was limited to H&E-stained slides, and it quantitatively characterized the range of variability found in these normal rat spleens. Performance of the segmentation model was evaluated by dice similarity coefficient (DSC). We found that the DL model could identify splenic compartments with high accuracy (overall DSC=93.3%) directly from the H&E-stained tissue. DSC for follicles was 89.4% and DSC for PALS was 91.8%, compared to pathologists' annotation of H&E slides with DSC of 75.8% and 77.6%, respectively. This level of model performance in abnormal spleens would support implementation of this DL algorithm in nonclinical toxicity studies to assist pathologists for gains in efficiency and accuracy.

P42 Deep Learning Anomaly Detection Method Applications in Toxicologic Pathology

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Abstract

The field of toxicologic pathology is poised to leverage the advancements in artificial intelligence (AI), specifically Deep Learning (DL), to transform pathology practice within the discipline. AI/DL enabled computer assisted diagnosis is emerging as a significant advancement in medical practices such as Medical Imaging, while in the field of pathology, similar approaches are being extensively researched and explored. However current real-world applications of these methods in pathology are more nascent, in part due to the complexity of the image data of whole slide images (WSI) as compared to radiographic images. Our team has taken a multipronged approach to develop DL algorithms that can highlight and, in some instances, classify abnormal tissue regions within standard toxicologic pathology WSI in rats. We have used anomaly detection approaches to identify areas of tissue with a high probability of being not-normal, and weakly-supervised multiple instance learning approaches to identify and classify lesions. These methods hold long-term promise to enable tissue-triage strategies to sort slides with abnormalities from those without and have immediate applications into current workflows to suggest regions with high probability of abnormal findings for pathologists' attention. Here we provide an overview of our computational approaches and select examples of their promise for near- and long-term implementation into toxicologic pathology workflows.



P43 Artificial Intelligence-Based Approach for Hepatobiliary Histopathology in Nonclinical Research

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Abstract

Recent applications of artificial intelligence (AI) via deep learning image analysis algorithms are poised to expand the pathologist's toolbox. Deep learning methods include unsupervised information generation to mimic the human brain by using large amounts of training data that fuel algorithms to cluster data and make predictions, allowing for high throughput, objectivity, and improved quantitative accuracy. Algorithms need to be carefully validated, however. We developed an algorithm based on advanced deep learning neural network for quantitative evaluation of portal inflammation and bile duct hyperplasia in Hematoxylin & Eosin-stained mouse liver sections. The algorithm was trained on a limited set of whole slide images (WSIs) scanned at 40X and annotated by a board-certified pathologist. Subsequently, it was tested on two sets of mouse liver WSIs generated in two different laboratories. In parallel, all liver slides (physical or WSIs) were blindly scored by 4 veterinary pathologists using consensus scoring criteria. The algorithm was able to detect and compute the changes with good sensitivity and correlation to the pathologists' scores. Importantly, we observed substantial differences in the algorithm's performance depending on the histopathologic parameter, interlaboratory variation in histologic staining, and slide scanner used. Iterative refinement, testing, and validation of the algorithm are in progress. AI-based histopathology algorithms, developed in close collaboration with pathologists, will be a helpful screening aid to research histopathology workflow. By using AI, many of the tasks that are manual and subjective can become more automated and standardized, with increased workflow efficiency, accuracy, and reproducibility.

P44 Efficiency Study to Evaluate Use of AI-Based Decision Support Tool for Toxicological Pathology

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Abstract

Introduction: An AI-based decision support tool (AI-DST) was developed to aid pathologists in the digital slide review workflow. The tool provides information on the distribution and severity of several common lesions in 5 potential target organs in the rat. The goal of this study was to compare the pathologist's experience in a digital pathology diagnostic workflow with and without AI-based decision support tools.

Methods: Twelve board certified toxicologic pathologists were provided de-identified study material that had the AI-DST classifiers available. A survey was completed by each pathologist to assess the impact of the AI-DST on target organ identification, lesion recognition, and to confirm the value of the AI-DST as a second opinion by confirming or updating the findings and targets reported previously.

Results: All pathologists made changes to their findings based on use of AI. Pathologists found the AI-DST had a positive impact on confirming findings, localising small lesions and subtle changes, identifying abnormal areas, and grading lesions. However, the effectiveness of the AI-DST varied depending on the organ being reviewed due to false positive detection, which could distract the pathologist's review. Pathologist feedback was included for targeted improvements of the tool.

Conclusion: The AI-DST helps the study review workflow to be more efficient and consistent. The tool is designed to be used as a support tool, whereby pathologists use the output similar to when they consult a professional colleague, or literature reference. The improvements identified during the study will be addressed for further generalisability of the AI-DST.

P45 The Division of Translational Toxicology Web-Based Global Toxicologic Pathology Training Program

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Abstract

Trainees in pathology, toxicology, and allied sciences across the globe will benefit from a new web-based Global Toxicologic Pathology Training Program being developed by the National Institute of Environmental Health Sciences Division of Translational Toxicology (DTT). The current model for training in toxicologic pathology relies heavily on on-the-job training. There are few locations that offer toxicologic pathology training to residents/trainees, resulting in a need for a new model of sharing training materials. To meet this need, the Global Toxicologic Pathology Training Program has created a new website-based training program created to share the experience and knowledge of the DTT (formerly known as the Division of the National Toxicology Program) with a broader, more diverse audience. The planned curriculum includes three core areas: basic concepts in toxicologic pathology, organ systems pathology, and test article cases. With a focus towards trainees and allied scientists, the Global Toxicologic Pathology Training Program website aims to increase the understanding and practice of toxicologic pathology worldwide via new educational and training materials. This training resource is free to access at <https://www.niehs.nih.gov/toxpathtraining>.

P46 Recommendations for the Utility of Equivalency and Concordance Studies in Digital Pathology

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Abstract

To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon. An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation in common use cases.

With regards to the utilization of comparative examinations of glass slide versus whole slide image; it was this group's opinion that a blinded concordance study (examination of glass slides and WSI, with an intervening wash-out period, and statistical analysis for concordance of the data generated from each media type) was primarily applicable as a proof of concept and not a prerequisite for GLP-compliant digital peer review or primary read. However, a need for published documentation of concordance studies from the toxicologic pathology industry to further support GLP digital primary read was identified.

The requirement for documented pathologist approval that WSI are fit for purpose (GLP digital peer review and primary read) was, therefore, considered to be met by an equivalency study (non-blinded comparison of glass slides and WSI to qualitatively confirm equivalence based on assessment of critical features and/or lesions).



P47 Prerequisites to the Conduct of a Non-GLP Digital Peer Review on a GLP Study

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Abstract

In recent years, working groups of toxicologic pathology societies, consortium-driven direct interactions with regulatory authorities, and efforts made by individual institutions have helped to develop a general understanding of the elements required for the GLP-conform use of digital pathology.

To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation.

One scenario discussed was not claiming GLP compliance for digital peer review on a GLP study. An in-depth assessment of the existing published regulatory guidance and their requirements was performed. The analysis suggested that not claiming GLP compliance for a digital peer review should be acceptable, as long as the overall integrity of the GLP study is maintained. It was considered this scenario represented a low regulatory risk. The poster summarizes the conclusion of the regulatory review, possible digital workflows with their respective stakeholders, and lists the mandatory and optional, site- and study-specific requirements that need to be in place before, during and after the conduct of the digital peer review. Finally, examples of wording for SOPs, study plans, and study reports are suggested.

P48 Deep Learning-Based Image Analysis Model for Evaluation of Testicular and Epididymal Lesions in Rats

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Abstract

Introduction: Spermatogenic staging and assessment of testicular toxicities in rat tissue sections are time-consuming and require well-trained pathologists. We present a deep learning (DL) based solution to analyze Whole Slide Images (WSIs) of rat tissue sections for detecting testicular and epididymal lesions in toxicology studies.

Materials and Methods: We trained an algorithm to classify the 5 stage groups (I-VI, VII-VIII, IX-XI, XII-XIV or unclassifiable) of spermatogenesis and to detect multiple lesions in WSIs of testis and epididymis in young Sprague Dawley (SD) rats. U-Net based convolutional neural networks and machine learning approaches were used for training the models. The training dataset was prepared from WSIs of testis and epididymis sections, scanned at 40x magnification using a scanner (NanoZoomer-S360). The trained algorithms were validated for several testicular and epididymal findings, including degeneration/necrosis of germ cells, tubular atrophy, tubular dilatation, vacuolation of Sertoli cells, multinucleated giant cells and so on.

Results: The models demonstrated high performance in classifying seminiferous tubules into stage groups and accurately detecting the multiple lesions. The models were validated by comparing the results with histopathological diagnoses made by JSTP-certified pathologists.

Conclusion and Impact Statement: This study suggests that the DL based models can classify seminiferous tubules into groups of spermatogenic stages and detect multiple lesions using the WSI of rat testis and epididymis. The solution could be an important supportive tool for histopathological evaluation, especially for primary testicular screening in early toxicity studies in rats.

P49 Digital Image Analysis (IA) of Cardiac Vasculature in Angiotensin II/ Phenylephrine Mouse Model of Heart Failure Administered Compound X

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Abstract

AngII/PE model of heart failure exhibits major vascular restructuring in capillaries and arteries. Here, we describe analysis of the total capillary and arterial vasculature in an AngII/PE mouse model administered compound X.

Groups with subcutaneously (SQ) implanted AngII/PE minipumps (n=13) were administered 4 weekly SQ injections of compound X, or negative control article, or angiotensin receptor blocker Losartan. Naïve control group was administered saline and did not have the AngII/PE minipump. IA was conducted on cardiac tissue cross-sections stained for α -smooth muscle actin (SMA) and endothelial marker CD31 using Halo software.

As expected, decreased area in capillaries $<500 \mu\text{m}^2$ and increased area/density in arteries $51\text{-}100 \mu\text{m}^2$ were observed in AngII/PE negative control group versus naïve control. A statistically significant increase in total bulk CD31 staining, without change in overall density of $5\text{-}500 \mu\text{m}^2$ capillaries, was observed in the Compound X group. When smaller capillaries were excluded, statistically significant increase was seen in density/area of $51\text{-}500 \mu\text{m}^2$ capillaries over negative control and Losartan. There was no effect of compound X on area/density of muscularized arteries $51\text{-}100 \mu\text{m}^2$ or any other artery sizes. Losartan had no effect on the examined parameters.

This IA approach to analyze the total cardiac microvasculature in the AngII/PE mouse model confirmed the inverse directionality for remodeling in capillaries versus arteries. The total capillary area and area/ density of larger capillaries was increased by Compound X, without an overall change in total capillary density.

P50 Deep Learning-Based Method for Quantification of Mast Cells in Toluidine Blue Stained Tissue Sections of Mouse Skin

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Abstract

Introduction: Mast cells play a significant role in skin immunity and pathogenesis of multiple skin diseases including atopic dermatitis, scleroderma, contact dermatitis, blistering cutaneous disorders and chronic graft versus host disease. A common characteristic across these diseases is increase in mast cells where mast cells undergo degranulation in the affected skin. Accurate quantification of mast cells is an important step in assessing efficacy study models for such skin diseases. We propose a Deep Learning (DL) method for quantification of mast cells in skin sections of mouse stained using toluidine blue.

Materials and Methods: U-Net model with an EfficientNet backbone was trained to identify mast cells in whole slide images (WSI) of mice skin sections. Patches of size 512×512 under $20\times$ magnification extracted from 10 WSI were used for training. A new set of 160 WSI from three different studies were used for evaluating the model performance.

Results: The algorithm for detecting mast cells performed with an IOU and f-score $>90\%$ with reference to ground truths. The results showed a high correlation with the pathologist's reports.

Conclusions: The proposed approach provides an automated method for detecting and quantifying mast cells in toluidine blue stained sections of mice skin.

Impact Statement: The proposed method automates the quantification of mast cells in the entire image thus avoiding selection bias, and improving on the current microscopic method of assessment on selected fields of view from a slide. The method has the potential to be applied in efficacy studies for improved mast cells quantification.



P51 Transcriptomic and Mutation Analysis of Pancreatic Acinar Tumors in Rats Exposed to Perfluorooctanoic Acid (PFOA)

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Abstract

Introduction: Perfluorooctanoic Acid (PFOA) is a legacy contaminant that is persistent and ubiquitous in diverse environmental compartments. It has been detected in surface waters globally, posing risks to human health. Rodent bioassays conducted by the National Toxicology Program to assess the carcinogenic potential of PFOA showed increased neoplastic responses in HSD rat exocrine pancreas and liver tissues. This study aimed to characterize molecular pathways and identify hotspot cancer gene mutations in pancreatic acinar adenomas (PAAs) associated with PFOA exposure.

Methods: Pancreatic tissues from vehicle control and PFOA-exposed (normal and PAA) rats were subjected to microarray profiling and Sanger sequencing for gene expression and mutation signature analyses, respectively.

Results: Compared to the vehicle controls, over 4000 genes were significantly dysregulated ($p_{\text{Valadj}} < 0.05$) in the PAAs. Interestingly, there were no significant alterations in gene expression between normal pancreatic tissues from vehicle controls and PFOA exposures. Ingenuity Pathway analysis on PAAs showed significant perturbations of canonical pathways related to NRF2-mediated oxidative stress response, TGF- β signaling, and IGF-1 signaling, and identified upstream regulators Gcg, Hnf4a and Hnf1a. Mutation analysis revealed hotspot mutations in Ctnnb1 but not in Kras, Hras, and Apc genes. Immunohistochemical staining showed increased nuclear expression of CTNNB1 and HNF-4a in PAA samples.

Conclusion: Molecular analyses revealed perturbations of Wnt/Ctnnb1 and HNF4a pathways in rat PAAs resulting from chronic PFOA exposure.

Impact statement: These data show that PFOA-induced rat PAAs share similar molecular alterations as human pancreatic acinar tumors especially those harboring CTNNB1 mutations.

P52 Application of Special Techniques in Toxicologic Pathology for Tumorigenicity Study of Cell Therapy Products

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Abstract

In the development of biopharmaceuticals such as cell therapy products, the importance of tumorigenicity evaluation has been increasing as test substance-related tumors can occur in unexpected regions in our body. However, it is challenging to determine whether the tumors present in the test substance-treated groups were induced directly by the treatment of test substance. As a way to reduce these difficulties in tumorigenicity study, special techniques in toxicologic pathology such as immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) can be usefully utilized although histopathologic examination using hematoxylin and eosin (H&E)-stained tissues still remains gold standard. In this study, BALB/c nude mice were administered with human colorectal carcinoma (HCT116) on subcutis and induced tumors for 13 weeks. At necropsy, the tumors from injection site, metastatic tumors, and major organs were collected and the evaluation of tumorigenicity in each tissue was performed by H&E staining, IHC, and CISH. In H&E, the tumors were definitely diagnosed by their morphology, but its origin was uncertain. In IHC, tumor cell origin (human) was identified by human-specific anti-mitochondria antibody, but it was difficult to select mouse-specific antibody. In CISH, the tumor cell origin for both human and mice was identified by human- and mouse-specific mRNA probes, and this was confirmed simultaneously in one tissue. However, the evaluation using CISH was relatively time- and cost-consuming works than other methods. Based on these results, special techniques like CISH can be useful for the evaluation of tumor origin in tumorigenicity study.

P53 Mutant IL7R Cooperates with RasGRP1 to Induce T Cell Acute Lymphoblastic Leukemia

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Abstract

Acute lymphocytic leukemia (ALL) is the most common cancer in children; of which, 15% are T-cell ALL (T-ALL). There is a need to develop targeted therapies to lessen side effects. Approximately 10% of T-ALL cases express gain-of-function, mutant IL-7R. Expression of the mutant IL-7R alone is insufficient for leukemogenesis. Sufficiency can be achieved with the addition of a secondary oncogene. Ras guanine nucleotide releasing protein 1 (RasGRP1), an activator of Ras that is overexpressed in 50% of T-ALL cases, was selected as a potential cooperating oncogene. The hypothesis is that the combination of mutant IL-7R and overexpression of RasGRP1 is sufficient for leukemogenesis. The thymocyte-OP9-DL4 co-culture system paired with retroviral transduction was used to test the hypothesis. Thymocytes were harvested from C57BL/6 mice. Retroviral vectors bearing *mutant Il7r-alpha* and *Rasgrp1* were used to transduce thymocytes. Transduced thymocytes were injected into *Rag-/-* mice. Blood, bone marrow, spleen, lymph nodes, and thymus were analyzed by histology and/or flow cytometry at endpoint. Histologically, the lymphoid organs were expanded by large numbers of neoplastic cells, which were CD8+, TCR-beta+, and pre-TCR-alpha-low, akin to thymocytes at the immature single positive (ISP) stage. There was also a TCR-beta-delta+ subpopulation. In conclusion, the combination of mutant IL-7R and overexpression of RasGRP1 in murine thymocytes is sufficient for leukemogenesis, and the neoplastic cells are arrested at the ISP stage with aberrant TCR-beta-delta expression. RasGRP1 is a potential therapeutic target in T-ALL. The mutant IL-7R-RasGRP1 driven leukemia may serve as a model to study the biology of the aberrant TCR-beta-delta.

P54 Poster Withdrawn

P55 Adenosquamous Carcinoma with Sebaceous Gland Differentiation in the Rat Mammary Gland

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Abstract

Introduction: The morphology and hormone receptor pattern of a rare type of adenocarcinoma of the rat was investigated. The two female rats were control animals from a 52 weeks and a 104 weeks carcinogenicity study respectively.

Experimental design: Control animals from carcinogenicity study with Sprague-Dawley [CrI:CD(SD)] and HanBrl:WIST (SPF) rats were investigated to generate historical control data for these strains.

Materials and Methods: Tumor tissue of a female control rat from a 104 weeks carcinogenicity study which died spontaneously after 728 days on study was investigated. Macroscopically, a 15 mm diameter cyst was noted in the right inguinal side of the mammary gland. In another 52 weeks carcinogenicity study a female control rat was sacrificed on Day 372 (terminal kill) on study and a dark mass was observed in the left ventrocaudal side (19 mm in diameter). Hematoxylin-Eosin stained slides and serial sections stained immunohistochemically (rat from 104 w study) with antibodies against estrogen receptor, progesterone receptor and androgen receptor were evaluated.

Results: A malignant mammary gland tumor with features of sebaceous gland differentiation is described with the expression pattern of nuclear hormone receptors in tumor cells. IHC was only performed on the tissue from the rat from the 104 weeks study.

Impact statement: A new diagnosis "Mammary Carcinoma, adenosquamous with sebaceous differentiation" in the rat is proposed to be included in the INHAND nomenclature as this tumor has now been observed in two different rat strains.



P56 Nonproliferative and Proliferative Lesions of the Rat and Mouse Female Reproductive System: New and Revised INHAND Terms for Ovarian Sex Cord Stromal Findings

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Abstract

The International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND) aims to establish a standard diagnostic lexicon with support of toxicologic pathology Societies of the UK (BSTP), Europe (ESTP), Japan (JSTP) and the US (STP). The nomenclature for the female reproductive system was originally published in 2014 (Dixon D. et al. *J Toxicol. Pathol.* 2014; 27 (3&4 Suppl): 1S–107S). In the meantime, challenges arose regarding sex cord stromal proliferative lesions. The organ working group has proposed refinement of these terms. Historically, sex cord stromal proliferative lesions have been separated and diagnosed based on the main cell type present (i.e., granulosa, theca, Sertoli); however, the majority of these lesions are mixed and occur in the context of other age-related changes. This has led to inconsistency in diagnoses, and we are proposing the use of “sex cord stromal” as the base term for hyperplasia and tumors. The default diagnoses for the majority of sex cord stromal lesions would be “sex cord stromal, mixed” but when a clear, predominate cell type is present, modifiers can be used (“sex cord stromal, granulosa cell” or “sex cord stromal, theca cell”). Also, previous terms such as “luteoma” and “granulosa cell tumor” will be replaced with “tumor, sex cord stromal, luteinized” and “tumor, sex cord stromal, granulosa cell,” respectively. In the case of tumors benign or malignant would be applied as appropriate. This simplified approach will provide consistency in diagnoses with a shared base term “sex cord stromal.”

P57 Chronic Toxicity and Carcinogenicity of Alpha-Glycosyl Isoquercitrin in Sprague-Dawley Rats by Dietary Exposure

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Abstract

Introduction: alpha-Glycosyl isoquercitrin (AGIQ), an antioxidative and tumor suppressive food and beverage additive, is currently marketed in Japan.

Experimental design: GLP and OECD TG453-compliant dietary exposures of male and female SD rats for two years at 1.5, 3.0 and 5.0% (up to 3149 mg/kg/day).

Results: Aside from yellow discoloration of bone, no AGIQ-related toxicity was observed. In the carcinogenicity study, a statistically significant increase in malignant gliomas of brain or spinal cord was observed in female rats exposed to 5.0% AGIQ compared to controls (5/49 [10.2%] vs 0/50, respectively) with absence of a dose response. Specific histomorphological features of the brains among all treated rats include absence of degeneration, hyperplasia, hypertrophy, cellular atypia, or preneoplasia. Based on cytological features and Iba1-positive immunohistochemistry staining, these gliomas are categorized as malignant microglial tumors. Recent historical control data (HCD) documents a glioma incidence in female SD rats of 4/550 (0.7%, range 0-3.3%).

Conclusion: The biological significance of these apparently rat-specific malignant tumors remains questionable since the current human neural tumor classification system does not include a “microglial tumor” category. An independent Scientific Advisory Panel concluded that these malignant microglial tumors are a rare, spontaneous, rat-specific neoplasm. Furthermore, overall assessment of AGIQ genotoxic potential is negative based on ICH S2(R1) guidance criteria.

Impact statement: HCD indicates that gliomas occur spontaneously in rats, thus supporting an interpretation that gliomas observed in the current study are likely unrelated to treatment with limited implications for predicting human cancer risk.

P58 Unique Mutation Signatures in B6C3F1/N Mouse Tumors Resulting from Chronic Inhalation Exposure to α -Pinene

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Abstract

Introduction: Chronic inhalation exposure to Alpha(α)-pinene, a component of turpentine, may occur in certain occupational and consumer settings. The cancer hazards due to (α)-pinene and its translational relevance are poorly understood.

Methods: Whole genome sequencing was performed on mouse hepatocellular carcinomas (HCCs, n=38) and alveolar/bronchiolar carcinomas (ABCs, n=12) arising spontaneously due to age or due to chronic inhalation exposure to α -pinene. Mutation signatures were compared to the Catalogue of Somatic Mutations in Cancer (COSMIC) and the Signal database to determine the mechanisms and translational relevance to human cancers. Additionally, cancer driver genes in these mouse tumors were also determined.

Results: α -pinene exposure in mice resulted in a dose-dependent increase in tumor mutation burden. Remarkably, tumors resulting from α -pinene exposure but not those arising spontaneously showed a unique mutation signature that was similar (cosine similarity ~0.85) to a newly identified human SBS signature (SBS118) reported in some hepatobiliary cancers. Mutations in known cancer drivers such as *Cttnb1* (common in human HCCs) and *Hras* (common in mouse HCCs) were identified but surprisingly, *Kras* mutations (common in human and mouse ABCs) were not observed in any of the examined ABCs. So far, no etiology-specific cancer driver genes were identified.

Conclusions: These data support a mutagenic mode of action of α -pinene that is likely mediated by its metabolite α -pinene oxide, a reactive mutagen.

Impact Statement: The mutation signature SBS118 may be used prospectively in molecular epidemiology studies to assess potential occupational exposures related to α -pinene.

P59 MEN-Like Syndrome: Does It Occur in Rats? An Analysis of Data from the RITA Database

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Abstract

Introduction: Multiple endocrine neoplasia (MEN) is a condition which encompasses several distinct syndromes featuring at least two tumors of endocrine glands, mainly of neuroendocrine origin. While proliferations of neuroendocrine origin in humans are related to a genetic background (MEN Syndromes), the background in domestic and laboratory animals in most cases is unknown (MEN-like Syndromes). An analysis of RITA data was performed to investigate whether the condition of a MEN-like syndrome may occur also in other rat strains. **Methods:** Substrains of control Sprague Dawley (SD) and Wistar rats were analyzed for proliferative neuroendocrine lesions from adrenals, pancreas, thyroid, parathyroid and pituitary. The diagnoses followed the criteria as described in INHAND. Animals with single organ versus multiple organs affected were counted and compared to expected values. In case of significantly increased multiple affections the combinations of organs known from MEN Syndromes were evaluated in search of MEN-like Syndromes. Co-occurrence in two organs versus one organ was analyzed as compared to expected values. **Results:** In male SD rats, significant results were observed in thyroid, adrenal, and pancreas. In male Wistar rats, similar results were seen for the pancreas and thyroid. An increased co-occurrence of adrenal and thyroidal lesions was clearly evident for both male SD and Wistar control rats. **Conclusion:** Even though no clear evidence for the existence of MEN1-like syndromes was given, the results from males of both breeds showed a MEN2-like pattern. **Impact Statement:** The analysis presented in this work indicates the value of detailed metadata of the RITA database.



P60 Transcriptomic Analysis of Pancreatic Acinar Tumors in Rat Exposed to Di(2-Ethylhexyl) Phthalate (DEHP)

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Abstract

Introduction: Rats developed pancreatic acinar adenomas (PAAs) and carcinomas (PACs) after chronic exposure to Di(2-ethylhexyl) Phthalate (DEHP) in rodent bioassays conducted by the National Toxicology Program. It is hypothesized that activation of peroxisome proliferator-activated receptor- α (PPAR- α) in the liver causes alterations in bile acid composition and cholestasis resulting in persistent increases in expression of cholecystokinin (CCK). CCK can act as a growth factor and induce proliferation of pancreatic acinar cells in rats. **Methods:** We examined transcriptomic alterations in PAAs and PACs from DEHP-exposed vs. normal pancreas from vehicle control rats using Ingenuity Pathway Analysis (IPA), and Gene Set Enrichment Analysis (GSEA). **Results:** IPA indicated significant overrepresentation of FXR/RXR, LXR/RXR and PXR/RXR pathways in both PAAs and PACs. These nuclear receptor pathways influence bile acid metabolism. In addition, IPA also indicated significant overrepresentation of Hepatic Cholestasis disease pathway as well as upregulated cholecystokinin B receptor (*Cckbr*) expression in PAC. Upstream transcriptional regulators *Hnf1a* and *Hnf4a* were also identified by IPA. GSEA indicated alterations in E2F transcription factor and cell cycle regulation in PACs. **Conclusion:** These data support the CCK hypothesis wherein chronic DEHP exposure causes alterations in several RXR-mediated nuclear receptor pathways that dysregulate bile acid homeostasis resulting in a CCK-mediated trophic response on pancreatic acinar cells. Additional mechanistic studies are in progress to examine these key events and build confidence in this adverse outcome pathway. **Impact Statement:** The translational relevance of DEHP-induced CCK mode of action in exocrine pancreatic tumors in rats needs to be determined.

P61 Immunohistochemical Characterization of the Ethyl Nitrosourea (ENU)-Induced Rat Gliomas

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Abstract

Introduction: The immunophenotype of rat glial tumors is poorly understood mainly due to the absence of significant GFAP reactivity in gliomas.

Methods: Ethyl Nitrosourea (ENU) (50 mg/kg) was intravenously administered to pregnant Sprague Dawley (SD) rats on gestation day 20. Gliomas (n=14) from male pups were examined in this study. Based on the H&E morphologic criteria described in the CNS INHAND document, gliomas in this study were diagnosed as astrocytomas, oligodendrogliomas, and mixed gliomas. Multiplexed immunofluorescence staining was performed to determine the cell composition in gliomas. The glial markers specific for astrocytes (GFAP, GLAST, Vimentin, ALDH1), Oligodendrocytes (Olig2, CC1, Nogo-A) and microglia (Iba1, P2RY12, TMEM119, CD68) were used in this study.

Results: Astrocytomas and mixed gliomas had greater abundance of Iba1+ cells than oligodendrogliomas. Interestingly, several of the Iba1+ cells were negative for P2RY12, a marker specific for microglial cells. Several astrocytomas had large number of Olig2+ cells that were comparable to mixed gliomas which argues for further characterization of Olig2+ specificity. Astrocyte progenitor markers, GLAST and Vimentin were present in greater abundance than GFAP in astrocytomas and mixed gliomas.

Conclusion: Majority of Iba1+ cells in gliomas were negative for P2RY12 suggesting that these may be tumor associated macrophages recruited from the periphery. Astrocytomas and mixed gliomas contain astrocytes in various stages of differentiation. These preliminary results need to be confirmed and validated in larger number of rat gliomas.

Impact statement: The multiplexed immunofluorescent glial panels may provide more insight into the cells comprising gliomas in rodents.

P62 Copper-Associated Chronic Hepatitis: The Tale of a Two-Year-Old Boston Terrier

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Abstract

A 2-year-old Boston Terrier uneventfully whelped five healthy puppies. She subsequently developed a distended abdomen and up to 710 mL of clear, yellow-tinged fluid was intermittently drained from the abdominal cavity. Exploratory surgery revealed a shrunken and nodular liver. She deteriorated and was euthanized, and an autopsy was performed at the Kansas State Veterinary Diagnostic Laboratory. On autopsy, the liver was diffusely tan and severely shrunken with variably sized nodules separated by fibrosis. Microscopically, there was distortion of the normal hepatic cord architecture and organization of the parenchyma into multiple micronodules that were separated by fibrous connective tissue that extended into periportal regions and bridged other portal regions. Hepatocytes within the micronodules were rounded, moderately swollen, and contained pale indistinct intracytoplasmic microvesicles and occasional brown to red granular pigment that was strongly positive for Rhodamine stain. The copper level in the liver was 642 ppm on a wet weight basis and 2247 ppm on a dry weight basis (threshold for established copper associated toxicosis is 1000 ppm on a dry weight basis). The clinical history, gross and microscopic lesions, and the presence of a toxic level of copper in the liver of this dog are diagnostic of chronic copper toxicosis.

P63 Comparison of High-Dose Systemic AAV Liver Toxicity in Nonhuman Primates Across Studies: Acute versus Subacute/Chronic Injury

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Abstract

High dose systemic adeno-associated virus (AAV) vector administration is associated with toxicity in nonhuman primates (NHP) and humans. The liver is implicated as the primary tissue involved in this toxicity due to the high vector concentration and expression achieved therein. Due to the limited amount of published data on this phenomenon, systematic review of toxicity findings in high dose NHP studies is essential to increase our mechanistic understanding and its translation to humans. NHP studies in this review utilized AAV9 or AAV9-related vectors with human-specific or reporter (e.g., GFP) transgenes administered intravenously at doses ranging 5x10¹³ - 2x10¹⁴ GC/kg to juvenile (1-3 years old) and adult (4-10 years old) Rhesus and Cynomolgus macaques. Observed toxicity profiles varied by chronicity and presented as acute (3-6 days), subacute (~2-4 weeks), or chronic (>4 weeks) cases that varied according to the suspected mechanism of action. The most severe clinical presentation (e.g., shock) was seen acutely, often required early euthanasia, and corresponded to massive liver necrosis upon histopathological examination. Immunomodulation had variable therapeutic effects on acute toxicity. Subacute and chronic presentations were less severe, often with delayed liver enzyme elevation, and did not typically require early euthanasia. Microscopic findings in the liver were milder with prominent inflammatory cell infiltrates suggestive of a host immune response. Understanding the possible clinical manifestations of systemic high dose AAV gene therapy (increasingly used to treat severe, often fatal, genetic diseases) will enable the development of treatment and mitigation strategies to enhance its safety in humans.



P64 Molecular Pathology Tools to Visualize Therapeutic Target Modulation for RNAi Delivery Systems

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Abstract

Introduction: Traditional approaches (e.g., tissue homogenate-based Western blot or PCR) lack spatial resolution to address critical questions related to pharmacological modulation of genes and proteins of therapeutic interest. Visualization of gene/protein modulation through siRNA therapy is critical to inform on efficacy (e.g., knockdown in disease-relevant cells) and safety (e.g., targeted delivery sparing cells/tissues not relevant to disease).

Objectives: We have developed molecular pathology immunohistochemistry (IHC) tools to visualize modulation of catenin beta-1 (CTNNB1) RNA and protein in the context of specific cell types resulting from siRNA pharmacology.

M&M: FFPE tissue sections from mice administered with siRNA conjugated to different molecules, were assayed with chromogenic IHC and multiplex immunofluorescence for CTNNB1, and cell markers to visualize cell-type specific modulation of β -catenin1 protein expression, which was analyzed by AI based image analysis.

Results: Shedding light on which cell types are targeted with these molecule/drug delivery systems is key to achieve therapeutic efficacy and develop life-changing drugs. The results obtained in these studies impacted selection of the most appropriate delivery system that can be leveraged to direct the siRNA cell tropism. These tissue-based techniques offer spatial information and can be applied to screen a wide array of organs of therapeutic interest (e.g., lung, eye, kidney, liver), thereby expanding our understanding of distribution and efficacy of candidate molecules.

Statement of Impact: Molecular pathology tools improve on traditional methods by providing microanatomical spatially resolved detection of target modulation, enabling greater understanding of distribution and mechanism of action of novel therapeutic modalities.

P65 Morphological Characterization of the Lymphocyte Rich Epithelial Proliferative Lesions in the Thymus from Wistar Rats

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Abstract

Introduction: The epithelial proliferative lesions in the rat thymuses are cellularity increased, epithelial cell; thymoma, benign and thymoma, malignant. However, in Wistar rats, a specific form of lymphocyte rich epithelial proliferative lesions (hyperplasia and thymoma) with the involvement of both epithelial and lymphocytic component have been reported. This study reports two further subgroups of lymphocyte rich epithelial proliferative lesions in the thymus in Wistar rats for the first time. **Methods and Materials:** Thymuses from two carcinogenicity studies involving two sub strains of Wistar rats (Wistar Rj: WI (IOPS HAN) R. Janvier – 473 thymuses, Crl: WI Han – 120 thymuses) were characterized in this project. The methods include H&E staining, Immunohistochemistry (cytokeratin, CD3 and CD45) and image analysis. **Results:** The rate of incidence of epithelial proliferative lesions was distinctly different in two different sub strains of Wistar rats. The females were affected more than males in both sub strains. Further morphologic characterization revealed that among the lymphocyte rich epithelial proliferative lesions, there are two subgroups with distinct subgroup of epithelial cells and lymphocytes. **Conclusion:** This study reports two subgroups of lymphocyte rich epithelial proliferative lesions in the thymus in Wistar rats. **Impact statement:** Proper regrouping of lymphocyte rich epithelial proliferative lesions in the thymus as described in this poster will be helpful in establishing the test item relationship, if any in the carcinogenicity studies using Wistar rats.

P66 Characterization of Spontaneous Subretinal Deposits in Minipig Eyes

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Abstract

Introduction: Minipigs are increasingly popular animal models for ocular and general toxicology studies. A previously unreported subretinal accumulation was identified and described.

Methods: A retrospective study of subretinal accumulations of 622 eyes from 311 minipigs documented the microscopic appearance, incidence, grade (0-3), and retinal localization (inferior, posterior, and superior) from dosed and control animals from 17 studies including general toxicology studies (n=7), intravitreal implant (n=2), intravitreal injection (n=3), and subretinal injection studies (n=5). Special stains and Rhodopsin immunohistochemistry were applied to selected slides, and concurrent retinal changes were noted.

Results: Subretinal accumulations were found in 100% of studies reviewed, and at least unilaterally in 40% of individuals. Accumulations were not associated with concurrent retinal degeneration and were found in 38% of unmanipulated globes, and 43% of globes with subretinal injections. Superior retina had the highest incidence (49%) and mean grade (1.85). Accumulations were less often associated with intravitreal injection studies, were eosinophilic to basophilic, finely granular, and elevated the photoreceptor layer from the retinal pigment epithelium (RPE). Accumulations were weakly positive for Alcian Blue only.

Conclusion: Subretinal accumulations occurred frequently in minipigs at this laboratory and did not have an association with dosing method. Histopathologic evaluation was incompatible with retinal or RPE pathology and were therefore most likely an artifact of tissue trimming or processing.

P67 Mineral Fiber-Reinforced Plate Implantation in a Sheep Model: Long Term Safety and Bio-Integration *In Vivo* Study

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Abstract

Introduction: Traditionally, orthopaedic implants were made of metal to provide mechanical strength and durability, essential for stable bone fixation. However, complications including implant migration, stress concentrations and bone loss, resulting in need for hardware removal, drove the use of non-permanent polymer-based implants, which carry other risks, of adverse inflammatory reactions to degradation by-products. In this study, we evaluated the biocompatibility and bio-integration profile of a new class of mineral-fiber reinforced plates in a sheep tibia model.

Methods: Plates, made of continuous reinforcing mineral fibers bound together by PLDLA (50%w/w), were implanted bilaterally over the medial surface of sheep tibiae. Left tibiae underwent periosteal elevation, and right tibiae had intact periosteum. MicroCT and histopathology were performed at 13,26,52,78,104,134-weeks(W) post-implantation. Overall cellular response, rate of bioabsorption (i.e., phagocytosis, M2-like macrophages/giant cell (MNGCs) infiltration), and mesenchymal ingrowth were graded according to ISO-10993-6(annex E).

Results: Mesenchymal ingrowth into the device wall was similar for both groups, and the cellular response consisted of anti-inflammatory M2-like macrophages and MNGCs. Adverse inflammation was not observed. Phagocytic activity started at 13W, peaked at 52W-78W, and resolved by 104W. Fibers were still evident at 78W and fully remodeled by 104W. By 134W, implants were fully bio-integrated.

Conclusion: This study demonstrates the safe use of fiber-reinforced implants in a bone plate application, with complete bio-integration with surrounding tissue and no toxicological adversity.

Impact Statement: This new bone-fixation technology offers a solution to metal hardware complications and removals, while avoiding adverse inflammatory reactions reported for existing non-permanent implants.



P68 Impact of Sexual Maturity Status on the Histological Interpretation of Xenobiotic-Related Findings in the Male Reproductive Tract of Rodents and Nonrodents

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Abstract

Potential toxicity in the male reproductive system during non-clinical toxicity testing is often first revealed during routine histopathologic evaluation of repeat dose toxicity studies. The relatively young age of animals available for non-clinical safety studies can impose challenges with the determination of xenobiotic-related findings in male reproductive organs of rodents and non-rodents. A toxicologic pathologist should be aware of the histological variations in immature or peripubertal male animals to avoid inaccurately attributing these changes to xenobiotic administration.

Whole slide images and tabulated data from multiple case examples from former CRL studies were collated to illustrate the morphologic patterns and variations in the male reproductive tract of immature/peripubertal animals.

Histologic changes present during puberty included tubular degeneration/atrophy, degeneration or exfoliation of germ cells, germ cell depletion/hypospermatogenesis, retained spermatids, multinucleated giant cells, tubular vacuolation; decrease or absence of sperm and presence of exfoliated germ cells or cellular debris in the lumen of epididymides; and/or atrophy of accessory sex glands, any of which can be mistaken for potential xenobiotic effects. Therefore, careful consideration of the possible correlations with maturity status, age, demographic data and organ weights were deemed essential during interpretation of xenobiotic-related effects in immature/peripubertal male animals.

Sexual maturity status can be a confounding factor in the histological interpretation of xenobiotic-related findings in the male reproductive system during non-clinical safety assessment.

An awareness of normal histologic variations in immature/peripubertal male animals will help to avoid false positive interpretations of xenobiotic-related effects in the male reproductive organs.

P69 Toxicological and Cardiovascular Effects of a Novel Kinase Inhibitor in Rats and Dogs

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Abstract

Cardiovascular toxicity was observed in toxicology studies in rats and dogs supporting the development of small molecules inhibitors of a tyrosine kinase (TKI-X) not expressed in heart or vasculature. Pharmacologic and toxicologic endpoints were evaluated to understand the mechanism of toxicity in both species. This TKI-X was administered in exploratory, 2-week, and 4-week toxicity studies in rats and dogs. Cardiac troponin, hemodynamic, and electrocardiographic endpoints were assessed in conscious and anesthetized animals. In rats, administration of TKI-X for 14 days induced acute decreases in mean arterial pressure associated with increases in heart rate and cardiac contractility and resulted in mild to moderate myocardial degeneration/necrosis with mononuclear cell infiltrate and few foci of minimal fibrosis. Similar findings were observed with short-term oral dosing of vasodilator Aprikalim and was attenuated by co-dosing TKI-X with beta blocker Atenolol. In dogs, administration of TKI-X induced significant and sustained increases in heart rate, cardiac contractility, and cardiac output. In repeat-dosing studies in dogs, myofiber degeneration/necrosis and/or fibrosis was observed in the heart and was associated with increases in serum cardiac troponin (cTnI). In both rats and dogs, decreases in tachycardia and/or decrease in mean arterial pressure were presumed to contribute to regional hypoxia in the papillary ventricular myocardium. Cardiac pathology findings accompanied by cardiac troponin elevations and associated with hemodynamic changes in two species highlights the importance of this physiologic-toxicologic mechanism in cardiovascular safety assessment. This mechanism has not been described with a xenobiotic that does not target the cardiovascular system.

P70 Investigation of the Liver Findings in Sprague-Dawley Rats Following Subcutaneous Administration of a Non-Binding Monoclonal Antibody

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Abstract

Introduction: Subcutaneous (SC) delivery of monoclonal antibodies is becoming a preferred route of administration when compared to IV infusion based on ease of use and improved patient compliance. To support clinical development, a local tolerability study was conducted in the rat following SC administration. The objective of this investigation was to characterize unexpected liver findings from this study.

Experimental Design: Rats (10/sex/group) were administered a recombinant humanized IgG1 monoclonal antibody by SC injection once weekly for four weeks. This antibody is directed toward an antigen not expressed in rats.

Methods: Parameters evaluated at study termination included clinical chemistry, hematology, organ weights and histopathology of limited tissues. Immunohistochemistry (IHC) for human IgG and rat IgG was performed on liver tissue sections from selected animals to further characterize microscopic findings.

Results: Test article-related minimal increases in liver weights and liver enzyme levels correlated microscopically with minimal to mild increased sinusoidal cellularity (predominantly due to macrophages) and increased hepatocellular mitoses. IHC evaluation of liver tissue for human IgG and rat IgG demonstrated concurrence of positively staining granular deposits in sinusoidal lining cells and Kupffer cells which was suggestive of immune complex deposition and clearance in these cells.

Conclusion: IHC staining pattern was consistent with an immune complex-mediated process due to dosing with a human antibody, which most likely contributed to the microscopic findings in the liver.

Impact statement: IHC data attributed liver findings to immune complexes suggesting lack of clinical relevance in humans.

P71 Acute Toxicity and Two-Week Repeated Dose Toxicity Studies of Perfluoropentanoic Acid in Sprague-Dawley Rats

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Abstract

Perfluoropentanoic acid (PFPeA), which is one of the short-chain PFAS, has been continuously detected in the environment, biota, and population of various ages, but the toxicity of PFPeA has not been sufficiently investigated. We conducted an acute oral toxicity study and a 2-week repeated oral toxicity study to investigate the short-term toxicity of PFPeA. To investigate the acute toxicity, PFPeA was orally administered to SD rats at doses of 300, 300, and 2000 mg/kg in three steps and mortality, clinical signs, body weight, and macroscopic findings were observed. In 2-week repeated dose toxicity study, PFPeA was repeatedly orally administered to SD rats at doses of 0, 100, 300, and 1000 for 2 weeks and general clinical observation, clinical pathology, organ weight, and macro/microscopic examination were performed. As a results of the acute oral toxicity study, all rats given PFPeA 2000 mg/kg (3rd step) were died on Day 1, so the LD50 cut-off of PFPeA was considered to be 500 mg/kg and it was classified as GHS category 4 (300-2000 mg/kg). In 2-week repeated dose toxicity study, dead or moribund rats were observed in 1000 mg/kg/day and histopathological changes in the liver (hepatocellular necrosis) and stomach (erosion/ulcer with acute inflammation) were noted. Therefore, a dose of 300 mg/kg or less is recommended as a high-dose for a subchronic repeated oral toxicity study.

Impact statement: These toxicity study results of Perfluoropentanoic acid are expected to be used to supplement the lack of toxicity information of short-chain PFAS and scientific basis for institutional regulation and risk assessment.



P72 Spontaneous Hepatic Steatosis and Glycogen Accumulation in a Colony of Captive Common Marmosets (*Callithrix jacchus*)

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Abstract

Common marmosets (*Callithrix jacchus*) are nonhuman primates utilized in many types of biomedical research. Their use as a spontaneous model of nonalcoholic fatty liver disease (NAFLD) with progression to nonalcoholic steatohepatitis (NASH) has been proposed. NAFLD is the leading cause of chronic liver disease in humans and progresses to NASH in 20-30% of patients, resulting in increased morbidity and mortality. We performed a retrospective analysis of fixed liver samples from adult marmosets necropsied from the MIT colony over 2 years. Hepatic steatosis and glycogen accumulation were noted in 77.6% of animals (52 out of 67). Among animals with fatty liver disease, a qualitative grade of mild was seen in 17 animals (32.7%), moderate in 11 animals (21.2%), and severe in 24 animals (46.2%). Animals with increased liver weight at necropsy had significantly higher likelihood of fatty liver disease compared to those with liver weight within the normal range (Fisher's Exact RR = 1, P = 0.01). Blood chemistry values proximate to necropsy were also evaluated when available, and animals with fatty liver had increased risk of hypertriglyceridemia (Fisher's Exact RR = 3.59, P = 0.0007). There were no correlations between fatty liver disease and ALK phos, AST, ALT, GGT, or blood glucose levels on chemistry. Cholecystitis was also significantly more common in animals with fatty liver (Fisher's Exact RR = 13.85, P < 0.0001). These findings indicate that common marmosets may be a useful animal model of NAFLD and NASH, and further prospective characterization of the model is warranted.

P73 Impact of Non-Fasting Before Necropsy on the Histopathology Evaluation of Liver of Control Rats and Nonhuman Primates in General Toxicology Studies

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Abstract

Introduction/Objectives – It has historically been common practice to withdraw food prior to necropsy in toxicology studies, although it is known this causes stress that impacts the study endpoints. The main concern of pathologists has been that elimination of fasting impedes the ability to discern subtle changes macroscopically and microscopically. Particularly, elimination of fasting may result in increased hepatic glycogen. The main objective of this study was to evaluate the impact on the histopathology evaluation of the liver. **Experimental Design/Methods and Materials** – We performed a retrospective analysis of control rats and control non-human primates from toxicology studies performed at the Charles River site in Edinburgh, which has not routinely fasted animals on study, in comparison with fasted animals from other Charles River sites, to determine the impact of non-fasting in the histopathological appearance of the liver. **Results**–glycogen vacuolation did not mask other cellular components. **Discussion**–As all pathology comparisons of study data are made with concurrent control or pre-treatment data from animals under the same feeding status, changing to non-fasted study designs will have no impact on data interpretation. **Conclusion**–Elimination of fasting is not jeopardizing histopathological interpretation. **Impact Statement** – Fasting will only be performed on studies run at Charles River when there is scientific or regulatory justification for specific study objectives. Charles River Labs strictly adheres to the three R's in animal testing and eliminating the stress of fasting fulfills a 4th 'R' of 'Responsible animal usage' and improves the animal welfare for all studies.

P74 Genotoxicity and Acute and Two-Week Repeated Dose Toxicity Study of Perfluoroheptanoic Acid in Sprague-Dawley Rats

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Abstract

Even the worldwide restriction on PFOS and PFOA, various PFASs are detected in high amounts in food, products, environments, and biological samples. However, Information on the toxicity of short-chain PFASs, including PFHpA is not sufficient. In the present study, to obtain data on the safety of PFHpA, we performed genotoxicity and, acute and 2-week repeated oral toxicity study. In genotoxicity, the Ames tests using *Salmonella typhimurium* and *Escherichia coli* and *in vivo* micronucleus tests in male SD rats were conducted. For acute toxicity, SD rats were orally treated with each 3 steps at dose 300, 300, or 2000 mg/kg and mortality, clinical signs, body weight gain, and gross observation were observed for 15 d. After 2 weeks of repeated dosing with PFHpA doses of 0, 50, 150, and 500 mg/kg in both sexes of SD rats, general toxicity was assessed using standard toxicological parameters including macroscopic/microscopic examination. Genotoxicity studies didn't show any abnormalities in the groups treated with PFHpA. In the acute toxicity study using female SD rats, the LD 50 cut-off of PFHpA was 500 mg/kg, and it was classified as GHS category 4(300-2000 mg/kg). In 2-week repeated toxicity study, all animals were died at 500 mg/kg, and PFHpA-related change were observed in liver (Centrilobular hepatocellular hyperplasia) and thyroid (follicular hyperplasia) at 50 and 150 mg/kg. A dose of 50 mg/kg or less of PFHpA for both sexes is recommended for further subchronic repeated oral toxicity research.

P75 Effect of Aqueous Extract of *Gastrodia elata* Blume on the Vancomycin-Induced Acute Kidney Injury in Rats

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Abstract

To investigate the preventive effects of *Gastrodia elata* blume (GEB) extract on vancomycin-induced acute kidney injury in rats, 5-week male Sprague-Dawley rats were randomly divided into the following three groups: control (CON) group, orally administered distilled water; vancomycin (VAN) group, orally administered distilled water; *Gastrodia elata* blume (GEB) group, orally administered GEB extract. The treatment period was 14 days. The VAN and GEB groups were intraperitoneally administered VAN after oral administration for the last 3 days of the 14-day treatment period. The kidney weight and the serum levels of blood urea nitrogen and serum creatinine levels in the GEB group were significantly lower than those in the VAN group. Pathological changes in the renal section, such as tubular damage and basement membrane damage of the VAN group were mitigated in the renal section of the GEB group. Expression levels of N-acetyl-D-glucosaminidase, myeloperoxidase, and tumor necrosis factor- α in the GEB group were decreased when compared with those in the VAN group. Compared with that in the VAN group, the number of TUNEL positive cells was significantly lower in the GEB group. The levels of total glutathione, an antioxidant, in the GEB group were significantly higher than those in the VAN group. Compared with those in the VAN group, the malondialdehyde levels were lower in the GEB group. The findings of this study suggested that GEB extract prevents VAN-induced renal tissue damage by exerting antioxidant and anti-inflammatory effects.



P76 Effects of Aqueous Extract of *Gastrodia elata* Blume on the Lung in Rat Allergic Asthma Model

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Abstract

In this study, we investigated the protective effects of GEB extract in allergic asthma model induced by ovalbumin (OVA) in rats. Rats were randomly divided into four groups: i) negative control (NC) group, ii) control (CON) group, iii) OVA group, iv) GEB group. Rats of OVA and GEB groups were sensitized by intraperitoneal injection of 0.3 mg OVA into 1 mL saline containing 30 mg aluminum hydroxide (alum) on every other day for 14 days. The CON group received 30 mg alum into 1 mL saline. OVA-sensitized rats were challenged with 2% OVA in saline (50 μ l) by intranasal inoculation on each side of the nose from days 29 to 35. The CON group received only saline. The GEB group received GEB extract (10 mL/kg, oral) from days 15 to 35 once daily. The CON and OVA groups received distilled water. All rats were sacrificed 24 h after the last administration. Level of total-IgE was decreased in the GEB group compared to the OVA group. Pulmonary edema, alveolar septa area, infiltration of eosinophils, fibroblast proliferation and collagen in lungs were decreased in the GEB group compared to the OVA group. In immunohistochemistry, GEB extract decreased the expression of interleukin-1 beta (IL-1 β), IL-4, IL-5, myeloperoxidase and CD206 in the GEB group compared to the OVA group. In conclusion, GEB extract showed protective effects in allergic asthma rats by reduction of infiltration of inflammatory cells and macrophages.

P77 Developmental Toxicity of Perchloroethylene (PERC) in Zebrafish (*Danio rerio*)

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Abstract

Introduction: Perchloroethylene (PERC) is a legacy environmental contaminant linked to neurotoxicity, developmental toxicity, and cancer. PERC is found at over half of US EPA Superfund sites and is a chemical of concern. **Experimental Design:** This study uses the zebrafish model to test the hypothesis that embryonic exposure to environmentally relevant concentrations of PERC results in developmental toxicity. **Methods and Materials:** A 120-hour post fertilization (hpf) LC50 for PERC was established. Zebrafish embryos were exposed to 0, 5, 50, or 500 parts per billion (ppb; ug/L) PERC in glass vials for 24 or 120 hpf. Embryo survival and hatching was checked every 24 hours for 120 hours. At 24-hpf, a Photomotor response assay was performed and at 120-hpf, morphology, cardiac function, and visual motor response assays were conducted. **Results:** The 120-hpf LC50 for PERC in zebrafish is 35,534 ppb. At 24-hpf, embryos exposed to 5 and 50 ppb demonstrated increased burst activity and total burst duration. At 120-hpf, heart rate was increased in the 5 and 500 ppb exposures. The 50 and 500 ppb exposures demonstrated decreased body length, while the 5 and 50 ppb exposures had decreased head width. The 50 ppb exposure had decreased head length. Eye and otolith diameter was decreased in all exposures. No significant differences were observed with the 120-hpf behavioral assay or in survival and hatching. **Conclusion:** Developmental exposure to PERC alters 24-hpf behavior, larval morphology, and heart rate. **Impact Statement:** The findings suggest PERC is associated with developmental toxicity and warrants additional investigation.