

Poster Setup and Presentation Times

Poster Setup

Sunday, June 19 12:00 Noon–5:00 PM
 Your poster must be set up by 5:00 PM on Sunday, June 19.

Poster Presentation Times

(Please plan to attend your posters during the following times.)
 Sunday, June 19 (Welcome Reception).....6:00 PM–6:30 PM
 Monday, June 20 10:10 AM–10:40 AM
and 3:10 PM–3:40 PM
 Tuesday, June 21 10:00 AM–10:30 AM
 Wednesday, June 22..... 9:50 AM–10:20 AM

Young Investigator Judging Times

Monday, June 20. 10:10 AM–10:40 AM
and 3:10 PM–3:40 PM
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Poster Teardown

Wednesday, June 22..... 11:30 AM–1:00 PM
 Posters not removed by 1:00 PM, Wednesday, June 22, will be taken to the Registration Desk for pickup.



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P01

Activation of the Fibroblast Growth Factor-21 Pathway Decreases Body Weight in Obese and Overweight Cats

Emily J. Brinker, Taylor J. Towns, Rie Watanabe, Adil Bashir, Robert C. Cole, Emily C. Graff

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Abstract

Introduction/Objectives: Obese cats are predisposed to metabolic dyscrasias such as hepatic lipidosis. Fibroblast Growth Factor-21 (FGF21) is an endocrine hormone that has beneficial metabolic effects in many species. The objective of this study was to determine the effects of activation of the FGF21 pathway in obese cats.

Experimental Design: In this cross-sectional study, 10 mg/kg/day LY2405319 (LY) or saline (control) was subcutaneously administered to a colony of ad libitum fed 6-year-old, male, neutered overweight and obese cats for 14 days. **Methods and Materials:** Body weight, food, and water intake were quantified daily. Metabolic parameters, serum liver enzymology, hepatic triglyceride content (proton magnetic resonance spectroscopy), and hepatic viscosity (elastography) were evaluated before and following treatment. **Results:** Treatment with LY resulted in significant weight loss (~5.99%) compared to saline (~0.31%; $p < 0.001$). LY-treated cats had a trend toward decreased liver lipid content (1.86% intrahepatic lipid decrease in LY-treated cats compared to a 2.89% increase in saline-treated cats; $p = 0.055$) and significantly decreased serum alkaline phosphatase ($p = 0.011$). No significant changes or trends were noted in liver viscosity, serum alanine aminotransferase activity, or serum metabolic parameters (insulin, glucose, NEFAs, triglycerides, and cholesterol). **Conclusion:** Treatment with an FGF21 analog can safely induce weight loss in overweight and obese cats fed an ad libitum diet but does not alter metabolic parameters. Furthermore, FGF21 pathway activation decreases liver lipid content, as reflected by the trend in decreased liver lipids and decreased serum ALKP. **Impact statement:** FGF21 analogs have potential as a therapeutic for feline hepatic lipidosis.

P02

The Evaluation of a Novel Training Method for Postmortem Cardiac Blood Collection in CD1 Mice Using an Inanimate Training Model

Glenn E. Brado, April George, Keith G. Nelson

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Abstract

Introduction: In this study, we evaluate and compare macroscopic findings at necropsy from CD1 mice that have undergone terminal cardiac blood collection to evaluate proficiency in trainee technicians. One group of technicians were trained using live animals (conventional) and the other via a novel training method using an inanimate training model (model) for cardiac blood collection. **Methods:** Macroscopic lesion data was collected following cardiac blood draw proficiency testing on 122 animals, 35 from the conventional and 87 from the model training groups. Cardiac and thoracic hemorrhage was scored based on severity (0-2), while the presence of lesions in the diaphragm, liver, pericardium, and lung were recorded for both groups. Comparison of lesion severity in the heart and thorax and lesion prevalence in the remaining listed organs were made between groups. **Results:** There was a significantly higher prevalence of gross lesions in the liver ($p = 0.0009$) and pericardium ($p = 0.02$) in the conventional group. There was no significant difference between the conventional and model groups when comparing severity scoring for cardiac and thoracic hemorrhage or lesion prevalence for the remaining endpoints tested. **Conclusion:** The use of an inanimate model for cardiac blood collection training in CD1 mice is a viable alternative to conventional live animal training. **Impact Statement:** Inanimate models can provide an effective, alternative training method for cardiac blood collection in mice. This training method can replace a number of live animals utilized for training programs, reducing animal usage while still serving to refine trainee skills prior to application in live animals.

P03**Systemic Overexpression of Tristetraprolin (TTP) Mitigates Inflammatory Responses in Allergic Asthmatic Mice**

Richa Lamichhane, Ishita Choudhary, Thao Vo, Dhruthi Singamsetty, Yogesh Saini, Sonika Patial

Louisiana State University, Baton Rouge, LA, USA

Abstract

Introduction: Allergic asthma is a chronic inflammatory disease characterized by immune cells infiltration, airway remodeling, mucus cell metaplasia (MCM), and airway hyper-responsiveness (AHR). Tristetraprolin (TTP) is an mRNA binding protein that binds to AU-rich elements within the 3' untranslated regions of certain transcripts for inflammatory genes and increases their rate of decay. Here, we tested the hypothesis that systemic TTP overexpression mitigates mixed allergen (MA)-induced allergic asthma.

Experimental Design: TTP-overexpression (TTP Δ ARE) and TTP-WT (TTPWT) adult mice from the same line were intranasally exposed to MA for 4-weeks. Lung injury, inflammation, and airways remodeling were assessed 48-hours post-last dose of MA. Additionally, bone-marrow transplantations were performed followed by administration of MA 8-weeks post-bone-marrow reconstitution.

Results: As compared to MA-challenged TTPWT mice, the MA-challenged TTP Δ ARE mice showed significantly decreased infiltration of immune cells in BALF, significantly mitigated consolidation and fibrosis in peribronchial regions, and inhibited MCM. Interestingly, irradiated TTPWT mice reconstituted with TTP Δ ARE hematopoietic progenitor cells (HPCs) exhibited significantly decreased inflammation while their reconstitution with TTPKO HPCs exaggerated inflammation. Furthermore, the reconstitution of irradiated TTP Δ ARE mice with either TTPWT or TTPKO HPCs significantly increased inflammation. However, the inflammation was more robust when TTPKO HPCs were given to TTPWT recipients compared to TTP Δ ARE recipients.

Conclusion: These data together suggest that the systemic overexpression of TTP protects mice against MA-induced asthma and that this effect is largely contributed by TTP overexpression in the hematopoietic lineage cells of the lung.

Impact: Our findings emphasize the role of TTP in the amelioration of MA-induced asthma.

P04**Assessment of Mouse Urinary Tract Pathology During Experimental Uropathogenic *Escherichia coli* Urinary Tract Infection**

Sarah C. Linn^{1,2}, Juan de Dios Ruiz-Rosado^{1,3}, Brian Becknell^{1,3}, John David Spencer^{1,3}

¹Nationwide Children's Hospital, Kidney and Urinary Tract Center, Columbus, OH, USA. ²The Ohio State University, Department of Veterinary Biosciences, Columbus, OH, USA. ³The Ohio State University, Department of Pediatrics, Columbus, OH, USA.

Abstract

Introduction: Urinary tract infections (UTI) are one of the most common infections in humans. There are a wide range of mouse models utilized to study UTI and many studies use bacterial burden alone as a predictor of outcome. We aimed to determine if urinary tract bacterial burden correlates with pathology during UTI. **Experimental Design:** Three common UTI model mouse strains (C57BL/6J, C3H/HeOJ, and C3H/HeN) were infected transurethrally with uropathogenic *Escherichia coli* for 6, 24, 48 hours, 1 week, and 4 weeks. Urine and one kidney were collected for bacterial burden assessment. The contralateral kidney and bladder were fixed in 4% paraformaldehyde, processed, sectioned, and stained with H&E. **Methods:** Urine and kidney burden were enumerated on LB agar. Kidney and bladder histopathology were scored utilizing a previously published scoring system and also scored by pertinent pathologic features identified by the authors. **Results:** C3H/HeOJ mice had high bacterial burden in the urine and kidney with moderate to marked pathology scores in the bladder and kidney. C3H/HeN mice had moderate bacterial burden and low pathology scores in both organs. C57BL/6J mice quickly cleared the infections with minimal kidney burden and exhibited low pathology scores in the bladder and kidney. **Conclusions:** C3H/HeOJ mice more accurately reflect clinical disease during UTI, especially within the kidney. C3H/HeN and C57BL/6J demonstrate only minor to no pathology despite having detectable bacterial burden which may reflect asymptomatic infection. **Impact Statement:** To adequately assess therapeutic outcomes in UTIs in specific mouse strains, both bacterial burden and histopathology should be performed.

P05

Out-of-Field Toxic Effects of Radiation Therapy

Kimberly Demos-Davies¹, Jessica Lawrence^{1,2}, Davis Seelig^{1,2}

¹Department of Veterinary Clinical Sciences, University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA. ²Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA.

Abstract

Introduction: Treatment-related toxic effects are commonly reported in cancer patients undergoing radiation therapy (RT). Radiation-induced toxicity typically affects tissue within and near the irradiated field. However, tissue changes are increasingly recognized in tissues that are distant from the irradiation field, such as the development of neurological signs despite undergoing hindlimb RT alone. Precise mechanisms for distant, out-of-field, radiation toxicity are unknown. **Objective:** To investigate the underlying mechanisms by which localized RT induces out-of-field effects. **Experimental Design:** Nine-to-eleven week old SKH1 mice were treated with a single dose of 20Gy or 30Gy radiation to the right hindlimb and underwent behavioral testing. Mice were euthanized at [6 hours (h), 24h, 5 days (d), 12d, 25d] post treatment. Plasma and irradiated tissues (skin, muscle, femur) were collected, along with left femur, brain, and spleen. All tissue except brain were processed for cytokine and immune cell analysis by flow cytometry and immunohistochemistry. Within brain, glial cell activation and neurogenesis were assessed by immunohistochemistry. **Results/Conclusion:** Hypocellularity and increased reticular fibers in the irradiated bone marrow was noted 5d post treatment compared to unirradiated mice. Distant effects including decreased splenic weight and lymphocyte populations and widespread microgliosis and astrogliosis in the brain. **Impact Statement:** This study is the first to evaluate temporal toxic effects in unirradiated tissues following local RT in mice. It provides evidence of clinically relevant distant tissue effects following focal RT and supports further work is needed to identify potential strategies to minimize the side effects in cancer patients.

P06

Preclinical Dog Model of Focal Prostate Cancer: Pathology and Novel Therapeutics and Diagnostics

Nathan K. Hoggard¹, Ramamurthy Gopalakrishnan², Xinning Wang², Eric T. Hostnik³, Matthew Joseph³, Reena Shaky³, Krishan Kumar³, Richard M. James³, Arijit Ghosh³, Dong Luo², Michael V. Knopp³, James P. Babilion², Michael F. Tweedle³, Thomas J. Rosol¹

¹Ohio University, Athens, OH, USA. ²Case Western Reserve University, Cleveland, OH, USA. ³The Ohio State University, Columbus, OH, USA

Abstract

Introduction: Dogs are the only laboratory animal that develops prostate cancer (PrCa) similar to men. We refined a model of PrCa using canine Ace-1 cells that mimicked early-stage pathology. **Design/Methods:** Dogs were immunosuppressed for 6-8-weeks. Ace-1 cells were transduced with canine or human gastrin-releasing-peptide-receptor (GRPr) or human prostate-specific-membrane-antigen (hPSMA) and implanted into both prostate lobes using ultrasound. An intravenous and intraarterial theranostic (therapeutic-diagnostic) were evaluated, 1) bombesin peptide analogs (BBN) for GRPr (n=20dogs) and 2) gold nanoparticles conjugated with a hPSMA ligand and near infrared (NIR) dye (for photodynamic therapy, PDT) (n=6dogs). **Results:** The PrCa model was repeatable and formed intraprostatic and intracapsular tumors (1-2cm). Tumors consisted of locally invasive PrCa cells that grew into and through the capsule and induced stromal cells. Some dogs developed intraperitoneal or subcutaneous tumors following percutaneous implantation. Extraprostatic tumors grew faster than intraprostatic tumors. BBN ligands were conjugated with an 800nmNIR fluorescent dye or Lutetium-177-labeled-chelate and administered to one lateral prostatic artery with an IV-catheter using fluorography. The BBN-NIR perfused the prostate and bound specifically to the PrCa within 2h. A dog with BBN-Lu-177 therapy had regression of PrCa confirmed by pathology. Systemic IV PDT therapy demonstrated *in vivo* internalization of the hPSMA ligand in the tumors. The tumors were treated with a 700 nm laser that induced superficial tumor necrosis in 2h. **Conclusion/Impact:** The experiments demonstrated refinement of a large animal model of PrCa with regulation of growth by tumor microenvironment, and proof-of-principle for novel therapies/diagnostics for focal PrCa.

P07**Effects of Prenatal and Lactational Exposure to Iodoacetic Acid on the F1 Generation of Female Mice**

Andressa Gonsioroski, Michael J. Plewa, Jodi A. Flaws
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Abstract

Introduction: Iodoacetic acid (IAA) is a disinfection byproduct that is unintentionally formed during water disinfection. IAA is a reproductive toxicant in rodents; however, it is unknown if prenatal and lactational exposure to IAA affects reproductive outcomes in female offspring. This study tested the hypothesis that exposure to IAA adversely affects reproductive outcomes in F1 female offspring. **Experimental design:** Female CD-1 mice were dosed with water or IAA in the drinking water for 35 days and then mated with unexposed males. Dams delivered naturally, and pups were continuously exposed to IAA until postnatal day 21. On postnatal day 21, females were subjected to measurements of anogenital distance, vaginal opening, and ovarian weight. Ovaries were subjected to histopathological evaluation and RNA sequencing analysis. Sera were collected to measure reproductive hormone levels. **Results:** IAA decreased vaginal opening rate, increased anogenital index, and increased the relative weight of the ovaries in the female pups compared to control. IAA decreased atretic follicles and increased testosterone levels in the female pups compared to control. RNA sequencing showed differentially expressed genes involved in the gonadotropin-releasing hormone, estrogen, and insulin signaling pathways. **Conclusion:** Collectively, these data demonstrate that prenatal and lactational exposure to IAA affects vaginal opening, anogenital index, weight of the ovaries, the percentage of atretic follicles, hormone levels, and ovarian gene expression in the F1 generation of female mice. **Impact statement:** IAA is a reproductive toxicant to F1 generation of female mice. Supported by NIH R21 ES028963, NIH T32 ES007326, and an Environmental Toxicology Fellowship.

P08**In Utero Cadmium Exposure Disrupts Development of the Placental Vasculature Leading to Growth Restriction in Male Offspring**

Danielle L. Kozlosky, Alexander Lu, Cathleen Doherty, Brian Buckley, Michael J. Goedken, Emily Barrett, Lauren M. Aleksunes
Rutgers University, Piscataway, NJ, USA

Abstract

Cadmium (Cd) is a ubiquitous environmental toxicant that causes fetal growth restriction (FGR) in mice and humans. Cd selectively accumulates in the placenta with little passing to the fetus. In this study, we sought to determine whether Cd alters the development of the placental vasculature as a mechanism contributing to FGR in mice. Pregnant female C57BL/6CrI mice (n=10/group) were administered a single dose of saline (5 ml/kg ip) or CdCl₂ (2.5 or 5 mg/kg ip) on gestational day (GD) 9. On GD 18, fetuses and placentas were collected, measured, and weighed. There were no changes in the total number of fetuses across treatments nor in the male:female ratio. Treatment with 5 mg/kg CdCl₂ reduced the weight and length of male fetuses and their placentas by up to 13% with little change in females. Surprisingly, the placentas of female offspring had 44% higher Cd concentrations compared to males. To identify mechanisms responsible for the selective sensitivity of male offspring to in utero Cd toxicity, we examined changes in placental vasculature. No histomorphological changes were observed in Cd-exposed placentas. Immunostaining for Cd34, a marker of endothelial cell differentiation, and β -catenin, a regulator of vascular integrity, was strong in the labyrinth zones of control placentas. Exposure to 5 mg/kg CdCl₂ reduced immunostaining of Cd34 preferentially in male placentas (37% of control) compared to female placentas (55% of control). Similar declines (~50%) in β -catenin immunostaining were observed in male and female placentas following CdCl₂ treatment. Further, significant reductions in the area of maternal and fetal vessels by one-third were detected in male placentas compared to vehicle-treated controls following exposure to 5 mg/kg CdCl₂. Vessel areas in female placentas were minimally affected by Cd treatment. Alterations in the development of the placental vasculature and secondary effects on the maternal transfer of nutrients to offspring may be a mechanism behind the sensitivity of male fetuses to Cd-induced FGR. Supported by F31ES032319, R01ES029275, T32ES007148, and P30ES005022.

P09

A NEW Approach for Characterizing Mouse Urinary Pathophysiologies

Hannah M. Ruetten¹, Gervaise H. Henry², Teresa T. Liu¹, Heidi M. Spratt³, William A. Ricke¹, Douglas W. Strand², Chad M. Vezina¹

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Abstract

Void spot assay (VSA) is a cost-effective method for evaluating mouse urination phenotypes. VSA has been used to differentiate voiding behaviors between experimental groups, but not as a diagnostic assay. To build toward this goal, we used the VSA to define urination patterns of male mice with diabetic diuresis (BTBR.Cg-Lepob /WiscJ mice), irritative urinary dysfunction (E. coli UT189 urinary tract infection), and obstructive urinary dysfunction (testosterone and estradiol slow-release implants) compared to their respective controls. Many studies compare individual VSA endpoints (urine spot size, quantity, or distribution) between experimental groups. Here, we consider all endpoints collectively to establish VSA phenomes of mice with three different etiologies of voiding dysfunction. We created an approach (normalized endpoint work through (NEW)) to normalize VSA outputs to control mice, and then applied principal components analysis and hierarchical clustering to 12 equally weighted, normalized, scaled, and zero-centered VSA outcomes collected from each mouse (the VSA phenome). This approach accurately groups mice based on voiding dysfunction etiology. We then used principal components analysis and hierarchical clustering to show that within a test group of aged mice (>24 m old) some develop a phenotype that groups with the obstructive or a diabetic diuresis VSA phenotype while others develop a unique phenotype that does not cluster with that of diabetic, infected, or obstructed mice. These findings support continued use of VSA to identify specific urinary phenotypes in mice. The VSA is a beneficial quick and cheap test used to identify urinary dysfunction in toxicologic pathology studies.

P10

Myeloid Cell-Specific IL4Ra Deletion Protects Against Mixed Allergen-Induced Lung Injury in Mice

Ishita Choudhary, Thao Vo, Dhruthi Singamsetty, Kshitiz Paudel, Yun Mao, Richa Lamichhane, Sonika Patial, Yogesh Saini
Louisiana State University, Baton Rouge, LA, USA

Abstract

Introduction: IL-4 and IL-13 play an essential role in the pathogenicity of allergic asthma via the common receptor, i.e., IL4Ra. However, the myeloid cell-specific role of IL4Ra-mediated signaling in allergic asthma has remained unclear. We hypothesized that myeloid cell-specific IL4Ra signaling is essential for granulocytic inflammation and associated pathological outcomes. **Experimental Design:** To test our hypothesis, we exposed myeloid cell-specific IL4Ra-deficient (LysMCRE/CRE/IL4Ra^{fl/fl}) and control (LysMCRE/CRE/IL4Ra^{WT/WT}) mice to mixed allergens for 4 weeks (3 doses/week) and examined the lung phenotypes at 24 hrs after last dose. **Methods:** Total cell counts, differential cell counts on bronchoalveolar lavage fluid, and histopathological analyses on unlavaged left lung lobes were performed. **Results:** In contrast to mixed allergen-exposed control mice that had exaggerated immune cell recruitment, myeloid cell-specific IL4Ra-deficient mice had significantly reduced immune cell infiltration into the lung airspaces. The reduction in immune cell infiltration in myeloid cell-specific IL4Ra-deficient mice was attributable to a significant decrease in eosinophils and lymphocytes. Additionally, while mixed allergen-exposed control mice exhibited pronounced peribronchiolar and perivascular lung inflammation, myeloid cell-specific IL4Ra deletion significantly ameliorates lung pathology. Mucous cell metaplasia (MCM), another key feature of allergic asthma, was unaltered in myeloid cell-specific IL4Ra-deficient mice suggesting its dispensable role in mixed allergen-induced MCM. **Conclusion:** Our data show that myeloid cell-specific IL4Ra signaling plays an important role in immune cell recruitment specifically eosinophils, and its deletion protects against mixed allergen-induced lung injury. **Impact Statement:** Findings from this study may be applied toward developing cell-specific therapeutics against allergic airway and other eosinophilic disorders.

P11

Investigating Plausible Mechanisms of Ovarian Endocrine Disruption Using Single-Cell Transcriptomics and a Translational Model Fish

Jennifer M. Cossaboon¹, Osman Sharif^{2,3,4}, Bruce W. Draper⁵, Yulong Liu⁵, Zachary Rabow⁶, Oliver Fiehn⁶, Janine LaSalle^{2,3,4}, Swee J. Teh¹

¹School of Veterinary Medicine, University of California Davis, Davis, CA, USA. ²School of Medicine, University of California Davis, Davis, CA, USA.

³Genome Center, University of California Davis, Davis, CA, USA. ⁴MIND Institute, University of California Davis, Davis, CA, USA. ⁵College of Biological Sciences, University of California Davis, Davis, CA, USA. ⁶West Coast Metabolomics Center, University of California Davis, Davis, CA, USA.

Abstract

Introduction/Objectives: Epidemiological studies reveal *in utero* exposure to dichlorodiphenyltrichloroethane (DDT) likely increases female reproductive cancer and infertility risk. However, exact causal mechanisms remain unclear. The Japanese medaka (*Oryzias latipes*) fish model has a short generation time and shared sex signaling pathways with humans. We are pairing medaka with cutting-edge single-cell transcriptomics (scRNA-seq) to test whether early life exposure to estrogenic o,p'-DDT induces transcriptional effects in ovarian cells, reducing fertility.

Materials/Methods: ScRNA-seq reveals heterogeneous cell responses and expression changes masked by bulk sequencing. Females (n=80/tank, 3 replicates/treatment) are exposed to physiologically-relevant 2 µg/L o,p'-DDT or control solutions from 0-30 days post-hatch (dph). This window aligns with critical ovarian cell differentiation in medaka and humans. Fish are grown out to 60 dph, then ovaries pooled, dissociated, and sequenced.

Results: No single-cell library for medaka ovary exists despite widespread use in endocrine disruption research. We optimized dissociations for a preliminary library of 2,232 cells from n=40 pooled ovaries (650 median genes/cell, 19K mean reads/cell, 93.6% mapped to genome). Cell clustering revealed 8 cell types (3 germ lineage, 5 somatic) expressing unique marker genes.

Conclusion: These genetic markers will streamline differential expression analyses in o,p'-DDT-exposed ovaries in the final experiment, which is currently underway.

Impact: Characterizing cellular-level responses may identify novel gene targets that could play roles in reproductive diseases. Further, this study evaluates combining medaka with cutting-edge scRNA-seq to enhance predictive toxicology screening for suspect endocrine disruptors and potential pathophysiological mechanisms.

P12

A Spontaneous Case of Uterine Choriocarcinoma in a Sexually Immature Cynomolgus Macaque

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Abstract

Introduction: Choriocarcinomas are rare neoplasms of trophoblastic lineage that most often arise in cycling or parous females. Published information of choriocarcinoma in sexually immature animals is limited. This case report highlights the development of choriocarcinoma in the uterus of a sexually immature cynomolgus macaque. **Methods:** A 2-year-old female cynomolgus macaque was part of a vehicle control group in a 3-month safety assessment study. Tissues were routinely processed and stained with hematoxylin and eosin (H&E) following a complete necropsy examination. **Results:** The uterine mass was comprised of a neoplastic population of polygonal cells arranged in sheets with moderate to abundant amounts of eosinophilic cytoplasm, vesiculate nuclei, and occasional binucleation. Anisocytosis and anisokaryosis were marked and rare mitotic figures were observed. The ovaries lacked corpora lutea and other tissues were unremarkable. **Conclusion:** The morphologic features based on H&E histologic evaluation were consistent with a choriocarcinoma. Further characterization of this neoplasm with immunohistochemistry is planned. While rare, choriocarcinoma in the female reproductive tract have been reported in sexually mature non-human primates, rabbits, and mice. This case is the first reported incidence of this neoplasm in a sexually immature animal. Spontaneous neoplasms are rare in nonrodents due to the relatively young age of these animals in nonclinical toxicity testing and knowledge of spontaneous background lesions is necessary to distinguish from potential test article-related effects.

P13

NRF2-Dependent Placental Effects Vary by Sex and Dose Following Gestational Exposure to Ultrafine Particles

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Abstract

Exposure to ultrafine particles (UFPs, PM0.1) during pregnancy triggers placental oxidative stress and inflammation, similar to fine PM (PM2.5). The Nrf2 gene encodes a redox-sensitive transcription factor that is a major regulator of antioxidant and anti-inflammatory responses. To investigate the role of NRF2 in regulating maternal antioxidant defenses and placental responses to UFP exposure, wildtype (WT) and Nrf2^{-/-} pregnant mice were exposed to either low dose (LD, 100 µg/m³) or high dose (HD, 500 µg/m³) UFP mixture or filtered air (FA, control) throughout gestation. Nrf2^{-/-} HD-exposed female offspring exhibited significantly reduced fetal and placental weights. Placental morphology changes appeared most pronounced in Nrf2^{-/-} LD-exposed offspring of both sexes. Glutathione (GSH) redox analysis revealed significant increases in the GSH/GSSG ratio (reduced/oxidized) in WT female placental tissue exposed to HD in comparison with Nrf2^{-/-} HD-exposed mice. The expression of inflammatory cytokine genes (Il1β, Tnfα) was significantly increased in Nrf2^{-/-} placentas from male and female offspring across all exposure groups. Genes related to bile acid metabolism and transport were differentially altered in Nrf2^{-/-} mice across sex and exposure groups. Notably, the group with the most marked phenotypic effects (Nrf2^{-/-} HD-exposed females) corresponded to significantly higher placental ApoA1 and Apob expression suggesting a link between placental lipid transport and NRF2 in response to high dose UFP exposure. Disruption of NRF2 exacerbates adverse developmental outcomes in response to high dose UFP exposure in female offspring.

P14

Lymphoma Characterization in Sprague-Dawley Rat: The Ramazzini Institute (RI) Approach

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Abstract

Introduction: Human lymphomas are described by WHO based on immunophenotype, morphology and molecular features, with the aim of connecting pathology to clinical outcome, treatment, and prognosis. In rodents, lymphoma classification is more focused on risk assessment than on clinical outcome and treatment. The INHAND classification of rat hematolymphoid lesions, follows closely the more detailed human WHO, although information about immunophenotypic markers is limited. Here, we outline the RI approach to validate lymphoma diagnosis, combining histologic findings with panels of immunophenotypic markers commonly used in humans. Experimental Design: 30 cases of lymphomas diagnosed in Sprague-Dawley rats from RI long-term experiments were included in the study. Methods: Lymphoma samples were subjected to immunohistochemistry (IHC) characterization following a multi-step process. Phase 1: identification of cell origin by a panel of markers including: leukocyte common antigen (CD45), B-cell markers (CD20, PAX5), T-cell marker (CD3), plasma cell markers (CD138). Phase 2: tumor diagnosis and subtypes identification by a panel of markers including: TdT, CD79a, Bcl-2, Bcl-6, CD10, CD4, CD30, CD19, Ki67 and Kappa chain. Results: The RI approach allowed us to identify and characterize according to INHAND, immunoblastic lymphoma, lymphoblastic lymphoma, lymphocytic lymphoma, pleomorphic lymphoma and plasmacytic lymphoma. Conclusions: IHC could be a useful tool providing solid and reproducible results for the identification of lymphoma subtypes and it is essential for pathologists to accurately characterize neoplastic lymphoid processes. Impact statement: Discovery of biomarkers not only enhances our understanding of pathogenetic mechanisms of lymphoma but also provides opportunities to refine classification improving the accuracy of diagnosis.

P15**SEND, The Standard for the Exchange of Non-Clinical Data, Had Been a Requirement for Studies Starting After December 17, 2016. The Objective of the Poster Is to Communicate the Future Proposed Microscopic Domain Additions or Changes to the SEND Implementation Guide***Daniel L. Potenta*

INSTEM, Mt. Arlington, NJ, USA

Abstract

Introduction/Objectives – SEND, The Standard for the Exchange of Non-Clinical Data, had been a requirement for studies starting after December 17, 2016. The objective of the poster is to communicate the future proposed Microscopic domain additions or changes to the SEND Implementation Guide. These additions and changes allow for a better representations of data collected during microscopy, organ measurement collection and necropsy. These changes and additions were implemented as a result of industry concerns submitted during the first 5 years of use of the SEND standard. These changes are seen as an improvement to the current standards and allow for better representation of Pathology data.

b. Experimental Design/Methods and Materials – New methods for parsing data within the Microscopic domain will be introduced here. These include biopsy data, sexual maturity in males, reproductive stage of cycle, special staining, MIRESMOD (result modifiers for microscopic findings), organ measurements, and deprecation of the tf.xpt domain.

c. Results – examples of the data modeling will be presented to show improvements made to the Microscopic domain and Organ measurements.

d. Conclusion – The future submissions of microscopic data submissions will be more clear and a number of improvements were made to allow for better use of domain data both internally for submitters and use for data parsing by regulatory agencies

P16**Historical Control Background Incidence of Spontaneous Nonneoplastic Lesions of Sprague-Dawley Rats Used in 104-Week Toxicity Studies***Marie M. Bockenstedt, Amit Kumar, Victoria A. Laast, Alok K. Sharma*
Labcorp Drug Development, Madison, WI, USA**Abstract**

Introduction: Historical control data are important tools used in toxicologic pathology for the evaluation and interpretation of neoplastic and nonneoplastic findings reported during rodent carcinogenicity studies. The aim of this poster was to provide the range and incidences of spontaneous nonneoplastic lesions reported in control rats from 104-week carcinogenicity studies.

Methods: A retrospective evaluation of the historical control data was performed on the recorded nonneoplastic findings in control Crl:CD(SD) rats used in 104-week studies completed between 2015 and 2021 at North American Labcorp Drug Development sites. Data from approximately 1800 rats were tabulated for the most common nonneoplastic findings. Diagnoses of various nonneoplastic lesions were intentionally grouped in a manner to provide a range of reported incidences of similar types of lesions. Crl:CD(SD) rats were obtained from Charles River (Portage, MI or Raleigh, NC).

Results: The most common reported nonneoplastic background lesions included chronic progressive nephropathy in the kidney (81% males, 54% females), cardiomyopathy of the heart (73% males, 45% females), prostate inflammation (69% males), brain compression (36% males, 63% females), hepatocyte vacuolation (50% males, 43% females) and bile duct hyperplasia (42% males, 30% females) in the liver, and alveolar macrophage infiltration in the lung (25% males, 16% females).

Conclusion: The incidence and range of nonneoplastic microscopic findings reported in SD rats provide support for the interpretation of findings encountered during 104-week carcinogenicity studies. This data is a useful tool to toxicologic pathologists in aiding in the interpretation and evaluation of noneoplastic background microscopic findings.

P17

Common Spontaneous Benign Neoplastic Findings in Sprague-Dawley Rats Used in Nonclinical Research Studies

Amit Kumar, *Marie M. Bockenstedt*, Victoria A. Laast, Alok K. Sharma
Labcorp Inc., Madison, WI, USA

Abstract

Introduction: Identification of spontaneous occurring neoplasm in Sprague Dawley (SD) rats in two-year carcinogenicity studies is important for proper assessment of the carcinogenic potential of the test article. This poster presents the common benign neoplastic historical control data of SD rats from 104 week studies over a 5-year period (2015 to 2021).

Methods: A retrospective evaluation of historical control data was performed on the recorded benign neoplastic findings in control SD rats in 104 week studies completed between 2015 and 2021 at different LabCorp Drug Development sites comprising approximately 1800 rats and were tabulated.

Results: Pituitary gland adenoma of the pars distalis was most common in both sexes. Benign neoplasm with > 1% incidence rates in males included skin lipoma, thyroid C-cell adenoma, benign pheochromocytoma, skin fibroma and keratoacanthoma, islet cell adenoma, mammary gland fibroadenoma, thyroid adenoma of follicular cell, adrenal cortex adenoma, interstitial cell tumor, benign follicular cell tumor, parathyroid adenoma, and acinar cell adenoma of pancreas. In females, benign neoplastic findings of > 1% incidence rate included mammary gland fibroadenoma, thyroid C-cell adenoma, hibernoma, uterine endometrial polyp, adrenal cortex adenoma, benign pheochromocytoma, and skin fibroma.

Conclusion: Knowledge of type and incidence rate of spontaneous background neoplastic findings in SD rats will help pathologist evaluating two-year carcinogenicity studies.

Impact statement: Presented information provides an important reference for the types, incidence, and range of spontaneous benign background lesions in SD rats beneficial to pathologists to discern spontaneous versus test article-related effects.

P18

Common Spontaneous Malignant Neoplastic Findings in Sprague-Dawley Rats Used in Nonclinical Research Studies

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Labcorp Inc., Madison, WI, USA

Abstract

Introduction: Identification of spontaneous occurring neoplasm in Sprague Dawley (SD) rats in two-year carcinogenicity studies is important for proper assessment of the carcinogenic potential of the test article. This poster presents the common malignant neoplastic historical control data of SD rats from 104 week studies over a 5-year period (2015 to 2021).

Methods: A retrospective evaluation of historical control data was performed on the recorded malignant neoplastic findings in control SD rats in 104 week studies completed between 2015 and 2021 at different LabCorp Drug Development sites in North America comprising approximately 1800 rats and were tabulated.

Results: Fibrosarcoma of skin/subcutis and thyroid C-cell carcinoma were the most common tumor type in males while mammary gland carcinoma and pituitary carcinoma were most common in females. The other malignant tumors of >1% incidence rate in males included pancreatic islet cell carcinoma, malignant glioma, mammary carcinoma, and pheochromocytoma. The malignant tumors of >1% incidence rate in females included thyroid C-cell carcinoma and clitoral gland carcinoma in females. Conclusion: Knowledge of type and incidence rate of spontaneous background neoplastic findings in SD rats will help pathologist evaluating two-year carcinogenicity studies.

Impact statement: Presented information provides an important reference for the types, incidence, and range of spontaneous malignant background lesions in SD rats beneficial to pathologists to discern spontaneous versus test article-related effects.

P19**Evaluation of LRRK2 Inhibitors: Alterations in Lungs of Nonhuman Primates**

Glen K. Miller, Sabu Kuruvilla, Lisa La-Franco-Scheuch, Binod Jacob, Chris DeMaula, Vasu Bakthavatchalu
Merck & Co. Inc., Kenilworth, NJ, USA

Abstract

Introduction: Inhibitors of leucine-rich repeat kinase-2 (LRRK2; a multi-domain protein regulating vesicular functions) are intended for potential treatment of Parkinson's disease. Tolerability of oral inhibitors of LRRK2 is presented from repeat-dose studies with nonhuman primates conducted at research laboratories of Merck & Co., Inc., Kenilworth, NJ, USA. **Results:** Monkeys exhibited physical signs during administration of compound A for 8 or 14 days. Levels of LRRK2 pSer935 were lower in lung and peripheral blood mononuclear cells. Histomorphologic and ultrastructural evaluation of lungs revealed hypertrophy of type II pneumocytes, due to increased size and number of lamellar bodies. Compound B elicited physical signs, as compound B in CSF reached 0.0795 μM on Day 8. Prominent changes were observed in peripheral regions of the lungs, as compared to central regions. Hypertrophy and hyperplasia of type II pneumocytes was associated with increased collagen deposition within alveolar walls as identified by histochemical staining and by ultrastructure. Hypertrophic and hyperplastic type II pneumocytes immunostained positive for pro-surfactant C while accumulation of intra-alveolar CD11c positive macrophages were admixed with neutrophils. Compound C was detected in brain, CSF, and plasma following 28-days administration. Hyperplasia of type II pneumocytes associated with collagen deposition within alveolar walls persisted among animals receiving compound C for 28 days followed by a 12-week treatment-free interval. **Conclusions:** These findings highlight the relevance of extensive sampling of lung to better characterize peripheral changes. This is the first report demonstrating hyperplasia of type II cells associated with alterations of alveolar walls following oral administration of LRRK2 inhibitors.

P20**Procedure-Related Vascular Lesions in a 13-Week Continuous Infusion Study in Minipigs**

Rossalin Yonpiam¹, Aaron M. Sargeant¹, David Rehagen², Christopher Houle³
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Abstract

Introduction: Evaluating the pathology of blood vessels (infusion sites) in intravenous continuous infusion studies requires not only the recognition of lesions induced by the test article but also the identification of lesions induced by the administration procedure. The aim of this report is to present procedure-related changes in peripheral blood vessels of control Gottingen Minipigs after receiving saline daily by continuous infusion for 13 weeks. **Methods/materials:** Catheters were placed aseptically in a peripheral vein in a total of eight Gottingen minipigs. These minipigs were then administered 3-hour continuous saline solution once daily for 91 days. Animals were designated for scheduled necropsies on Day 92. The infusion sites were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, and H&E-stained sections were examined. **Results:** Procedure-related microscopic findings at the intravenous infusion sites after 13-weeks of continuous infusion included minimal to marked thrombosis, minimal to marked intimal proliferation, minimal to marked vascular/perivascular mixed cell inflammation, and minimal to marked medial thickening of blood vessel walls. **Conclusion:** These findings were consistent with reported infusion procedure-related changes anticipated in continuous intravenous infusion studies in other animal models. **Impact statement:** This is the first report to our knowledge of microscopic findings associated with peripheral catheterization/infusion in Minipigs.

P21

Simultaneous Characterization of CNS Major Cell Types Using Multiplex Fluorescence Platform

Sandra Diaz Garcia, Yan Ren, Nathaniel Roscoe, Kelsie Smith, Joycel Nadonga, Janina Gooco, Mathieu Marella, Vinicius Carreira
J&J, San Diego, CA, USA

Abstract

Introduction

The brain is one of the most complex organs comprising assemblage of multiple cell types. Each cell has molecular, morphological, and spatial signatures that define its type, sub-type, and functional state. Knowing the status, localization, and interaction between these cell types in their native anatomical context is vital for understanding healthy and pathological processes, and to potentially derive pertinent treatments for a wide array of neurological disorders.

Methods and Materials

Conventional immunohistochemical (IHC) approaches often underrepresent the brain complexity. We use Opal™ for simultaneous detection of 8 tissue biomarkers plus DAPI, within a single image. We use Vectra Polaris to scan the slides, visualize and analyze our markers in our tissue sections.

Experimental Design

We present a scalable multiplex method that generates readouts for a comprehensive panel of biomarkers characterizing all major brain cell types.

Results

A total of 6 different markers (NeuN, Iba1, GFAP, ptau, bamieloid, olig2) were combined in iterative cycles of optimized immunostaining to accumulate spatially resolved datasets from individual brain sections. Specific fluorescent signals associated with each marker was computationally deconvolved and analyzed through a bioinformatic software suite creating a phenotypic map with subcellular resolution.

Conclusion

The cell type panel with 6 different markers allow contextualization with specific therapeutic targets and the characterization of inflammatory processes, proteinopathies and other pathophysiological states in the CNS.

Impact Statement

This approach promises to accelerate pre-clinical drug development, novel biomarker detection and characterization of system-level brain physiology by simultaneously profiling multiple biological targets in their physiological and anatomical context.

P22

Assessment of the “Biological or Toxicological Relevance” of Clinical Pathology Changes—Results from the 9th ESTP International Expert Working Group Virtual Workshop, April 5–6, 2022

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Abstract

In an effort to better align the positioning of clinical pathology findings in reports and regulatory documents amongst the global clinical pathology toxicologic pathology community, a European Society of Toxicologic Pathology (ESTP) Working Group was formed to discuss the inconsistent utilization of terms such as “biological relevance” and “toxicological relevance”.

This working group built upon previous ESTP workshops and published literature that addresses some, but not all, clinical pathology terminology. Global recommendations for more consistent microscopic terminology and nomenclature use in toxicologic pathology exists (i.e., INHAND), however terminology in clinical pathology has not been a primary focus.

Twenty-four international experts in clinical pathology spanning the pharmaceutical and chemical industries, contract research organizations, and regulatory authorities met for 12 preparatory videoconferences and an online interactive workshop webinar to address the discrepant use of these and similar terms.

Videoconferences combined individual presentations and group discussions. Topics included biological variation, appropriate comparisons, statistics, reporting, anatomic pathology correlations, nonstandard biomarkers including immunophenotyping, indirect (secondary) clinical pathology findings, and a weight of evidence approach. More than 150 international scientists participated in the interactive workshop webinar which sought to drive a more consistent use of clinical pathology terminology to better align the global clinical pathology community.

The unique intricacies of each clinical pathology endpoint in relation to additional study aspects, including study design and other endpoints, test system, and variability were considered and incorporated into discussions regarding alignment and positioning of clinical pathology findings and will be integrated into the final output of the working group.

P23**The Impact of Storage on Rat Activated Partial Thromboplastin Times Using Two Different Reagents**

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Abstract

Background/Objective: Activated partial thromboplastin time (APTT) in rat plasma exhibits greater instability compared to other species without a defined causal mechanism. Our objectives were to evaluate the stability of rat APTT using STA-C.K.Prest[®]5 or HemosIL[®]SynthASil reagents on a Stago[™] coagulation analyzer and investigate the sensibility of both reagents on plasmas with different coagulation factor deficiencies. **Methods:** APTT assay stability was evaluated with both reagents on pooled rat plasma after storage at room temperature (RT), 4°C and at -80°C. Different dilutions of pooled rat plasma (2, 10, 20, 50, 100% in buffer) were mixed 1:1 with human plasma deficient in coagulation factors (XII, XI, IX, X or VIII). Each plasma combination was assessed in duplicate using STA-C.K.Prest[®]5 or HemosIL[®]SynthASil before and after storage. **Results:** APTT in rats was more stable with SynthASil reagent at 4°C and RT, and not different at -80°C. APTT was prolonged with factor VIII, XII, and X-deficient plasmas up to 50 seconds with SynthASil compared to C.K. Prest. C.K. Prest APTT was prolonged up to 27 seconds with factor XI-deficient plasmas compared to SynthASil APTT. Storage at RT for 6 hours had no effect on factor deficient plasmas, except for a prolonged APTT with FVIII-deficient plasmas up to 14%. **Conclusions/Impact:** Significant coagulation time differences were observed between tested reagents due to sensitivity differences to single factors that are present in variable concentrations among species. Different stabilities were not explained by the degradation of specific factors during storage.

P24**Qualification of the Automated Hematology Analyzer Sysmex[®] XN-1000V for Whole Blood Evaluation in Rabbits and Comparison to the ADVIA[®]2120 and Microscopic Evaluation**

Tiffany N. Scott¹, F. Poitout-Belissent², F. Kong Kaw Wa², L. Bau-Gaudreault²

¹Charles River Laboratories, Mattawan, MI, USA. ²Charles River Laboratories, Senneville, Quebec, Canada.

Abstract

Introduction: The Sysmex[®] XN-1000V generates complete hematologic data using low volume samples, providing a useful alternative when assessing species from which larger volume samples can be challenging to obtain. Its utility for laboratory rabbit hematology has not been widely documented. **Objective:** To evaluate hematology parameters in EDTA blood from rabbits using the Sysmex[®] XN-1000V and compare it to the ADVIA[®] 2120i analyzer, including manual assessment. **Experimental Design:** Ear vein-derived samples were collected from nineteen clinically healthy New Zealand White rabbits and assessed using both analyzers. Two smears from each sample were prepared for manual leukocyte and reticulocyte assessment. Bias and total error observed (TEobs) were compared. **Results:** The erythrocyte parameters generated by the Sysmex[®] exhibited acceptable error when compared with the ADVIA[®] with the greatest TEobs for lower hemoglobin (TEobs=6.29%) and related variables (MCH, MCHC) and lower reticulocyte counts (13.62%). Platelet counts exhibited an acceptable degree of error when using the Sysmex[®] PLT-F channel (12.6%). Higher leukocyte counts were generated by the Sysmex[®] (14.6%) compared with the ADVIA[®] with similar differential percentages in all subpopulations, except for higher monocytes (115.00%) with the Sysmex[®], matching more closely to the manually-derived counts than the ADVIA[®] (bias=11.25% versus 54.65%). **Conclusion:** The Sysmex[®] XN-1000V provides reliable data for the assessment of rabbits, however this data is different from that generated by the ADVIA[®]2120i. Consequently, these analyzers cannot be used interchangeably in the context of one particular experiment. **Impact Statement:** Our data provides evidence justifying the usage of the Sysmex[®] XN-1000V in rabbits.

P25

Deep Learning-Based Method for Identification and Quantification of Non-Proliferative Findings in Rat Testes

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¹Janssen, Beerse, Belgium. ²Aira Matrix, Mumbai, India.

Abstract

Introduction

Recognizing the 14 stages in the rat testis cycle is a difficult task [1]. We [2] have developed a deep learning (DL) based method to automate the staging which is easy to use, and provide results comparable to an expert on normal testes, and to historical data [3]. Taking this ahead, we propose a DL-based method for identification and quantification of findings in rat testes.

Materials and Methods

We trained customized U-NET based DL models using data from 35 WSI for semantic-segmentation of degenerated tubules, vacuolation and giant cells. A rule-based approach was followed for detecting stage-specific abnormalities viz. spermatid retention and degeneration/necrosis of spermatids. For validation, slide level findings provided by pathologists on data from two studies were compared with algorithm results, and statistical analysis was performed.

Results and Conclusion

Quantitative outputs of the algorithm correlate with semi-quantitative grades provided by pathologists. The proposed method can accurately detect and quantify spermatid retention, degenerative tubules, atrophic testes and other findings in normal or abnormal testes.

Impact Statement

Such quantitative DL methods bring quantification and throughput to the pathology evaluation and can change the paradigm in the pathologist's workflow (results analyzed with statistical methods; toleration of some errors by the DL method once statistical significance is attained; need to improve the slide quality). Further, this method can be generalized and extended to other species.

[1] Creasy (2021). [Rat testes DL staging] doi: 10.1177/0192623320969678

[2] Hess (1990). [Rat stage frequency] doi: 10.1095/biolreprod43.3.517

[3] Russell (1990). [Testis evaluation]. ISBN 0-9627422-0-1

P26

Quantification of Olney Lesions Using Deep Learning

Erio Barale-Thomas¹, Fauve Versaevel¹, Fetene Tekle¹, Jogile Kuklyte²

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Abstract

Introduction

Detection of Olney lesions (neuronal vacuolation and necrosis in retrosplenial cortex (RC)) is an important part of evaluating NMDA receptor antagonists. Previously, we showed that brain levels 3 & 4 (preparing sections according to [1] and correlating them to specific planes in [2]) allowed to detect both changes accurately, on H&E glass slides with a semiquantitative scale.

Methods

We describe a deep learning (DL) method combining 4 independent approaches (brain level identification; RC selection; pixel segmentation for vacuoles at 6 hours PD and necrosis at 7 day PD; quantification) which allow a semi-automatic quantified assessment of the changes.

Results

We also show the pitfalls that affect the accuracy of the results (slide artefacts...) and provide the methods to overcome them. We explain how we compared the results of the glass slide evaluation ("slide-level ground truth") to the results of the DL method using a statistical analysis approach.

Conclusion

We conclude by discussing the advantages of deep learning methods for toxicologic pathology (quantification and throughput); and the change of paradigm in the pathologist's workflow (analysis of results with statistical methods; toleration of some errors by the DL method once statistical significance is attained; need to improve the slide quality). Lastly, we present the potential for the generalization of the approach to detect necrotic neurons in other brain areas; detect vacuoles in different organs; and apply the developed approach to other species.

[1] Bolon & al. (2013). [Brain Sampling and Processing] doi: 10.1177/0192623312474865

[2] Paxinos & al. (2014). [Rat Brain Atlas] <https://www.elsevier.com/books/the-rat-brain-in-stereotaxic-coordinates/paxinos/978-0-12-391949-6>

P27**Enabling IHC-Guided Ground Truth Establishment to Support Deep-Learning-Based Toxicopathology Assessment on Bone Marrow H&E Slides**

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Abstract

Introduction: Assessing bone marrow cell type composition directly from routine histologic samples could enhance insight generation from routine histologic studies. However, manual assessment of bone marrow is time consuming and subject to inter-observer variation, requiring ancillary techniques (e.g., IHC) as cell type identification on histologic samples of bone marrow is challenging due to the paucity of distinguishing morphologic features in histologic compared to cytologic preparations. We seek to develop deep learning (DL) computational methods to identify and enumerate cell types of interest on whole slide images (WSI) of hematoxylin and eosin (H&E) stained bone marrow slides. To build these models, we are first developing methods to collect the ground-truth image annotations needed for algorithm training by integrating the hematoxylin and eosin (H&E) and immunohistochemistry (IHC) methods to enable IHC guided cell type identification on H&E WSI.

Methods/Experimental Design: Archival paraffin embedded decalcified rat sternum samples were utilized to generate paired WSI of H&E and IHC preparations performed sequentially on single tissue sections. Paired WSI were aligned to allow IHC guided ground truth generation for future algorithm development.

Results and conclusion: We developed methods to augment H&E WSI with IHC marker expression results from the same section to guide ground truth generation. These methods increase pathologists' confidence in annotating cell types of interest, and create a convenient interface to speed their work. **Impact:** Future efforts developing DL models using this ground truth promise to enable greater insight generation from routine histologic assessment of bone marrow.

P28**Spatial Protein Expression Profiling: Application of Synthetic Antigen Controls in IHC Assay Development and Qualification**

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Abstract

Introduction: Synthetic antigen controls (SAC) are pure peptides at different concentrations matrixed into a gel with BSA to create a control that may be processed into FFPE and stained with IHC. SAC provide a controlled gradient of staining to be used to check assay performance over numerous runs within the same lab, or between different labs. **Experimental Design:** SAC with "peptide A" at various concentrations ranging from 0 µg/mL to 120 µg/mL were generated and cored into a tissue micro-array (TMA). SAC TMAs were stained with different detection systems in triplicates and on different instruments. **Methods:** SAC were generated by combining equal parts of BSA and formalin with varying concentrations of "peptide A". The peptide mixture was heated to form a gel and left to cool at room temperature overnight. Pellets were then processed and embedded into FFPE blocks and stained with an antibody specific for "peptide A". IHC were performed on three different Leica Bond RX autostainers. Staining intensity analysis was done using HALO software to measure optical density. **Results:** SAC IHC reproducibly generated a low-to-high optical density gradient that strongly correlated with the different peptide concentrations. **Conclusion:** SAC are robust tools that enhance IHC assay development by enabling tighter sensitivity qualification and assessment of inter-lab reproducibility during assay transfer. **Impact statement:** Tissue section-based protein expression studies are critical to inform pathobiology investigations in pharmaceutical R&D. IHC sensitivity and assay transferability are key elements in assay qualification and can be uniquely facilitated by using SAC.

P29

Peripheral Nerve G-Ratio Assessment Using a Supervised Deep Learning Image Analysis Approach

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Abstract

G ratio assessment (inner to outer diameter myelinated nerve fiber ratio) in preclinical models is a relevant aspect of peripheral nerve assessment and correlates with nerve conduction velocity data, histopathological and clinical observations. Current methodology can be time consuming; yield low fiber counts and be potentially biased. We present an automated solution using rat resin embedded peripheral nerve samples stained with toluidine blue and digitally scanned for image analysis. A commercial artificial intelligence-based image analysis platform (Aiforia[®]) was used to train a convolutional neural network (CNN)-based supervised deep learning model. Training included: semantic segmentation of the nerve fascicle, instance segmentation of nerve fibers and semantic segmentation of the myelin sheet and axons. Measurements obtained were highly consistent and amenable to continuous statistical analysis and thus deep learning image analysis is a valuable tool in acquiring pivotal quantitative and non-biased myelinated fiber data to aid in decision making during preclinical development.

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Assessment of Preclinical Developmental Neuropathology Using Image Analysis

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Abstract

The assessment of toxicological effects in the developing brain is a regulatory requirement for the pharmaceutical, industrial and agrochemical industries. These data are used, in conjunction with histopathology, to evaluate developmental neurotoxicity. Current methodology relies on a limited number of manual linear morphometric measurements that is intrinsically associated procedural variability and bias. We present an automated solution using digitally scanned PND 21/22 and PND 71/73 rat brain samples. A GLP (Good Laboratory Practices)-validated commercial artificial intelligence-based image analysis platform (Visiopharm[®]) was used to train a convolutional neural network (CNN)-based supervised deep learning model to automatically detect and measure relevant brain regions: neocortex, caudate putamen, corpus callosum, hippocampus and cerebellum. This method is highly reproducible and precise. It increases and the number of quantitative endpoints collected, decreases analytical turn-around time, and improves the ability to detect morphometric changes in homologous sections, providing an invaluable regulatory compliant asset for decision-making across industries.

P31**Automated Identification and Quantification of Pancreatic Pathology in Rodents**

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Abstract**Introduction**

Drug-induced pancreatic injury is a serious liability in preclinical toxicity studies. Islet cell hyperplasia, acinar cell apoptosis, and atrophy are the commonly observed abnormalities in pancreas. We present a Deep Learning (DL) based method to detect and quantify histopathological changes in rodent pancreas from preclinical safety assessment studies.

Materials and Methods

Whole Slide Images (WSI) of 200 (33 training, 167 testing) Hematoxylin & Eosin(H&E) stained sections from Wistar rat pancreas, obtained from three different laboratories, were included in this study. Using images acquired at 40x magnification, three independent DL-models were trained for three different parameters: islets of Langerhans, acinar cell apoptosis, and acinar atrophy. The DL-models were built using a customized U-Net segmentation design. Each islet of Langerhans region was first detected. Islet cell hyperplasia was then classified using a threshold size ranges from 350-700µm. For apoptosis, counts >100 cells were considered abnormal. A separate DL-model was used to locate acinar atrophy.

Results

The proposed technique effectively identified the islets of Langerhans, apoptosis, and acinar atrophy regions, with recall of 90.30, 96.12, and 100% & a precision of 88.35, 92.55, and 100%, respectively.

Conclusions

The proposed algorithm provides a sensitive and precise method for identification and quantification of islet cell hyperplasia, acinar cell apoptosis, and acinar atrophy, in H&E-stained sections of Wistar rat pancreas.

Impact Statement

We present a DL method that can be utilized to identify and quantify pancreatic pathology in rodent safety assessment studies.

P32**Quantification of Fibrotic Changes in Hematoxylin and Eosin-Stained Wistar Rat Liver Sections Using Deep Learning**

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Abstract**Introduction**

Fibrosis is usually linked to chronic liver parenchymal injury and is considered irreversible. The most common method for histopathological determination of fibrosis is to stain liver slices with Masson's Trichrome or Picrosirius Red (PSR). However this method is resource intensive. Because regular histological evaluation is performed in Hematoxylin and Eosin (H&E) stained images, we provide a Deep Learning (DL) based technique to identify fibrotic changes on H&E-stained liver images of Wistar rats.

Materials and Methods

For identifying and quantifying fibrotic alterations, we employed a modified form of the U-Net DL model that was trained using data from 27 Whole Slide Images (WSI). The algorithm's performance was measured by comparing the results with annotations provided by pathologists on 10 WSI. Additionally, the results of 100 H&E-stained sections were compared with the fibrotic region/s in serial sections stained with PSR (Ramot et al, 2021).

Results

The algorithm had a recall of 97.35% and precision of 90.78% with reference to ground truths provided by the pathologist. The results of the algorithm identifying fibrosis on H&E sections strongly correlated (>85%) with fibrotic regions in serial sections stained with PSR.

Conclusions

The proposed algorithm provides a sensitive and precise method for detecting and quantifying fibrotic alterations in H&E stained sections of Wistar rat liver.

Impact Statement

The proposed technique eliminates the requirement for specialized stains like PSR for measuring fibrotic changes in liver sections of Wistar rats. It has the potential to be used in routine nonclinical efficacy studies.

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Deep Learning-Based Image Analysis Algorithm for Classification and Quantification of Multiple Histopathological Lesions of the Rat Liver and Kidney

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Abstract

Introduction

Recently, AI-based image analysis has been intensively investigated in the field of healthcare diagnostics. The development and implementation of this technology is still in progress for preclinical safety studies in the pharmaceutical industry. In this study, we present a DL-based method for classification and quantification of multiple histopathological lesions in rodent kidney and liver.

Materials and Methods

A modified form of the U-Net DL model was trained using data from Whole Slide Images of 120 liver sections and 55 kidney sections. The trained model was used for identifying and quantifying 7 types of histopathological findings in both liver (bile duct hyperplasia, single-cell necrosis, microgranuloma, EMH, necrosis, and hypertrophy) and kidney (vacuolation, basophilia/degeneration/regeneration tubule, dilatation, hyaline cast, mineralization, mononuclear cell infiltration and cyst). The algorithm was validated by comparing the results with pathologists' findings on 255 liver sections 285 kidney sections.

Results

Algorithms showed consistent performance across all lesions with a balanced accuracy of above 90% for most of the findings. The results of quantitative analysis and classification of the diagnosis based on the threshold values between "no findings" and "findings" correlated well with diagnoses made by the pathologists.

Conclusion

The proposed algorithm provides an accurate method for classification and quantification of multiple findings in tissue sections of rodent liver and kidney. The algorithm can be used as a supportive tool for histopathological evaluation, especially for the early screening of rat toxicity studies when a faster turnaround on go/no-go decision making is paramount.

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Using AI-Based Nuclear Segmentation to Infer Toxicologic Pathology Outcomes Such as Liver Hypertrophy from Cell Density Maps

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Abstract

Background: Reliable identification of liver hypertrophy within toxicologic pathology workflows, informed by subtle differences in individual hepatocyte size and extent, is challenging. Application of digital pathology tools can help with reliable identification of such lesions, enabling detection of their presence in an unsupervised fashion.

Methods: Whole slide H&E images containing representative lesions were reviewed within the Patholytix Study Browser. StarDist, a well established cell segmentation AI algorithm was applied to the images, resulting in nucleus detection. Features including area, shape, and coordinates of each cell were calculated at the point of detection, permitting derivation of cellular density metrics.

Results & Conclusions: Following validation of nuclear segmentation performance against manual counts, we developed a "Cell Density" overlay permitting visualization of changes in cellular density across the tissue. We then applied this cell density overview to example regions of either hypertrophy (i.e. a decrease in nuclear density within an area), or immune infiltrate (revealed by an increase in nuclear density within an area).

Impact Statement: Here we present a methodology for the visualization of changes in cellular density within tissues, which can provide a pathologist reviewer with a heatmap visualizable at low resolution that captures changes happening at the cellular level, which would normally require higher magnification views to observe directly. This view can enable the toxicologic pathologist to more rapidly screen the tissues of interest, to highlight the presence of tissue abnormalities in such a way as to increase the speed of screening of tissues for the presence of such lesions.

P35**Identification of Hemolymphoreticular Neoplasias in Radiofrequency Study on Sprague-Dawley Rats of the Ramazzini Institute (RI) Using a Morphological and Immunohistochemical Approach**

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Abstract

Introduction: RI has developed a IHC protocol that allows to discriminate between the presence of hemolymphoreticular tumors (HLRTs) or non-neoplastic lesions, and the characterization of the immunophenotype of tumor. Here are presented the results regarding the diagnosis of HLRTs on the RI radiofrequency study on Sprague-Dawley rats, using a combined morphological and immunohistochemical approach. **Experimental design:** 330 cases of neoplastic and non-neoplastic lesions of the haemolymphopoietic system from the RI radiofrequency study were evaluated. **Methods:** Sections from alcohol-fixed paraffin-embedded samples were obtained: one stained with HE, to characterize morphologically the lesion according to INHAND and others used for IHC markers expression with a panel of antibodies including CD3, CD20, CD45 and CD68. **Results:** Of the 330 cases analyzed, 267 (80%) were HLRTs, while 63 (20%) were diagnosed as lymphoid hyperplasia, lung inflammation, or other non-neoplastic lesions. All HLRTs expressed at least one clonal marker from the panel and the most frequently observed tumors were lymphoblastic / immunoblastic lymphomas type B; followed by leukemias mostly of the myelo / monocytic type, and histiocytosarcomas. No statistically significant differences were observed in the incidences of total HLRT among the different groups for both males and females. **Conclusion:** The 4 antibody-base-panel used in this study enabled us to identify and characterize all HLRTs and discriminate from non-neoplastic lesions. **Impact statement:** The use of a simple IHC panel in support of morphological diagnosis allows to discriminate HLRTs from non-neoplastic lesions in Sprague-Dawley rats.

P36**Later-Life Effects of Developmental Trichloroethylene Exposure in Zebrafish (*Danio rerio*)**

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Abstract

industrial solvent and is considered a legacy environmental contaminant of public health concern. TCE is a known carcinogen and has been linked to congenital defects, reproductive dysfunction, and central nervous system abnormalities. This study utilizes zebrafish (*Danio rerio*) to test the hypothesis that developmental exposure to ecologically relevant levels of TCE causes delayed reproductive and neural toxicity in adult life. Zebrafish embryos were exposed to 0, 5, 50, or 500 parts per billion (ppb; $\mu\text{g/L}$) TCE from 1 to 120 hours post fertilization (hpf). Following exposure, larval zebrafish were rinsed and reared under normal conditions until 6 months post fertilization (mpf). At 6 mpf, adult zebrafish were bred in dose matched pairs to evaluate reproductive performance. No significant differences were observed in embryo production or survival across exposure groups. Adult behavior and locomotion were evaluated at 7 mpf via a novel tank test and an open field test. In the novel tank test, no differences in locomotor endpoints were observed in either sex and no changes in anxiety-related behavior was observed in males; however, female zebrafish with developmental exposure to 5 ppb TCE demonstrated increased time to reach top arena ($p=0.01$). In the open field test, no locomotor or anxiety-related endpoints were altered in either sex. The results suggest developmental exposure to TCE does not cause later-life reproductive toxicity but may be associated with changes in later-life behavior.

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Retinal Nerve Fiber Myelination in an Asian Cynomolgus Monkey (*Macaca fascicularis*)

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Abstract

Introduction: Myelinated retinal nerve fibers (MRNFs) occur due to an abnormal intraocular myelination of the nerve fiber layer in the retina. There is a paucity of reports in non-human primates that fully characterize this finding with standard histopathology, histochemical staining, and ophthalmic examination. **Materials and methods:** Both eyes and optic nerves were collected from a 4-year old Asian (Chinese origin) male cynomolgus macaque (*Macaca fascicularis*) from a 39-week oral gavage toxicity study, embedded in paraffin, sectioned at 5µm, and stained with hematoxylin and eosin. Additional sections were stained with Luxol Fast Blue (LFB) stain for myelin. Photomicrographs were prepared from digitally (Aperio Scanscope XT2) captured images. **Results:** Ophthalmoscopic examination revealed focal to multifocal, slightly raised white patches in both eyes, that were tentatively diagnosed as exudative optic neuropathy. Microscopic examination of the eyes revealed a fairly well demarcated, monomorphic population of spindle cells arranged in fascicles at the optic disc that were identified as myelinated nerve fibers via positive LFB staining. **Conclusion:** The bilateral occurrence of multiple foci of well-differentiated MRNFs is consistent with a potential congenital or developmental origin and not an induced change, and underscores the importance of the accurate classification of this incidental, spontaneously occurring finding in toxicity studies. **Impact statement:** MRNFs are an uncommon spontaneous background finding in the eye of Asian cynomolgus monkeys that should be differentiated from potential test article-related findings.

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Sampling of Vitreous at Necropsy in Ocular Toxicology Studies: Can Vitreous Sampling and Histopathology Evaluation Be Performed on the Same Eye?

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Abstract

Sampling of different volumes of vitreous at necropsy and the impact on sections' quality was tested in the non-human primate (NHP) and New-Zealand white rabbit (NZW).

Following enucleation, samples of 50, 100, 500 or 1000µL or entire vitreous volume were taken with an 18G needle. Replacement by an equal volume of Davidson's fixative was tested on additional eyes.

Needle size enabled easy sampling of both the fluid and gel phases of the vitreous. Main iatrogenic artefacts included misshapen eye, retinal detachment, retinal tears and lens rupture and their incidence increased with increasing volume sampled. Histological quality of 1 section/eye in both species tested was deemed adequate up to 100µL sampled, inconsistent at 500µL and considered inadequate for microscopic evaluation when 1000µL or more was withdrawn. NZW eyes appeared to be more prone to artefacts than NHP eyes for the same volume sampled. Subsequent replacement by Davidson's fixative is not recommended due to additional iatrogenic artefacts.

In conclusion, vitreous sampling at necropsy with subsequent histopathological evaluation can be performed in NHP and NZW eyes with minimal artefacts when the volume sampled does not exceed 100µL. Therefore, the decision to sample vitreous in ocular toxicology studies in the same eye allocated for histopathology should always be weighed against the study objectives, reduction of animal use and the representativity of sampling a small volume of vitreous, which is inherently heterogeneous. Replacement by an equal volume of Davidson's fixative is not recommended and will not significantly improve quality of sections.

P39**Background Outer Plexiform Layer Atrophy in the Retina of Wistar Han Rats from Different Origins with In-Life Correlates***Typhaine Lejeune*

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Abstract

Wistar Hannover rats are seldom used in ocular toxicology studies, therefore there is not much in-life ocular data available in this strain. We report a newly described histological ocular background change observed in eyes of Wistar Han rats from 3 different origins, with in-life correlates.

For an oral gavage toxicity study with ocular endpoints, two batches of 44 Wistar Han rats were ordered from 2 North American suppliers. Following pre-treatment abnormal electroretinogram (ERG) results, eyes of rats rejected from study were examined microscopically. Additionally, Optical Coherence Tomography (OCT) was performed on eyes of a subset of these rejected animals and additional histological data was gathered from 2 other systemic studies.

ERG of rejected rats (22% of animals) showed decreased response to light stimuli. On OCT images, a loss of continuity of the outer plexiform layer (OPL) of the retina was noted and histologically, there was diffuse bilateral retinal OPL atrophy. This background retinal change appears to be present in the North American population of Wistar Han rats regardless of origin. ERG would be the in-life exam of choice as a screening tool for this retinal change.

In conclusion, background OPL atrophy was found in the retina of Wistar Han rats from 3 different North American origins. Given the apparent high incidence of this background retinal change, this strain appears less suitable for ocular toxicity studies unless pre-screened with ERG, as the response of retinas with diffuse OPL atrophy to ocular toxicants, regardless of administration route, is currently unknown.