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STUDENT RESEARCH DAY

2024

MARCH 22, 2024

University of Pennsylvania School of Veterinary Medicine

Vernon & Shirley Hill Pavilion

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COVER PHOTOS:

Student Research Day 2023
Photo Credit: John Donges

PROGRAM

11:15 a.m.	**BOXED LUNCHES AND REGISTRATION**	H132 & Hill Lobby
12:00 p.m.	**OPENING REMARKS**	Andrew M. Hoffman, DVM, DVSc *The Gilbert S. Kahn Dean of Veterinary Medicine* Elizabeth M. Woodward, PhD *Chair, Student Research Day Organizing Committee*
12:15 p.m.	**STUDENT PRESENTATIONS** (10 min with 3 min Q & A) *Introduction of short-term project oral presenters*	H130 Marookian Auditorium Introduction by Samantha Lackeyram-Owen
12:20 p.m.	*Evaluation of biomarkers for prediction of equine palmar/plantar osteochondral disease* Mentor: Dr. Mary Robinson	Kayla M. Even
12:33 p.m.	*Efficacy of CART cells targeting meningeal B cells in an animal model of progressive multiple sclerosis* Mentor: Dr. Jorge Íván Alvarez	Sara Hernández Suárez
12:46 p.m.	*A Study on the UpTick of Disease: Pathogen surveillance in ticks and white-tailed deer (Odocoileus virginianus) in Pennsylvania using a combination of QPCR and metagenomic sequencing* Mentors: Drs. Erica Miller, Daniel Beiting, and Julie Ellis	Shelby Monnin
1:00 p.m.	**BREAK**	Hill Lobby
1:30 p.m.	**STUDENT PRESENTATIONS** (10 min with 3 min Q & A) *Introduction of long-term project oral presenters*	H130 Marookian Auditorium Introduction by Katherine Morucci
1:35 p.m.	*Sample pooling as a CWD surveillance strategy in free range white-tailed deer using RT-QuIC as a diagnostic tool* Mentors: Drs. Michelle Gibison and Erick Gagne	Lindsay Dwyer
1:48 p.m.	*Abolishment of an intracellular retention signal in the cytoplasmic tail of the SFTSV glycoprotein Gc improves its incorporation onto the vaccine vector VSV* Mentor: Dr. Paul Bates	Philip Hicks
2:01 p.m.	*An atlas of primate-specific Alu exons across human tissues* Mentor: Dr. Yi Xing	Emerson Hunter
2:14 p.m.	*Evidence for chemogenetic control of manual dexterity in a macaque model* Mentors: Drs. Sebastien Tremblay and Kristin Gardiner	Antonina Kalkus

PROGRAM CONT'D.

2:27 p.m.	**POSTER SESSION** **Poster Slam**—*a video presentation played on a loop* **Poster Crawl** **2:30—3:00 p.m.** Session I: Odd-numbered posters **3:00—3:30 p.m.** Session II: Even-numbered posters	Introduction by Erin DeNardo H130 Marookian Auditorium Hill Lobby
3:35 p.m.	**THE CLASS OF 1966 ENDOWED LECTURE** *Planning One Health in Policy and Action* **Catherine K. Brinkley, VMD, PhD** Associate Professor, Department of Human Ecology Faculty Director, Center for Regional Change *University of California, Davis*	H130 Marookian Auditorium 40 min with 10 min Q & A Introduction by Erin DeNardo
4:30 p.m.	**AWARDS AND RECEPTION** Awards presented by **Phillip Scott, PhD,** Vice Dean for Research and Academic Resources, and Student Research Club representatives **Erin DeNardo** and **Samantha Lackeyram-Owen**	H130 Marookian Auditorium and Hill Lobby

THE CLASS OF 1966 ENDOWED LECTURE

IN 1991, A GIFT FROM THE CLASS OF 1966 ESTABLISHED AN ENDOWED FUND TO SUPPORT MAJOR LECTURES AT THE SCHOOL OF VETERINARY MEDICINE.

Catherine K. Brinkley, VMD, PhD

CATHERINE K. BRINKLEY, VMD, PhD

Associate Professor, Department of Human Ecology
Faculty Director, Center for Regional Change
University of California, Davis

Planning One Health in Policy and Action

Combining design and quantitative sciences, Dr. Catherine Brinkley's research focuses on planning for One Health. Her early work included a Watson Fellowship designing zoo exhibits to shape public perceptions about the value of conserving wildlife. Following that, she obtained a master's degree in virology, tracking the spread of a novel zoonotic disease along the seam of the urban-wildlife interface. As a student at the University of Pennsylvania, Dr. Brinkley embarked on a joint veterinary medical degree and PhD in urban planning program to bridge her design and health interests. The Penn VMD-PhD program allowed her to combine expertise on the intertwined health of humans, animals, and environmental systems with a focus on land-use planning that leverages public values to create policies that set aside land for wildlife.

Now, as the Director of the University of California, Davis Center for Regional Change, she is using machine learning to read across hundreds of local plans to understand the many approaches communities are using to reach their shared climate and health goals. In addition, she is working in partnership with scholars across the country to build a National Zoning Atlas, which maps planned development and conservation efforts. Her research seeks to understand whether the many local efforts will sum up to meet the global challenge of climate mitigation—stemming the loss of biodiversity. The participatory action aspect of her work aims to help build local capacity to meet these challenges.

Recently, the 2021 National Climate Task Force recommended a ten-year, locally-led campaign to conserve 30% of terrestrial and marine habitat by 2030. Known as the Thirty-by-thirty (30×30) promise, over 190 countries have adopted the Kunming-Montreal Global Biodiversity Framework at COP15. The aim of these national and global promises is to safeguard environmental and animal health to safeguard human health. Yet, practically, nations can only achieve these promises through local actions. Findings from Dr. Brinkley's recent research and public-facing tools reaffirm the leading efforts of several communities while building evidence-based pathways and hope for the future of our planet.

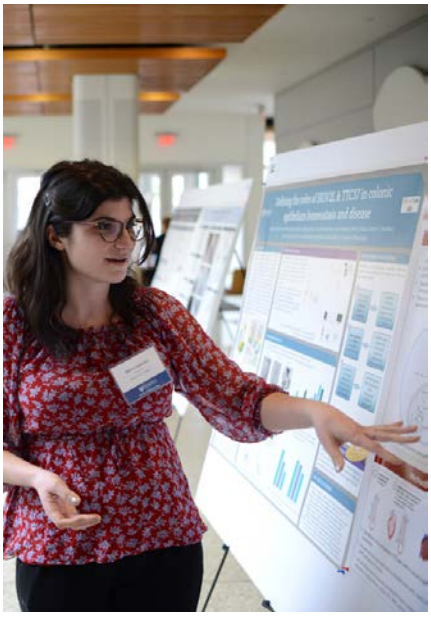
ACKNOWLEDGMENTS

THE UNIVERSITY OF PENNSYLVANIA SCHOOL OF VETERINARY MEDICINE IS GRATEFUL TO THE SPONSORS, DONORS, AND FAMILIES OF ITS PATIENTS WHO MAKE RESEARCH POSSIBLE.

NIH/Boehringer Ingelheim Summer Research Program
We acknowledge the NIH/Boehringer Ingelheim Summer Research Program, which is designed to expose students in their first or second year of veterinary school to all phases of biomedical research.

Richard O. Davies Fund
Established in 2007 by former students and colleagues of Dr. Richard O. Davies, this fund provides support for an award or fellowship to be given at the discretion of the Department of Biomedical Sciences.

The Class of 1966 Endowed Lectureship
A gift from the Class of 1966 established an endowed fund in 1991 to aid and support major annual lectures at the School of Veterinary Medicine.



PHOTOS:

Student Research Day 2023
Photo Credit: John Donges

SPECIAL THANKS

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Samantha Lackeyram-Owen
Katherine Morucci

Abstract and Poster Judging

ABSTRACT AND POSTER JUDGES:
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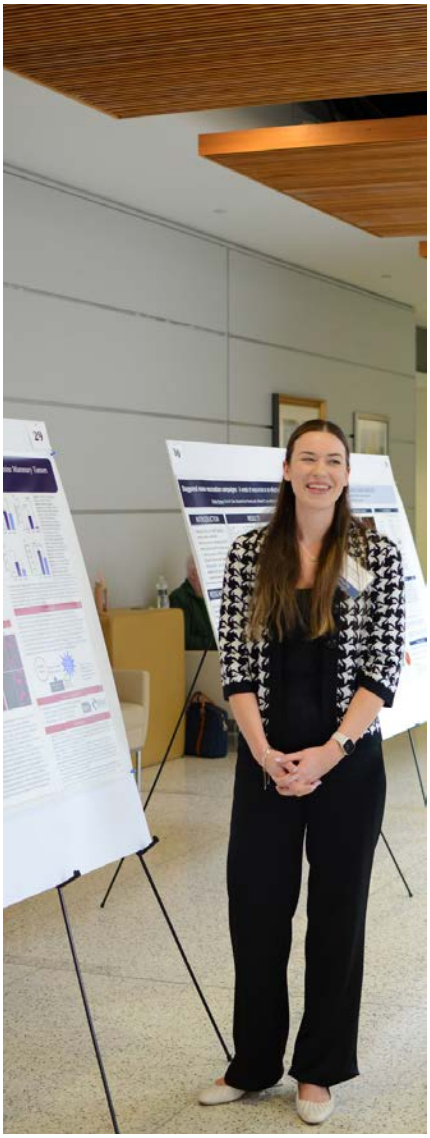
Thank You

Heartfelt thanks to **Associate Dean of Education Kathryn E. Michel** for her foundational efforts in establishing, growing, and professionalizing Student Research Day.

We are grateful to **Dr. Christopher J. Lengner**, Chair, Department of Biomedical Sciences, for generously offering the Richard O. Davies Fund to be used for student awards.

Our thanks to the **Student Research Club, John Donges, Ashley Hinton, Steven Atchison, Stephen Hawkins, Penn Vet Facilities Services**, and **Penn Vet Information Technology**, who provided much-needed organizational, technological, photographic, promotional, and logistical assistance.



PHI ZETA

Phi Zeta was founded in 1925 by a group of senior veterinary students in the New York State Veterinary College at Cornell University. With the assistance of a group of faculty members, including the dean of the college, Dr. Veranus A. Moore, the Society was formally organized, and Dean Moore was elected as the first president of the Alpha Chapter. The Society of Phi Zeta was organized in 1929 at a meeting in Detroit, Michigan, and Dean Moore became the first president of the Society. Also, in 1929, a charter was granted to the School of Veterinary Medicine at the University of Pennsylvania, and the Beta Chapter was established. In 1931, the Executive Committee approved the petition of a group from Iowa State College, and the Gamma Chapter was established. Since then, twenty-four chapters have been chartered, bringing the total number of chapters to twenty-seven. Chapters of the Society may be formed at any recognized veterinary medical college or at any other institution of higher learning.

NAME AND SYMBOLS OF THE SOCIETY

The organizers of the Society, when seeking a suitable name, sought the help of a learned Greek scholar, Professor George P. Bristol, of Cornell University. Professor Bristol suggested a Greek word, which in the Latin form is spelled PHILOZOI and means "love for animals." The abbreviation of Phi Zeta was adopted as the name of the Society.

UNIVERSITY OF PENNSYLVANIA OFFICERS

Beta Chapter
Dr. Margret Casal, President

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Kara Anderson, Rebecca Brisman, Lauryn Cooper, Suna Li Cranfill, Tryssa deRuyter, Nicole DeRogatis, Alyssa Gargagliano, Kaitlin Murphy, Jacob Rabin, Katherine Reilly, Alyssa Marie Silverman, Jaclyn Soulas, Julia Supino, Bridgette Zerbe

ABSTRACT NUMBERS

Abstracts are ordered alphabetically by the presenting student's last name.

Oral Presentations: Indicated by an asterisk*

Poster Presentations: Abstract numbers correspond to the poster board number.

1. Fern Akkrawong
2. Elena Anderson
3. Theresa Astmann
4. Jillian Bastidas
5. Estefania Benavides
6. Breezy Brock & Katie Larsen
7. David R. Buckwalter
8. Bridget Cincotta
9. Robert Z. Cochran
10. Erin K. DeNardo
11. Giovanna DiStefano
12. Jessica F. DiStefano
13. Lindsay Dwyer*
14. Kayla M. Even*
15. Taylor Frownfelter
16. Nathalie Fuhrman
17. Sabrina Garcia
18. Abigail Hamilton
19. Rachel Harrell
20. Sara Hernández Suárez*
21. Philip Hicks*
22. Sabina I. Hlavaty
23. Emerson Hunter*
24. Annie M. Jones
25. Antonina Kalkus*
26. Nicholas N. Laganelli
27. Elisabeth A. Lemmon
28. Elisabeth A. Lemmon
29. Keara Monaghan
30. Shelby Monnin*
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32. Clare Munroe
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40. Martha E. Stone
41. Rachel Tevere
42. Elissa Williams
43. Tiffany C. Wu
44. Caroline Zagoren

ABSTRACTS

Abstracts are ordered alphabetically by the presenting student's last name.

1. THE POTENTIAL USE OF PREDATORY BACTERIA IN THE TREATMENT OF CANINE OTITIS EXTERNA.

Fern Akkrawong[1], Stephen Cole[2], Daniel Kadouri[3], and Jaclyn Dietrich[2].

[1]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; [2]Department of Microbiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; [3]Department of Oral Biology, Rutgers School of Dental Medicine, Newark, NJ.

Purpose: To assess the effect of two predatory bacteria strains (*B. bacteriovorus* HD100 and *B. bacteriovorus* 1095) on well-characterized, diverse, and clinically relevant collection of gram-negative (*P. aeruginosa* and *P. mirabilis*) isolates from canine ears.

Methods: To evaluate the killing efficacy of two *B. bacteriovorus* strains (HD100 and 109J) against canine otitis pathogens, we will perform a percent kill study assessed at 4 time points marked at 0, 24, 48, and 72 hours. In total, 20 genetically distinct isolates of *P. aeruginosa* and 10 genetically distinct isolates of *P. mirabillis* cultures submitted to the Penn Vet clinical microbiology lab will be used.

Results: Overall, the strain 109J seemed to be the most effective predator at preying upon both *Pseudomonas aeruginosa* and *Proteus mirabilis*.

Conclusion: While 109J proved to be more efficient at reducing the population of bacteria, both were equally adequate in log reduction elimination of both prey species.

2. INFLUENCE OF BRANCHED-CHAIN AMINO ACID RESTRICTION ON PYROPTOTIC CELL DEATH IN BONE MARROW-DERIVED MACROPHAGES.

Elena Anderson, Mikel Haggadone, and Sunny Shin.

Department of Microbiology, Perelman School of Medicine, Philadelphia, PA.

The innate immune system acts through a suite of pattern recognition receptors (PRRs) to serve as the host's first line of defense against pathogens. Inflammasomes comprise an important class of cytosolic PRRs triggered during infection. Oligomerization of these multiprotein complexes in response to pathogen sensing and/or cellular damage drives an inflammatory form of cell death called pyroptosis, coupled with interleukin (IL)-1 cytokine release. Our lab is interested in the functions of two major inflammasomes, the canonical NLRP3 inflammasome and the noncanonical caspase-11 inflammasome. NLRP3 requires a priming signal driven by Toll-like receptor (TLR) signaling. A secondary activation signal, mainly a potassium efflux for NLRP3, promotes inflammasome assembly and proteolytic cleavage of the pore-forming protein gasdermin D to drive pyroptosis and IL-1 release. It is known that macrophages undergo metabolic rewiring after PRR activation to promote their proinflammatory functions. However, much remains unknown about the metabolic drivers of inflammasome priming and activation. Here, we have studied the role of branched-chain amino acids (BCAAs) in regulating NLRP3 inflammasome biology in macrophages. Our data suggest that metabolic stress invoked by restricting BCAAs dampens pyroptotic cell death driven by the NLRP3 inflammasome. Mechanistically, BCAA restriction post-translationally blunts NLRP3 inflammasome activation in response to various TLR ligands. Here, we have unveiled a new role for BCAAs in metabolically licensing NLRP3-driven pyroptosis.

3. PANGENOMIC ANALYSIS OF SECONDARY BILE ACID-PRODUCING CLOSTRIDIA.

Theresa Astmann[1], Elliot Friedman[2], and Daniel Beiting[1].

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Division of Gastroenterology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

In the mammalian gut, the transformation of host-derived primary bile acids is a complex metabolic process mediated by the gut microbiome. Interestingly, only a very small subset of gut microbes harbors the complete *bai* operon encoding the 7α-dehydroxylation pathway required for the production of secondary bile acids (SBAs). Numerous studies suggest that SBAs play an important role in modulating host immunity and driving colonization resistance against a diverse range of pathogens. In our previous work, *Clostridium* (*Peptacetobacter*) *hiranonis* was identified as a diet-responsive SBA producer significantly associated with remission in a canine model of inflammatory bowel disease. More precise analysis of the molecular mechanisms underlying these observations, however, is currently limited by the extremely low abundance of these organisms in the gut and a relative scarcity of published genome sequences. To address this knowledge gap, we mined publicly available metagenomic datasets from over 500,000 samples representing a range of host species for reads mapping to SBA producer query genomes. Our results from this initial screen highlight that *C. hiranonis* is uniquely enriched in canine samples, while the closely-related SBA producer *C. scindens* is primarily found in human and pig metagenomes. Abundance of SBA producers across this massive set of public data was then used to prioritize samples to construct metagenome-assembled genomes (MAGs) for both *C. hiranonis* and *C. scindens*. In total, 76 high-quality MAGs were assembled *de novo* from human, pig, and canine stool metagenomes and used to construct a massive-scale SBA producer pangenome. Our *in silico* analysis of estimated metabolic potential across all genomes revealed that multiple pathways for amino acid biosynthesis are largely restricted in *C. hiranonis* compared to *C. scindens,* suggesting an important role for amino acid availability in survival and host adaptation in this species.

Student Support: University of Pennsylvania NIH T32 Training Grant

4. THE EFFECTS OF PRIMING ON EXTRACELLULAR VESICLES DERIVED FROM EQUINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS.

Jillian Bastidas, Lauren Olenick, Alexandra Usimaki, Mana Okudaira, and Kyla Ortved.

Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Despite its high prevalence in the equine industry, osteoarthritis (OA) currently lacks an effective treatment. OA is a complex disease driven by intra-articular inflammation, making the development of an intra-articular biotherapeutic that targets and suppresses inflammatory cascades highly beneficial. The objective of this proposal is to investigate the effects of priming on the production of extracellular vesicles (EVs) derived from equine bone marrow-derived mesenchymal stem cells (BM-MSCs). *In vitro* and *in vivo* studies have shown that MSCs can inhibit inflammation and show promising biotherapeutic potential in OA treatment. Priming stem cells has emerged as a recent strategy to enhance their immunomodulatory potential by exposing them to various microenvironments, including pharmacological agents and biomaterials. Priming has the potential to increase the production of an anti-inflammatory secretome, including modification of EV biocargo. EVs are small, membrane-bound vesicles released by parent cells into the extracellular environment. They contain proteins, lipids, and nucleic acids that play crucial roles in cell-to-cell

signaling. We hypothesize that priming equine BM-MSCs with interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), or a combination of both will result in an increased yield and immunodulatory signature of EVs. The aim of this study is to optimize an *in vitro* priming protocol for BM-MSCs using IFN-γ, TNF-α, or a combination, while maintaining cell proliferation and enhancing EV production. Cell proliferation will be quantified over a 96-hour culture period and cell culture supernatants will be collected for cytokine analysis using a multiplex immunoassay. Additionally, EV isolation will be conducted through stepwise ultracentrifugation, and nanoparticle tracking analysis (NTA) and Western blot analysis using anti-CD9, anti-CD81, and anti-TSG101 antibodies will be employed to confirm the presence of EVs. In a secondary study, this optimized priming protocol will be utilized to produce EVs with a potentially enhanced immunomodulatory microRNA (miRNA) profile. The objective of the secondary study is to compare the miRNA profiles of EVs derived from primed and non-primed equine BM-MSCs. Overall, this research aims to explore the potential of priming equine BM-MSCs to enhance EV production and modify their immunomodulatory properties for use as a cell-free intra-articular biotherapeutic. By investigating these factors, we hope to contribute to the development of novel therapies for the treatment of OA in horses with potential for translation to human patients.

Research Grant: Raymond Firestone Trust and Raker/Tulleners Fund
Student Support: NIH grant T35 OD010919-25

5. ADVANCING INSIGHTS INTO MENINGOENCEPHALOMYELITIS OF UNKNOWN ORIGIN.

Estefania Benavides[1], Kevin D. Woolard[2], Jorge I. Alvarez[1], and Molly E. Church[1].

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Integrative Pathobiology, School of Veterinary Medicine, University of California at Davis, Davis, CA.

Meningoencephalomyelitis of unknown origin (MUO) is a group of progressive, fatal neuroinflammatory diseases for which ante-mortem diagnosis is difficult. Specific MUO subtypes, including granulomatous meningoencephalomyelitis (GME) and necrotizing meningoencephalitis (NME), are only definitively diagnosed with post-mortem histopathology. Previous investigations into the demographics and clinical presentation of GME and NME have largely relied on the presumed antemortem diagnosis of each subtype. Thus, there is a pressing need for an in-depth clinical, pathological, and epidemiological understanding of MUOs. The primary aim of this study is to determine the patient demographics and antemortem diagnostic test results that define each MUO subtype by retrospectively examining medical records from different locations across the US. We will correlate these demographic and clinical data, including therapy given, with disease progression. We performed a search of postmortem records (2013 to 2023) from pathology departments at two US veterinary schools. A total of 84 records from MUO patients have been preliminarily reviewed and reveal an inverted proportion of GME and NME cases on the east coast compared to the west coast: 75% of cases at Penn Vet were diagnosed with GME and 25% with NME, whereas 41% of cases at University of California Davis were diagnosed with GME and 59% with NME. Overall, there was a 1.15:1 sex ratio of males to females for GME whereas the ratio for NME was 2:1. In PennVet, 43% of patients diagnosed with GME were greater than 8 years old whereas 44% of NME patients were between 0-4 years old. For GME, there is a near equal frequency of small to medium-large breed dogs with a ratio of 1.3:1. In contrast, this ratio in NME is 8:1. Future studies will expand the data collected to include cases of other MUO subtypes as well as cases from veterinary hospitals across the country.

6. USING CANINE SINGLE CHAIN TRIMERS TO PREVENT NATURAL KILLER CELL-MEDIATED LYSIS OF MHC CLASS I-EDITED ALLOGENEIC T CELLS.

Katie Larsen, Breezy Brock, Lauren Olenick, and Nicola Mason.

Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Although chimeric antigen receptor T cells (CARTs) show promise in treating humans with hematological malignancies, generating autologous T cells is time-consuming, expensive, and may not be feasible in some patients. To mitigate these issues, CARTs can be derived from healthy donors and cryopreserved so that these allogeneic CARTs would be immediately available for use. However, transfer of allogeneic T cells may elicit an adverse immune reaction. In response, universal CARTs (UCARTs) are generated by removing the donor T cell receptor, as well as beta-2 microglobulin (B2M), a key component of MHC class I (MHCI). However, MHCI null cells such as UCARTs are susceptible to NK cell-mediated lysis. To enable UCARTs to evade NK cell recognition, a non-classical MHCIb molecule can be expressed on their surface in the form of a single chain trimer (SCT). In humans, SCT consist of the HLA-E heavy chain, B2M, and a HLA-E binding peptide linked together and expressed on the MHCI null cell's surface. Relatively little is known about canine MHC (DLA complex); the functional class I gene DLA-88 associates with B2M. We hypothesized that a DLA-88 SCT using canine self-peptide K11 could inhibit NK cell-mediated lysis of MHCI null target cells. K11-DLA88-SCT will be synthesized and used to transduce MHCI null target T cells. Luciferase-based cytotoxicity assays will be performed using canine NK cells co-cultured with T cells for the following conditions: MHCI positive, MHCI null, or expressing K11-DLA88-SCT. If we find that an MHCI-based SCT prevents NK cell-mediated lysis, UCART therapy could provide a safe, effective method to treat patients that may not have the money, time, or functional T cells to generate an autologous product.

Research Grants: NIH Grant T35 OD 010919-25 and MSTP Grant GM07170

Student Support: NIH Boehringer Ingelheim, Armour-Lewis Foundation, Rattner Scholarship

7. GROSS LESIONS IN LAME VERSUS NON-LAME FINISHER PIGS.

David R. Buckwalter[1], Meghann K. Pierdon[2], Julie B. Engiles[2,3], and Nathan Fanzone[2,3].

[1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; [3]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Lameness represents a widespread issue affecting growing pig welfare and has economic implications. The objective of this study was to use gross pathological examination to compare lesions in lame and non-lame growing pigs to better understand the etiology of lameness in growing pigs. The hypothesis of this study is pigs identified with clinical lameness will have a higher prevalence of gross lesions and positive cultures than non-lame pigs. Two production companies enrolled 5 farms each for a total of 10 farms. On each farm 2 pigs were chosen, a single lame pig (L) and a single non-lame control pig (C). Pigs were euthanized by chemical euthanasia and transported to a diagnostic lab for complete postmortem evaluation. There were 16 times greater odds of having multiple lesion types in the L pigs compared to the C pigs (OR =16 95% CI 1.3 to 239.5). All 10 of the L pigs had at least one synovial lesion whereas only 30% of the C pigs had synovial lesions. Lesions were evenly distributed between front and hind limbs with 16% of locations scored in the front limbs having a lesion and 14% of the locations in hind. Only one L pig joint (11 swabs) was found to be positive for *M. hyosynoviae* by PCR and none of the C pigs tested positive (9 swabs). L pigs had more lesions, and were more likely to have synovial lesions, than C pigs though only one pig was found positive for

M. hyosynoviae so the cause of the lesions is unclear. Determining the cause of lameness in these animals remains challenging though bacterial pathogens that cause lesions to the synovium like *M. hyosynoviae* may be more likely than other causes based on these findings.

8. NEURONAL PATTERNS MAY MEDIATE STRESS-RELATED OPIOID DEPENDENCY.

Bridget Cincotta, Emma Tyner, and Julie Blendy.

University of Pennsylvania, Philadelphia, PA.

While humans experience various forms of stress each day, how individuals respond to such stress, even the same stressors, differ greatly. Research suggests that individuals can be categorized into those who demonstrate a susceptible phenotype and those who demonstrate a resilient phenotype following stress (Cathomas et al., 2019). Despite work supporting this classification, little research has explored the underlying mechanisms for such a distinction and how these phenotypes may relate to affective and substance abuse disorders that can arise as part of a maladaptive response to stress, such as opioid dependency. In the present study addressing this knowledge gap, we used a murine model to examine if differences in neuronal patterns can help account for differences in the stress response phenotypes. Sixteen male MOR-Cre mice with a floxed GFP-reporter, allowing for visualization of MOR containing neurons, were subjected to social defeat stress for 10 days to model chronic social stress in humans. At the end of the 10-day paradigm, mice underwent a social interaction test with video analysis to determine the stress response phenotype of each mouse. All mice were then administered an acute injection of morphine and anesthetized and perfused 90 minutes later to allow enough time for protein expression of FOS, a marker for neuronal activity. The mouse brains were then extracted, frozen, and sliced into 30-micron sections on a cryostat. Immunohistochemistry staining for FOS was performed, and image analysis conducted on the mounted brain slices to examine FOS expression within the paraventricular nucleus of the thalamus (PVT), particularly within mu-opioid receptor containing cells. Susceptible mice were seen to have greater FOS expression in the PVT compared to resilient mice, though this trend was not significant; however, resilient mice showed significantly greater colocalization of FOS in mu-opioid receptor-containing neurons.

Student Support: NIH Grant T35

9. IDENTIFYING THE ROLE OF GATA6 IN HUMAN ADRENOCORTICAL DEVELOPMENT USING A HUMAN IPSC-DERIVED ORGANOID SYSTEM.

Robert Z. Cochran, Michinori Mayama, and Kotaro Sasaki.

Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

In humans, organogenesis of the embryonic adrenal cortex results in the establishment of two distinct steroidogenic ($NR5A1^+$) zones: the peripheral definitive zone (DZ) and the central fetal zone (FZ). Previous studies of fetal adrenals (FAds) using rodent models showed reduced translatability. To overcome this, the Sasaki lab has developed a novel human fetal adrenal organoid (hFAO) system generated via stepwise induction of human-induced pluripotent stem

cells. Despite reduced translatability, several murine studies have revealed data that can be applied to the hFAO model. Murine studies show that the GATA family of transcription factors play a key role in specification of steroidogenic lineages. One member of this family, *GATA4,* has been shown to work synergistically with NR5A1 to confer gonadal specific steroidogenic gene expression. However, studies show that *GATA6,* but not *GATA4,* is expressed in the developing adrenal cortex in humans and mice, and *GATA6* mutant mice developed hypoplastic adrenals. In humans, homozygous *GATA6* mutation has not been reported, likely due to embryonic lethality. We hypothesize that homozygous knockout (KO) mutations of *GATA6* in hFAOs will markedly decrease the level of steroidogenesis and subsequently downregulate *GATA6* targeted genes. Using *GATA6* mutant hiPSC lines and their isogenic control lines, we induced hFAOs, measured steroid synthesis, and completed qPCR at various stages of development to identify target genes of *GATA6.* Results show *GATA6* KO hFAOs have reduced steroid production. Moreover, steroidogenic enzyme gene expression is reduced in *GATA6* KO compared to controls, while expression of steroidogenic gene transcription factors show no difference between *GATA6* KO and control.

Research Grant: NIH T35 OD 010919-25
Student Support: NIH/Boehringer Ingelheim

10. INVESTIGATING THE ROLE OF IFN-γ-ACTIVATED MACROPHAGES IN CONTROLLING STAPHYLOCOCCUS AUREUS INFECTION.

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Cutaneous leishmaniasis (CL) is a parasitic infection that causes a wide spectrum of clinical presentations ranging from single, self-healing lesions to chronic, non-healing infections despite treatment. We found that lesions from CL patients exhibit an altered skin microbiome and higher levels of *Staphylococcus aureus* promote increased IL-1β-dependent immunopathology and healing time. Similarly, skin colonization with *S. aureus* enhanced disease in *Leishmania braziliensis* and *major*-infected mice. Since IFN-γ plays a role in clearing *S. aureus* in macrophages in bloodstream infection, we expected to find an inverse relationship between IFN-γ levels and *S. aureus* burden. In contrast, we found that high levels of IFN-γ were associated with an increased *S. aureus* burden in the skin and invasion of the draining lymph nodes in *L. braziliensis*-infected mice. Therefore, we tested if IFN-γ-activated macrophages could limit *S. aureus in vitro*. We first infected macrophages with a *S. aureus* isolate from CL patient lesions and compared the growth of S. aureus in control and IFN-γ-activated cells. Surprisingly, *S. aureus* grew better in IFN-γ-activated macrophages. To confirm the activation of macrophages, we use the Griess Assay, as macrophages increase production of nitric oxide, which is important for pathogen killing, in response to IFN-γ. We then tested a panel of *S. aureus isolates* from CL patients to determine that enhanced bacterial replication in macrophages is a common property of *S. aureus* isolated from patients. These results suggest that IFN-γ has a dual role in leishmaniasis: killing parasites while also promoting increased *S. aureus* levels that contribute to heightened immunopathology. We are now investigating the mechanisms involved in the enhanced growth of *S. aureus*.

Research Grant: NIH R01 AI43790
Student Support: NIH/Boehringer Ingelheim

11. VALIDATION OF ACCELEROMETER BASED MONITORING INTERPRETED BY A THRESHOLD ANALYSIS TO DETECT LIMB MOVEMENTS IN HORSES.

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Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Supporting Limb Laminitis (SLL) occurs in horses with persistent severe unilateral lameness and causes serious complications during recovery from conditions like fractures. SLL has been related to increased weight bearing for compensation of the painful contralateral limb. Thus, causing altered weight distribution and movement of the limbs. The objective was to train and test a threshold analysis that detects steps and offloads of horses sound at the walk. The hypothesis is that threshold analysis could be applied to accurately detect walking steps and static offloads. Characteristic offloading patterns have been observed in past cases that have developed SLL. The goal is to apply accelerometer is to potentially identify that pattern and apply the knowledge to assist with diagnosis. Using Pearson correlation it has been determined there is strong correlation between steps detected by threshold analysis and visual counts. There is a weak correlation between the two methods for offloads. When considering fore and hindlimbs independently, there is a moderate correlation between both offload methods for hindlimb and no correlation for forelimb offloads. This indicates that it is necessary to develop separate threshold values for fore and hindlimbs respectively to derive a more accurate analysis due to the variability of motion.

Student Support: Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania

12. DETERMINING THE ORIGINS OF THE PIGLET GUT MICROBIOME USING STRAIN-RESOLVED LONGITUDINAL METAGENOMICS.

Jessica F. DiStefano[2], Lisa Mattei[2], Clara Malekshahi[2], Erin DeNardo[2], Jalisa D. Zimmerman[1], Andrew Shulman[1], Alexander Berry[2], Meghann K. Pierdon[1], and Daniel P. Beiting[2].

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The piglet gut microbiome has been linked to numerous aspects of swine health and productivity, including growth and development, fat content of tissues important in meat quality, diarrhea in neonates, and even swine welfare. Despite this association, little is known about the factors that contribute to the initial colonization of the neonatal gut. The goal of this study was to chart the assembly of the piglet gut microbiome in the three weeks following birth, while simultaneously sampling maternal and environmental microbes and using strain-tracking to identify potential sources of early life colonization. Ten farrowing units, each consisting of one sow and at least 6 piglets were housed in individual farrowing stalls in the same room. Sow feces and piglet rectal swabs were collected on days 2, 4, 7, 14, and 21 post-gestation, and sow vaginal swabs were collected pre- and post-gestation. Environmental samples, including sow feed, piglet feed from a creep-feeder, and floor swabs, were used to monitor the farrowing environment for microbes. To profile the microbiome in these samples, we extracted DNA using the Qiagen PowerSoil Pro kit, used this DNA to prepare sequence-ready libraries, and carried out shotgun metagenomic sequencing. This data may help identify 'keystone' bacteria that are beneficial for promoting healthy, fast-growing piglets, thus potentially setting the stage to develop probiotics that can improve animal health and production in swine farming.

Student Support: NIH/Boehringer Ingelheim

ORAL PRESENTATION

13. SAMPLE POOLING AS A CWD SURVEILLANCE STRATEGY IN FREE RANGE WHITE-TAILED DEER USING RT-QUIC AS A DIAGNOSTIC TOOL.

Lindsay Dwyer, Maria Pansulla, Roderick B. Gagne, and Michelle Gibison.

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Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy (TSE), or prion disease, in cervids due to the misfolding of normal cellular prion proteins (PrP^{C}) into a pathogenic form (PrP^{SC}). This fatal disease is currently detected using enzyme-linked immunosorbent assay (ELISA) and confirmed with immunohistochemistry (IHC). An additional option for detecting CWD is real-time quaking induced conversion (RT-QuIC), which has been shown to detect CWD earlier in the disease progression than other approaches. We validated the RT-QuIC assay by running 599 samples of retropharyngeal lymph nodes (RPLN) of free ranging white-tailed deer in Pennsylvania in triplicate. Samples were initially tested using BioRad ELISA and positive samples were run in duplicate and confirmed by a board-certified veterinary pathologist with IHC. The RT-QuIC results showed a 98.10% sensitivity and 99.10% specificity at a 25-hour cut off. An additional two blind sets of 40 samples were run and returned a 100% sensitivity and specificity at a 25-hour cut off. The results indicate that RT-QuIC can be a reliable diagnostic tool for testing RPLNs of white-tailed deer using a commercially available substrate. Runs longer than 25 hours were associated with an increase in false positives due to spontaneous substrate conversion events. Sample pooling on RT-QuIC allows a larger number of samples to be included in a single run to increase surveillance in low prevalence areas for CWD. From the 599 samples ran on ELISA, IHC and RT-QuIC, 40 positive and 40 negative samples were selected for pooling. Pools of 10 samples with varying ratios of CWD negative and positive sample were plated to test the sensitivity of the tool. This experiment was repeated at 10^{-2} — 10^{-5} dilutions and replicated. The results showed that RT-QuIC was able to pick up positive samples when pooled with additional samples in a single well.

ORAL PRESENTATION

14. EVALUATION OF BIOMARKERS FOR PREDICTION OF EQUINE PALMAR/PLANTAR OSTEOCHONDRAL DISEASE.

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Palmar/plantar osteochondral disease (POD) is a common injury of the distal condyles of the third metacarpal/tarsal bone in racehorses caused by fatigue of the subchondral bone due to repeated overload. POD is thought to precede metacarpal/tarsal condylar fractures. A biomarker test using equine whole blood could be offered as a clinical diagnostic screening tool to identify horses at risk of injury. The objective of this study was to evaluate changes in candidate biomarkers over one racing/training season in Thoroughbred horses. We hypothesized that racehorses that developed POD would have characteristic changes in pro-inflammatory cytokines and markers of bone metabolism prior to clinical manifestation of the disease compared to healthy horses. Monthly whole blood samples were

collected prospectively. Plasma and mRNA were harvested and stored until further analysis. Samples were selected from 4 racehorses that developed POD diagnosed using standing robotic computed tomography (CT)(RI), 4 healthy Thoroughbred horses exercised on a high-speed treadmill to simulate race training with no pathology diagnosed by standing robotic CT after 6 months (TMH), and 4 healthy racehorses that continued to race successfully in the ensuing season (RH).Trends in the plasma proteins, C-terminal telopeptide of type I collagen (CTX-I) and osteocalcin, and white blood cell mRNA biomarkers (IL-1α, IL-RA, and TGF-β) were evaluated using ELISA and PCR, respectively. CTX-I plasma levels did not change significantly over time or between groups. Osteocalcin levels did not change significantly over time in any group. However, levels in RH (p>0.10) were statistically significantly different from RI (p>0.06). RH osteocalcin levels were not significantly different from TMH and TMH osteocalcin levels were not significantly different from RH or RI groups. RH group circulating IL1RA levels decreased significantly over time during the study period (p<0.001). TMH (p>0.99) horses showed statistically significant differences in overall IL-1RA gene expression compared to the RI (p>0.06) and RH (p<0.001) groups. Gene expression levels of TGF-β and IL-1α in circulating WBCs showed no statistically significant difference between either group and no statistically significant changes over time. Population studies of osteocalcin and IL1RA will be pursued.

15. PREVALENCE OF ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERALES IN SHELTER DOG AND CAT POPULATIONS.

Taylor Frownfelter, Stephen Cole, and Jaclyn Dietrich.

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Antimicrobial resistance remains a global crisis and one of the medical field's greatest current challenges. The increasing prevalence of infections with extended-spectrum beta lactamase (ESBL) and carbapenemase producing Enterobacterales (CPE) is threatening the future use of drugs commonly used in veterinary practice. In human medicine, the presence of ESBL producing Enterobacterales (EPE) is known to increase mortality risk compared to other causes of bacteremia. Since companion animals are serving as reservoirs for these organisms, it is imperative that we gain a better understanding of the potential sources of infection and improve surveillance. These infections were previously thought to be nosocomially acquired, but increasing reports of EPE and CPE in companion animals demonstrated a need for a more inclusive study to establish baselines. This study aimed to determine the prevalence of EPE and CPE colonization in sheltered dogs and cats using 4 different shelter populations in the Philadelphia region. Since sheltered and unhoused dogs and cats have lower incidences of antimicrobial exposure compared to housed dogs and cats receiving veterinary care, we hypothesized that sheltered animals would have a lower prevalence of colonization. Fecal samples were collected from sheltered animals within 1 week of intake to the facility. Each specimen was selectively cultured to identify EPE and CPE. Positive samples underwent whole genome sequencing to characterize the molecular mechanism of resistance and genetic lineage. We found that the prevalence of fecal colonization with EPE and CPE are 3.8% and 0% respectively in sheltered cats and dogs. This was consistent with our hypothesis that the shelter population would have a lower prevalence compared to housed animals. The importance of β-lactams in veterinary practice highlights the need for continued surveillance among companion animals. This data not only helps to inform future surveillance studies, but also helps refine preventative and biosecurity measures in shelters.

16. ROLE OF FAP PROTEASE ACTIVITY ON COMPOSITION, STRUCTURE AND BIOLOGY OF FIBROBLAST-DERIVED MATRICES.

Nathalie Fuhrman, Leslie Todd, Katherine Pelland, Zebin Xia, Pranidhi Baddam, and Ellen Puré.

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Fibroblast activation protein (FAP) is a serine protease and member of the dipeptidyl aminopeptidase family with collagenase activity *in vitro*. FAP, and specifically its proteolytic activity, is upregulated on fibroblasts in the presence of Transforming Growth Factor Beta (TGF-β). FAP has been demonstrated to play important roles in ECM remodeling, by increasing the accumulation of collagen and fibronectin, and in tumor development, making it a potential therapeutic target. But, a comprehensive look at how FAP itself, either through or independent of its enzymatic activity, impacts the composition and structure of stroma has yet to be conducted. We address this gap by comparing the biophysical and biochemical properties of fibroblast derived ECM (FDMs) generated in the presence and absence of FAP and FAP proteolytic activity to better understand the impact of FAP in matrix production and remodeling. Fibroblasts were isolated from congenic C57BL/6 wild-type, FAP-null (FAP-KO) and FAP^{S624A} (enzymatic dead mutant of FAP) mice and were cultured in either control media or media supplemented with 2ng/ml of exogenous TGF-β for eight days. Flow cytometry was used to characterize their phenotype and the matrix produced was visualized using second harmonic generation imaging microscopy. The cells cultured in a TGF-β enriched environment are expected to have a higher expression of FAP and more fibrillar collagen matrix, as has been seen in human fibroblasts. Regarding the different genotypes, we hypothesize that the wild-type cells will produce a more fibrillar matrix. Comparing the wild type to both a global FAP knock out and the FAP enzymatic dead mutant will allow us to tease out FAP's role in driving the fibrogenic phenotype.

Student Support: NIH/Boehringer Ingelheim
Research Funding: Capstan Therapeutics

17. CONTROLLING NEURAL ACTIVITY IN A PRIMATE BRAIN USING OPTOGENETIC TECHNIQUES.

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Recent advancements in neurotechnologies have propelled our current understanding of the complex circuitry of the brain and the etiologies and possible treatments of numerous neuropathies. One such tool, optogenetics, presents a powerful method for targeting and manipulating individual neurons using light1. In this study, the viral vector AAV1-CaMKIIa-SwiChR++-eYFP was injected into the primate brain and its genetic cargo incorporated into target neurons. The SwiChR motif is an inhibitory opsin activated by blue light (437 nm) and deactivated with red light (635nm). As such, neurons expressing this opsin can be deactivated by blue light, and reactivated by red light. Through electrophysiology techniques, we are completing preliminary testing to ensure that the infected neurons respond as hypothesized to blue and red light stimulation. The applications of this research are most obvious when looking at neuropathies such as epilepsy where the electrical functioning of the brain is compromised. Through targeted light pulsation, individual neurons could be activated or inhibited in order to regulate misfiring events in neurological patients.

18. APOPTOSIS AND NON-APOPTOTIC LAMELLAR CELL DEATH ARE FEATURES OF SUPPORTING LIMB LAMINITIS.

Abigail Hamilton[1], Julie Engiles[1-2], Jeaneen Kulp[1], and Andrew van Eps[1].

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Supporting limb laminitis (SLL), a disease of the equine hoof lamellae, is a significant barrier to successful treatment of orthopedic injuries. Although not fully understood, there is evidence that it is an ischemic process. Experimental models of prolonged preferential weight bearing relevant to SLL showed increased TUNEL positive, caspase negative (non-apoptotic) epithelial cell death adjacent to the keratinized axis of the lamellar epithelium. This unique pattern differs from models of endocrinopathic and septic laminitis and is undocumented in natural laminitis. There is no published evaluation of lamellar epithelial cell death in clinical cases of SLL, our study aims to characterize this. Archived mid lamellar tissue sections of multiple limbs (injured, contralateral, and one additional) from clinical SLL cases (n=8) and matched controls (n=8) were utilized. Formalin-fixed, paraffin embedded sections were stained for DNA fragmentation using TUNEL method (a non-specific cell death marker) and using immunohistochemistry for cleaved Caspase-3 (apoptosis specific). Digital image analysis was used to quantify number of positive cells per primary epidermal lamellae (PEL) and counts were tested for normality then statistically compared between cases and controls. Both caspase-3 and TUNEL were significantly increased in the supporting limb compared to control ($p<0.05$). Although it did not reach significance, there appeared to be an increase in positive cell counts in the non-supporting limbs (injured and alternate limbs) for both markers. The increase in both TUNEL and caspase-3 positive cell counts confirms an overall increase in apoptosis in SLL; however, the distinct pattern of TUNEL positive/caspase-3 negative staining specifically observed in parabasal cells confirms the importance of non-apoptotic parabasal cell death/dysfunction in SLL.

19. MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF CESTODES IN NORTH AMERICAN MUSTELIDS.

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Mustelids are considered the largest, most diverse group of carnivorous mammals and are known to harbor several species of tapeworm. The family Taeniidae includes clinically important tapeworms in both human and veterinary medicine, which have become increasingly intertwined due to urbanization. Among Mustelidae, mink, fishers, and weasels are known definitive hosts of genera within the family Taeniidae that have been implicated in zoonotic infections. There is limited knowledge of the prevalence and distribution of cestodes in North American mustelids, and the risk posed to humans and domestic animals. We hypothesized that morphological and molecular approaches on tapeworms harvested from these mustelids would aid in understanding prevalence and distribution and allow us to identify phylogenetic patterns among the Taeniid species. Mustelid carcasses were legally harvested and donated, and necropsies were performed with a focus on collecting gastrointestinal contents. Adult cestode species were found in the small intestine of five hosts. Specimens were cleared in lactophenol and examined under an optic microscope at different magnifications for morphology. Molecular data was acquired by PCR amplification and sequencing of the cox-1 gene, a highly conserved region of the cestode genome. A phylogenetic analysis was performed using the Maximum Likelihood method in MEGA X 10.1 using 1,000 bootstrap replicates. Morphological and molecular data on the tapeworm

recovered from the mink host were consistent with *Mesocestoides sp.* Identification of the tapeworms recovered from the fisher hosts were inconclusive, with molecular data of tapeworms from one host revealing the closest relation to *Taenia pisiformis*. These preliminary results could signify genetic variation within the species. Morphological and molecular analyses on other harvested tapeworms are ongoing.

Research Grant: Institute of Infectious and Zoonotic Diseases
Student Support: Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania

ORAL PRESENTATION

20. EFFICACY OF CART CELLS TARGETING MENINGEAL B CELLS IN AN ANIMAL MODEL OF PROGRESSIVE MULTIPLE SCLEROSIS.

Sara Hernández Suárez[1], Hania Kébir[1], Michael J. May[2], and Jorge I. Alvarez[1].

[1]Department of Pathobiology, and [2]Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Multiple Sclerosis (MS) is an autoimmune inflammatory disease in which the myelin sheath of the Central Nervous System (CNS) is attacked, leading to neurodegeneration and long term sequalae. Despite devastating clinical outcomes and decades of research, progressive (chronic) forms of the disease remain difficult to treat. In progressive MS (PMS), meningeal aggregation of B cells is predictive of poor clinical outcomes and rapid progression of disability. The gold standard animal model for MS, experimental autoimmune encephalomyelitis (EAE), is unable to mimic the pathophysiology seen in PMS. The Alvarez lab has developed a progressive EAE (pEAE) model that presents leptomeningeal B cell accumulation associated with underlying pathology as described in PMS. Thus, we aim to interrogate the causal relationship between meningeal B cell responses and progressive disease using a novel therapeutic intervention: the Chimeric Antigen Receptor T cell (CART) therapy. CART are re-engineered T cells that recognize and kill specific aberrant cells. To target B cells in pEAE mice we injected CART cells directed against CD19 (CART19), a marker expressed on the surface of B cells. Mice were euthanized 2- and 10-days post-CART administration. The CNS and spleen were then processed for flow cytometry and immunofluorescent (IF) imaging. Flow cytometry revealed no significant differences in the absolute number of B cells within the spleen after CART19 treatment, but there was a significant decrease in the control mice at both time points. We found similar numbers of B cells in the CNS of control and experimental mice on day 2. However, by day 10 CART19 administration led to a significant reduction in B cell numbers. IF analysis demonstrated no decrease in spleen B cells on both days for either group while CNS tissues showed significant reduction in leptomeningeal B cell clusters on both time points as compared to the control group. Our findings indicate that CART19 specifically target inflammatory B cells in the CNS and suggest this therapy holds promise as a therapeutic intervention to treat PMS.

ORAL PRESENTATION

21. ABOLISHMENT OF AN INTRACELLULAR RETENTION SIGNAL IN THE CYTOPLASMIC TAIL OF THE SFTSV GLYCOPROTEIN GC IMPROVES ITS INCORPORATION ONTO THE VACCINE VECTOR VSV.

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging bunyavirus that carries a high case fatality rate in human and feline infections. Despite the public health risk posed by this and genetically related viruses, there is no approved vaccine for use in any patient population. Recently we demonstrated that a recombinant vesicular stomatitis virus (rVSV) expressing the SFTSV glycoproteins Gn and Gc in place of the cognate VSV glycoprotein (rVSV-SFTSV) protected mice from lethal SFTSV challenge. However, like many rVSVs encoding bunyavirus glycoproteins, rVSV-SFTSV was challenging to launch from a plasmid recovery system and grew inefficiently in cell culture relative to wild type VSV. The mismatch between the cell surface assembly site of VSV virions and the intracellular retention sites of SFTSV Gn/Gc complexes were responsible for this phenotype. To improve incorporation of SFTSV glycoproteins onto VSV particles, we pursued a genetic strategy to redistribute Gn/Gc complexes to the cell surface. We identified a lysine residue near the C-terminus of Gc through sequence homology that was predicted to function in the intracellular retention of Gn/Gc complexes. Substitution of this lysine with alanine (K-3A) redistributed a recombinant fluorescent protein chimera containing the transmembrane domain and cytosolic tail of Gc from the ER to the cell surface. In addition, K-3A increased expression of full length Gn/Gc on the surface of transfected A549 cells as measured by flow cytometry. K-3A also improved incorporation of fluorescent protein chimeras onto the surface of fluorescent VSV particles as measured by spectrophotometry. Finally, K-3A improved the recovery kinetics of infectious replication-deficient VSV particles pseudotyped with the full-length glycoproteins of SFTSV or the genetically-related Heartland bandavirus. These data suggest rVSVs expressing bunyavirus glycoproteins may be improved by genetically redistributing the glycoproteins to the cell surface. This change in biological behavior may translate to more efficacious vaccines.

22. ACSS1-DEPENDENT ACETATE UTILIZATION REWIRES MITOCHONDRIAL METABOLISM TO SUPPORT AML AND MELANOMA GROWTH AND METASTASIS.

Sabina I. Hlavaty[1,2], Kelsey N. Salcido[1,2], Katherine A. Pniewski[1], Weili Ma[3], Toshitha Kannan[1], Dzmitry Mukha[1], Joel Cassel[1], Yellamelli V.V. Srikanth[1], Qin Liu[1], Andrew Kossenkov[1], Joseph M. Salvino[1], Qing Chen[3], and Zachary T. Schug[1].

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Tumor development involves metabolic changes, such as shifting to using alternative nutrient sources to support vital metabolic pathways. One such nutrient is acetate, which is metabolized into acetyl-CoA by two enzymes: acetyl-CoA

synthetase 1 and 2 (ACSS1 and ACSS2) of the mitochondrion and cytosol, respectively. The resulting acetyl-CoA can enter the tricarboxylic acid cycle, as well as be used to synthesize lipids required for growth. While ACSS2 is a known therapeutic target in a variety of cancers, ACSS1 is understudied. We show that ACSS1 and ACSS2 are differentially expressed in cancer. Melanoma, breast cancer, and acute myeloid leukemia cells expressing ACSS1 readily use acetate for acetyl-CoA biosynthesis and to fuel mitochondrial metabolism *in vitro*, and loss of ACSS1 expression reduces acetate utilization for acetyl-CoA production. ACSS1-dependent acetate metabolism decreases the relative contributions of glucose and glutamine to the TCA cycle. ACSS1 knockdown suppresses acute myeloid leukemia and melanoma tumor growth *in vivo* and inhibits melanoma from metastasizing to the kidney and liver. Our study highlights a key role for ACSS1-derived acetyl-CoA metabolism for cancer growth, raising the potential for ACSS1-targeting therapies in cancer.

ORAL PRESENTATION

23. AN ATLAS OF PRIMATE-SPECIFIC ALU EXONS ACROSS HUMAN TISSUES.

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Transposable elements (TEs) are genomic parasites that insert themselves into animal genomes over evolutionary time, now comprising nearly 50% of the human genome. Historically undervalued, TEs were thought of as "junk DNA." With recent advancements in 'omics technology, TEs are increasingly implicated in health and disease, although their exact roles often remain elusive. Here, we focus on a class of primate-specific TEs, called Alu elements, that arose within the primate lineage ~70 million years ago. With more than 1 million insertions in the human genome, Alus have and continue to shape primate genome architecture. One way Alu elements continue to contribute to genome evolution is through the formation of novel exons in mRNA transcripts. Such Alu exonization may represent an evolutionary strategy by which host genomes can modulate protein expression and protein behavior. Long-read sequencing technology has allowed for more comprehensive detection and quantification of TEs. We have developed ESPRESSO-TEA, a computational pipeline to profile locus-specific TE expression from long-read RNA-seq. ESPRESSO-TEA characterizes TE exons within full-length transcripts, thereby capturing the complex, spliced structures of different TE-containing transcript isoforms. Using this platform, we characterize the effects of Alu exons in the transcriptome across 30 human tissues. We highlight two mechanisms by which Alus act as novel regulatory agents that may drive tissue-specific and species-specific differences in gene expression and protein production: (1) Alus that form poison exons, altering mRNA stability, and (2) Alus that form upstream open reading frames, altering mRNA translation. This exploration delineates the regulatory impact of Alus, showing that Alus are major players in gene regulation and persist as key contributors in primate genome evolution. Lastly, we establish the transcriptional landscape of TE insertions across human tissues. We expect our research will shed light on evolutionary and functional innovations that distinguish human and non-human primates.

24. THE ROLE OF NDR KINASES IN RETINAL VASCULATURE DEVELOPMENT AND RETINAL MAINTENANCE.

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Nuclear Dbf2-related (Ndr) 1 and 2 are paralogous protein kinases known to function in a noncanonical Hippo tumor suppressor pathway. Recent data suggest Ndr kinases are important for maintaining retinal health. Notably, an Ndr2 loss of function mutation causes retinal degeneration in young dogs, and in mice, deletion of Ndr1 or Ndr2 causes multiple retinal phenotypes including decreased amacrine cell abundance and aberrant cell cycle activation in a subset of retinal neurons. However, these studies are limited in that they do not reveal the full scope of Ndr1/2 function in the retina, as expression of one Ndr kinase increases in the absence of the other. Moreover, is unknown if the aberrant mitotic retinal neurons in Ndr mutants are caused by developmental defects or due to cell cycle activation in terminally differentiated neurons. Therefore, we generated conditional double Ndr1/2 KO mice (Ndr1/2 cKO), in which floxed Ndr1 and Ndr2 alleles are simultaneously deleted via a tamoxifen-inducible Cre recombinase. We induced Cre expression in neonate mice and conducted IFM on retinal flat mounts to determine how Ndr1/2 deletion influences retinal vasculature development and observed a marked decrease in vascular outgrowth in Ndr1/2 cKO mice when compared to similarly treated control mice. To surveil retinal degeneration and determine whether Ndr deletion reactivates the cell cycle in fully differentiated retinal neurons, we induced Cre expression in adult Ndr1/2 cKO mice and conducted H&E and IFM analyses with retinal sections. Our initial data reveal a critical role for Ndr kinases in retinal vasculature development and help elucidate mechanisms that control retinal neuron proliferation and maintenance.

ORAL PRESENTATION

25. EVIDENCE FOR CHEMOGENETIC CONTROL OF MANUAL DEXTERITY IN A MACAQUE MODEL.

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Numerous neurological conditions involve disruptions in motor control. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) present a promising avenue for treating fine motor impairments, like those seen in epilepsy, by reversibly modulating neuronal activity. DREADDs are specially engineered receptors that can influence neuronal activity when exposed to specific ligands. Here we explored the application of DREADDs in a macaque model to inhibit fine motor control. Our goal was to comprehend the neural mechanisms governing precise motor movements in macaques, closely mirroring human motor skills, and demonstrate the efficacy of DREADDs in modulating those pathways.

To achieve this, we utilized an adeno-associated viral vector to introduce inhibitory DREADDs into the primary motor cortex. These inhibitory DREADDs decrease neuronal excitability upon administration of the synthetic ligand

Deschloroclozapine (DCZ). We evaluated the impact of DCZ administration on fine motor performance using the Brinkman Board, a behavioral task requiring precise finger movements. With DCZ administration, the macaque exhibited diminished ability to perform the task and increased variability in movements that ceased within the hour of administration. The declining difference in performance between DREADDs activation and no activation over time implies potential neuroplasticity in motor pathways. Varying the interval between DCZ testing also revealed lower task scores with longer intervals, suggesting more effective recovery from transient lesions in neural pathways. Finally, histopathological analysis of the motor cortex confirmed DREADDs expression, indicating successful surgical inoculation.

These findings underscore the feasibility of using DREADDs to selectively inhibit fine motor control in macaque models, offering valuable insights into the neural circuits governing precise motor movements. The demonstrated utility of DREADDs as a tool for investigating the neural basis of motor control opens up avenues for future research and potential therapeutic applications in the realm of fine motor control, with a less invasive mechanism than traditional neurologic studies.

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26. MICROBIOTA-DERIVED METABOLITES PROVIDE PROTECTION FROM SEVERE CLOSTRIDIODES DIFFICILE INFECTION.

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Clostridiodes difficile infection (CDI) is a major public health threat resulting in over 220,000 hospitalizations and 13,000 deaths annually in the United States. *C. difficile* is a gram positive, anaerobic, spore-forming bacterium. Following disruption of the intestinal microbiota, *C. difficile* produces toxins that damage the intestinal epithelial lining. In preliminary studies, our group has found that mice fed two different standard mouse lab diets: Purina LabDiet 5010 & Purina LabDiet 5053 (Diet 1 & Diet 2, respectively) exhibited differences in susceptibility to CDI. Mice fed Diet 1 succumb to severe CDI while mice fed Diet 2 are protected. This protection was independent of *C. difficile* burden and toxin production. Furthermore, germ free mice are susceptible to severe CDI regardless of diet, suggesting that the protective nature of Diet 2 is acting through the host microbiota. To investigate the role of microbial metabolites produced from these diets in the susceptibility of infection, we compared the growth of *C. difficile* co-cultured with cell-free cecal supernatants from mice fed Diet 1 and Diet 2. This study was done to confirm the findings from our *in vivo* study showing that diet-mediated protection is independent of the pathogen replication. In addition, we explored the role of cecal supernatants derived from mice fed Diet 1 or Diet 2 in modulating intestinal epithelial integrity by measuring the transepithelial electrical resistance across a monolayer of cultured colonic epithelial cells. These findings highlight the importance of microbial metabolites in the protection of mice fed Diet 2 and may inform future studies of diet-based therapeutics.

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27. A SMALL MOLECULE TGF-β AGONIST DRIVES FIBROUS TISSUE FORMATION IN MENISCUS TISSUE AFTER INJURY.

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The meniscus, a crucial load-bearing fibrocartilaginous structure in the knee, is commonly injured and has a poor innate healing capacity due to the loss of matrix at the tear interface. The pleiotropic cytokine TGF-β, while capable of stimulating meniscal fibrochondrocytes (MFCs) matrix production, has the propensity for loss of biologic activity when incorporated into drug delivery systems. To address this challenge, we evaluated whether the small molecule TGF-β signaling agonist, SRI-011381 hydrochloride (referred to as SRI), could promote anabolic matrix production in MFCs. Micromechanical (atomic force microscopy, AFM) properties of surgically induced porcine meniscus tears were evaluated over 3 weeks to identify optimal treatment windows. The ability of SRI to promote myofibroblastic conversion of MFCs was evaluated in vitro on both a stiff substrate (glass) and a softer 3D fibrin adhesive commonly used in meniscus tear repair. Micromechanical testing showed decreased stiffness at the meniscus tear edge within 1 week of injury with a similar trend seen at both 2- and 3-weeks. On a stiff substrate, MFCs responded to SRI by increasing their aSMA:actin correlation coefficients and cell area, comparable to TGFβ-3 treated MFCs. In 3D culture, fibrin + MFCs supplemented with SRI exhibited increased expression of genes downstream of TGF-β, including DCN, SOX9, YAP, COL1A1, and ACTB. These findings suggest that meniscus defects rapidly lose stiffness within 1 week of injury, emphasizing the need for early intervention. In vitro, MFCs treated with SRI showed myofibroblastic conversion and increased expression of TGF-β-induced genes. Collectively, these data suggest that SRI holds promise as a small molecule for site-specific delivery to meniscus injuries by promoting matrix deposition at the tear interface. Future work will evaluate the efficacy of SRI when delivered to meniscus tears in a large animal (porcine) injury model.

Research Grants: NIH (R01 AR056624 and T32 GM007170) and the VA (I01 RX003375 and IK1RX003932-01A1).

28. INTERLEUKIN RECEPTOR THERAPEUTICS ATTENUATE SYNOVIAL INFLAMMATION IN CANINES FOLLOWING CRUCIATE LIGAMENT INJURY.

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Tears in the cranial cruciate ligament (CCL) of dogs, equivalent to the anterior cruciate ligament (ACL) in humans, contribute significantly to knee joint disease in both species. Current treatments primarily focus on joint stability, often overlooking the synovial pathology that perpetuates widespread inflammation. In this study, we investigated the structural (histology), micromechanical (atomic force microscopy, AFM), and transcriptional (RNA-sequencing) profiles of healthy and CCL-injured canine synovium. Using our RNA-sequencing findings, we assessed the effectiveness of IL1 and IL6 receptor therapeutics in reducing inflammation in synovial organ cultures from dogs with CCL tears. CCL tear synovium had increased histologic scores for inflammation, vasculature, fibrosis, and hyperplasia, and higher

indentation moduli on micromechanical testing compared to healthy controls. RNAseq revealed that CCL synovium had enriched immune response and cell adhesion pathways, with increased expression of interleukins (IL1 and IL6). In addition, genes associated with negative regulation of Rho protein signaling were reduced. In CCL synovium explants, expression of PRG4 (lubricin) decreased while inflammatory markers IL1β, IL6, and PTGS2 increased. When CCL synovium was treated with an IL1 receptor antagonist or an IL6 receptor inhibitor, both restored PRG4 expression to control levels while decreasing expression of IL1β, IL6, and PTGS2. This study highlights that structural and mechanical synovial disease progression in dogs with spontaneous CCL tears closely mirrors that in humans with ACL tears. Notably, both RNAseq and AFM showed heterogeneity across donors, suggesting the need for a tailored approach based on the stage of disease progression to enhance the clinical efficacy of treatments. Ongoing clinical trials are evaluating the efficacy of interleukin receptor therapeutics applied intra-articularly to client-owned canines with CCL tears. Overall, this study emphasizes the value of canines with spontaneous CCL tears as a clinically relevant model to evaluate novel therapeutics targeted at knee joint inflammation after injury.

Research Grants: NIH (R01 AR056624 and T32 GM007170) and the VA (IK6 RX003416 and I21 RX004628).

29. DIVERSITY OF GASTROINTESTINAL HELMINTHS AND LUNGWORMS ISOLATED FROM MUSTELIDS IN PENNSYLVANIA AND NEW YORK.

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Wildlife parasitology is an important field of research as many parasites have a significant impact on wild animal, domestic animal, and human health. Mustelidae is a diverse family of carnivorous mammals that includes weasels, mink, fishers, otters, badgers, and others. Many of these species commonly inhabit regional forests and waterways, yet research is limited on the helminth species that infect these animals. The goal of this study was to determine the diversity of gastrointestinal (GI) and respiratory parasites infecting mustelids in Pennsylvania and New York and potential risks to domestic animals and humans. We collected harvested and donated mustelids from across both states and extracted feces and intestinal contents postmortem. Feces were examined for parasite eggs and cysts using centrifugal fecal flotation with Sheather's sugar solution. Lungworm first-stage larvae were identified using zinc sulfate flotation on intestinal contents. All parasitic life stages were identified and described morphologically using an optic microscope. We discovered a wide variety of GI helminths including *Metorchis conjunctus, Aonchotheca putorii, Uncinaria* sp., and *Physaloptera* sp., along with one protozoal species *Cystoisospora* sp. Additionally, we found four lungworm species *Eucoleus boehmi, Eucoleus aerophilus, Crenosoma vulpis* and *Filaroides* sp. *Metorchis conjunctus, Uncinaria* sp., and *E. aerophilus* are zoonotic helminths that pose a public health concern, and all isolated parasites possess the ability to infect domestic dogs and cats. These results give us insight to the prevalence, host range, and environmental niches of several GI and respiratory parasites in Pennsylvania and New York and provides novel information on parasitic disease implications veterinarians and medical doctors in the area should be aware of.

Research Grant: Institute for Infectious and Zoonotic Diseases
Student Support: Institute for Infectious and Zoonotic Diseases

ORAL PRESENTATION

30. A STUDY ON THE UPTICK OF DISEASE: PATHOGEN SURVEILLANCE IN TICKS AND WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN PENNSYLVANIA USING A COMBINATION OF QPCR AND METAGENOMIC SEQUENCING.

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The state of Pennsylvania is home to lush forests and mountains that provide an ideal habitat to support large populations of white-tailed deer, *Odocoileus virginianus.* Cervids frequently harbor multiple tick species and their associated pathogens, which can pose a significant health risk to deer and humans alike. This study sought to identify known zoonotic pathogens in ticks and matched deer blood samples collected from across the state of Pennsylvania. 184 ticks and host blood samples were collected from 62 deer. Ticks were identified by sex, genus, species, age, and extent of engorgement, and total nucleic acids were extracted using the Qiagen DNA/RNA AllPrep kit. Nearly all of the ticks we identified were either *Ixodes scapularis* or *Haemaphysalis longicornis*, the latter being an introduced species in the state of Pennsylvania for which little is known about distribution and contribution to pathogen transmission. All ticks and matched deer blood samples were tested by QPCR using primer-probe assays for pan-*Ehrlichia,* pan-*Rickettsia,* pan-*Apicomplexa,* pan-*Borrelia,* and Powassan virus lineage II. Our results identified a prevalence of 40% (73/184) for *Apicomplexa* spp., 25% (47/184) for *Ehrlichia* spp., 38% (28/74 non-Ixodes ticks) for *Rickettsia* spp., 10% (18/184) for *Borrelia* spp., and 0% for Powassan virus. QPCR analysis of deer blood samples is ongoing. Finally, we selected 48 of the 184 ticks for 'shotgun' metagenomic sequencing to determine sensitivity for detecting known tickborne pathogens, as well as discovering potential novel tickborne pathogens. Continued research on these samples include RT-QuIC for prions among ticks from Chronic Wasting Disease suspect cases. This study on the prevalence of tickborne diseases gives researchers an evolving look into the landscape of zoonotic and infectious pathogens present in ticks and their deer host in verdant Pennsylvania.

Research Grant: Richard King Mellon Foundation

31. SPATIAL ASSOCIATION OF DOG MOBILITY AND DEVELOPMENT OF A SPILL-OVER MODEL OF HYDATID DISEASE IN AN ECHINOCOCCOSIS-AFFECTED AREA IN JUNÍN, PERU.

Katherine Morucci[1,2], Lizzie Ortiz Cam[3], Elvis Diaz[3], Guillermo Porrás-Cotrina[3], Javier Bustos[3], Cesar Gavidia[4], Dustin Brisson[5], and Ricardo Castillo-Neyra[1,3].

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Cystic Echinococcosis (CE) is a parasitic disease that is caused by *Echinococcus granulosus* and poses significant zoonotic risk to humans. While dogs serve as the definitive host for *E. granulosa,* eggs that are shed in dog feces hatch upon consumption by an intermediate host, where parasite larva contribute to slow-growing cystic lesions. Humans

that consume *E. granulosa* eggs can become infected and develop such lesions, which often go undetected for years until cysts grow large enough to contribute to life-threatening disease that warrants surgical intervention. While the prevalence of CE in the Central highlands of Peru has previously been estimated around 5-7%, disease experts agree that the true prevalence is likely higher given the protracted period of asymptomatic disease, reduced medical access of at-risk populations and poor understanding of local disease epidemiology.

This study sought to quantify the epidemiological behavior of CE in Pachacayo, Peru, with the goal of developing and measuring the efficacy of future intervention programs. To address this goal, this study implemented three distinct strategies: 1) the development of an epidemiological model of regional CE dynamics, 2) placement of GPS-collars to tract dog mobility to understand the movement of the definitive host of *E. granulosa* on the landscape, and 3) detection of *E. granulosa* positive dogs using copro-ELISA. Preliminary results suggest that dogs are visiting a local slaughterhouse, which may provide access to infected viscera from slaughtered sheep. Future directions include examining whether dogs are moving in a goal-directed manner to and from the slaughterhouse, or if this movement is random. Results from the copro-ELISA indicate that approximately 75% of the dogs tested are suspected to be positive for *E. granulosa.* These preliminary results are being used to inform our understanding of the local disease ecology and the development intervention strategies targeted to reduce human cases.

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Student Support: VMD-PhD Program; Medical Scientist Training Program; Field costs were provided by the Department of Biostatistics, Epidemiology and Informatics.

32. LINEAGE PLASTICITY OF YY1-NULL PRO-B CELLS ENABLES THEM TO DEVELOP FEATURES OF NK CELLS.

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The ability to direct cell development from one lineage to another has numerous applications in regenerative medicine. Preliminary data from the Atchison laboratory indicate that knock out (KO) of transcription factor YY1 in pro-B cells enables them to develop features of T lineage cells in appropriate culture conditions. To determine if YY1-null pro-B cells can develop into additional hematopoietic lineages, I cultured them in conditions conducive to natural killer (NK) cell differentiation. I optimized the conditions for NK cell development using hematopoietic progenitor cells, which I exposed to various cytokine and stromal environments. To determine the phenotype of the resulting cells, I used flow cytometry to assess the presence of NK cell surface markers. Using conditions selected based on ease of maintenance, overall cell yield, and generation of cells with NK cell surface markers, I cultured $yy1^{f/f}$ (wild-type (WT)) or Mb-1CRE$yy1^{f/f}$ (YY1-null) pro-B cells. After 3 weeks, more YY1-null pro-B cells were beginning to acquire NK cell surface marker NK1.1 compared to WT pro-B cells; however, the fraction of cells expressing NK1.1 was still very low. Ongoing experiments will allow the WT and YY-null pro-B cells to differentiate for longer, and I expect the YY1-null cells to continue to gain NK1.1 positivity, while expression remains low in WT cells. Future experiments will investigate whether the cells generated from YY1-null pro-B cells demonstrate NK cell cytotoxic effector function. If YY1 KO allows cells to develop features of NK cells as well as T cells, it may be possible to direct cells from one lineage to another by manipulating YY1.

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33. GENERATION OF SWINE GAMMA DELTA CAR T CELL LINES.

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Chimeric antigen receptor (CAR) T cell therapies use T cells engineered to target antigens expressed in cancer cells. Research has been developed to optimize this technology using animal models such as rodents. Unfortunately, rodent models have demonstrated poor clinical predictive value, limiting the translatability to man. Swine share many anatomical, physiological, and immune characteristics with humans, making them ideal for clinical translation. Gamma delta T cells (GDCs) are a subset of CD3+ cells that can be found in the peripheral blood and mucosal tissues and have strong anti-tumor properties and no MHC restriction. This makes them an attractive cell type as there is minimal risk for inducing graft-versus-host disease compared to alpha-beta T cells. We sought to take advantage of both CAR technology and GDC characteristics to generate a large animal CAR GDC. Using swine peripheral blood mononuclear cells, we isolated and expanded GDCs for transduction with a characterized CAR molecule. Optimization of expansion techniques are being studied using zoledronic acid, IL-2, and artificial antigen presenting cells (aAPCs) based on a K562 cells line expressing human CD86 and CD64 loaded with anti-swine CD3. To date, using 1uM ZA, 1000 IU/IL2 and a 10:1 aAPC:GDC ration lead to a >4-fold expansion of absolute GDCs within 8 days. Flow cytometry data suggests that the majority of these GDCs are CD4- and CD8-. Lentiviral transduction of GDCs with an anti-HLA A2 CAR, characterized previously in human and swine T cell effectors, yielded up to a 24.8% CAR transduction making them a viable new cell line for clinical application upon further refinement of the protocol.

Research Grant: ITMAT
Student Support: Internal funds

34. ACQUIRED IMMUNITY TO SECONDARY PENETRATION BY STRONGYLOIDES RATTI IS DEPENDENT ON SKIN BARRIER INTEGRITY ALTERATIONS DRIVEN BY THE ALARMIN CYTOKINE IL-33.

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Soil-transmitted helminth (STH) infections affect approximately 1.7 billion individuals, and while humans typically experience recurrent infections, data has shown that mice are less susceptible to worm penetration upon secondary challenge. Historically, STH infection research focuses on larval activity and protective immune responses at mucosal barriers, such as the lung and gastrointestinal tract. However, the architecture and immune cell repertoire of the skin in the context of re-exposure to helminth infection has not been studied. Data shows that skin penetration of *Strongyloides ratti* is significantly reduced upon secondary exposure to infectious third stage larvae (iL3). We hypothesized that a major component to secondary resistance is IL-33-dependent modulation of extracellular matrix (ECM) molecules and adhesion factors which promote a robust skin barrier. A Qiagen RT^2 Profiler PCR Array that focused on murine ECM genes and cell adhesion markers was performed on skin homogenates from secondary infected IL-33-deficient mice to determine genes of interest (GOI) that may operate in an IL-33 dependent manner. Additionally,

immunofluorescence assays (IFA) were performed on naïve and secondary-infected skin from the footpad to visualize how IL-33 controlled immune cell localization at the site of infection. Col6a1, Ccn2, and Itgb1 were three GOIs whose expression found to be markedly increased in wild-type secondary-infected tissue; this upregulation was absent from all other cohorts. IFA revealed dense clusters of M2-differeniated macrophages in the stroma of wild-type infected skin. Future experiments will focus on investigating how GOIs are expressed in an IL-33 dependent manner and whether their depletion affects acquired resistance. IFA will be performed to investigate the density of these ECM molecules and adhesion factors following secondary infection.

35. WHAT MAKES THEM STICK?: UNDERSTANDING THE ROLE OF CYCLIC AMP IN THE ADHESION MECHANISM OF TRYPANOSOMATID PARASITES.

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Trypanosoma cruzi is the parasitic agent of Chagas disease, a high-mortality, zoonotic illness growing in prevalence in the southern United States. Trypanosomatids, including *T. cruzi,* adhere to the gut of their insect vectors as a mode of transmission. Once established in the canine, *T. cruzi* causes irreparable damage to the patient's myocardioctyes. This harsh pathophysiology, coupled with a lack of reliable treatments for Chagas disease, highlights the need for new control measures: potentially one focused on decreasing the transmission of *T. cruzi* by its insect vector, the triatomine bug? In this project, we utilized a close, non-infectious relative of *T. cruzi*, *Crithidia fasciculata,* as a model to study trypanosomatid adhesion. Using an in vitro assay previously confirmed to mimic *C. fasciculata* adhesion to its insect vector, we investigated the effects of cAMP concentrations on the parasite's ability to adhere. An established phosphodiesterase inhibitor in trypanosomes, NPD1, raised cAMP levels in *C. fasciculata* and subsequently lead to a blockage of cell adhesion. A heterozygous mutation in the receptor adenylate cyclase 1 (RAC1) protein increased adhesion in *C. fasciculata*, which we attribute to an anticipated decrease in cAMP levels. Therefore, we believe that cAMP plays a regulatory role in *C. fasciculata* adhesion as its concentration is inversely related to the level of parasite adherence. Additionally, both adherent and swimming RAC1 mutant cells showed a decreased rate of growth, suggesting another potential role of this protein in cell division.

36. DEVELOPMENT OF LYMPHOHEMATOPOIETIC TUMOR CELL LINES FOR THE ESTABLISHMENT OF A PORCINE MODEL OF CANCER.

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Animal models of human disease have always played a central role in biomedical research, but the predictive value gap between humans and rodent models have hindered the applicability of these discoveries. Swine are ideally positioned as a cancer model as their anatomy, physiology, and immunology closely resemble that of humans. However, the current lack of transplantable hematologic cancers limits the use of swine as a model for cancer studies. The Oncopig

model is a transgenic swine model that recapitulates human cancer through development of site and cell specific tumors following Cre recombinase induced expression of heterozygous $KRAS^{G12D}$ and $TP53^{R167H}$, mutations that have been demonstrated to be key drivers of human cancer. We have previously determined lymphoid leukemias can be generated in vivo by injection of Adenovirus Cre-recombinase into secondary lymphoid organs surgically in three Oncopigs. We found that this procedure resulted in lymphadenopathy, suggestive of lymphoma. Flow cytometry data from tissue samples showed a massive predominance of CD8+ T cells. We are now transforming isolated Oncopig PBMCs and CD3+ T cells *in vitro* with the goal of generating T cell specific leukemias to later be transplanted back into the host swine. To date, we have isolated CD3+ T cells from Oncopigs and transformed them by Cre-mediated oncogene activation. Preliminary flow cytometry data from these samples have also shown a predominance for CD8+ T cells to expand, corresponding with the results seen *in vivo*. The overall goal is to establish a reliable and reproducible swine cancer model and increase the predictive value of pre-clinical cancer studies.

Research Grant: ITMAT, Internal funds
Student Support: NIH Grant T35 OD 010919-25

37. EFFECT OF MUTATIONS IN THE GLYCOPROTEIN OF DABIE BANDAVIRUS (SFTSV).

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Dabie bandavirus is an emerging tick-borne virus found predominantly in Southeast Asia. It is also referred to as Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) due to the clinical symptoms associated with infection. Some infected patients exhibit mild flu-like symptoms, but approximately 12-30% of patients develop severe hemorrhagic disease and succumb to infection. Currently, there are no FDA-approved therapeutics or vaccines for the treatment or prevention of SFTSV. Due to this and the high mortality rate of infected patients, the World Health Organization has designated SFTSV a priority pathogen. Recent research has focused on developing a recombinant VSV vaccine that expresses the SFTSV glycoprotein (rVSV-SFTSV). This is because VSV can readily incorporate foreign glycoproteins onto its surface and can induce an effective immune response to these foreign glycoproteins. Moreover, VSV is nonpathogenic and has a low seroprevalence in humans, making it a very safe and effective vaccine candidate. However, the rVSV-STSV vaccines that have been developed are very attenuated in cell culture, making it difficult to produce the vaccine in quantities large enough for vaccination trials. Recent studies by our lab and others have identified mutations in the glycoprotein of SFTSV that increase the titer of rVSV-SFTSV in cell culture, but these mutations have never before been combined into one vaccine candidate. We sought to determine the effect of three different mutations on incorporation of the SFTSV glycoprotein onto VSV pseudoviruses, and we discovered that each mutation alone increases the titer. However, the mutations appear to have a synergistic effect when combined together, significantly increased the titer of pseudoviruses. Future work will elucidate the mechanism(s) by which these mutations increase the titer.

38. DEFINING THE ROLES OF SKIV2L & TTC37 IN COLONIC EPITHELIAL HOMEOSTASIS AND DISEASE.

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Very Early Onset Inflammatory Bowel Disease (VEO-IBD) is a classification of inflammatory disorders that occur in children under 6 years of age. Like adult chronic IBD such as Crohn's Disease, the number of pediatric cases has increased over the past years. Tricho-Hepato-Enteric Syndrome (THES) is a rare, monogenic form of VEO-IBD with a heterogeneous clinical presentation. For example, alongside characteristic intractable diarrhea, some patients may present with cardiac defects, liver and hair abnormalities and facial dysmorphia. THES has been linked to mutations in two genes, *TTC37* and *SKIV2L* which encode subunits of the Ski complex, a cofactor of the cytoplasmic RNA exosome. Current literature suggest that the Ski complex may not only be involved in post-transcriptional RNA processing but also in translation given its purported interaction with the ribosome. The contribution of these gene variants to THES pathogenesis, and particularly to epithelial barrier dysfunction or homeostasis, is poorly understood. Furthermore, current therapies for THES tend to only address immune dysregulation rather than primarily the impaired epithelial barrier. Therefore, a more concrete understanding of the role of these gene variants in the intestinal epithelial barrier during both disease and healthy states is necessary if we are to address THES and other monogenic VEO-IBD. The following study utilized 3D colonoids derived from patients with THES to model the disease *in vitro* and characterize how the gene variants described contribute to disease pathogenesis. Furthermore, the study sought to define the roles of SKIV2L and TTC37 in colonic epithelial homeostasis and disease, as well as expand our current understanding of the role of the Ski complex in RNA degradation and processing pathways. Preliminary data shows that colonoids from patients with THES grow at a reduced frequency compared to healthy control colonoids, and that there is altered gene expression observed in these *in vitro* models.

39. IMMUNOMODULATION USING A CELASTROL-LOADED CYCLODEXTRIN NANOPARTICLE ON CARDIAC TISSUE IN MICE AFTER ISCHEMIA REPERFUSION INJURY.

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Cardiac ischemia reperfusion (IR) injury causes sterile inflammation that can lead to cardiac remodeling and decreased function. Modulation of the immune response following ischemia can have improved long-term outcomes. Celastrol is a compound known to convert pro-inflammatory M1 macrophages to pro-healing M2 macrophages. The aim of this study was to characterize the macrophage immune response after IR injury in mice and to determine the immunomodulatory

potential of celastrol in cardiac tissue following IR. In this study, a macrophage-targeting cyclodextrin nanoparticle (CDNP) was utilized to deliver celastrol to inflamed cardiac tissues. Adult C57BL/6 mice underwent a left thoracotomy, and the left anterior descending (LAD) artery was ligated for 45 minutes and then released to allow for reperfusion. To characterize the immune response, animals were separated into Day 1, Day 3, Day 5, and Day 7 post injury cohorts. The left ventricular infarct and border zone tissue was processed for flow cytometry, which demonstrated peak numbers of M1 macrophages on Days 2 and 3 post IR injury. To assess the biodistribution of the CDNP, live near-infrared imaging studies on animals treated with labeled CDNP were performed on the day of peak M1 expression (Day 2). Imaging demonstrated an accumulation of CDNP in the inflamed heart within 24 hours of injection (Day 3) and retention for up to 72 hours (Day 5). Using this information, animals were treated with 10 mg/kg IV of celastrol-loaded CDNP on Day 2 following IR injury to coincide with peak inflammation. Flow cytometry post treatment showed a decrease in M1 numbers compared to control animals, suggesting favorable immunomodulation following IR injury in treated animals.

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40. OREXIN REGULATION OF ANESTHETIC STATE STABILITY.

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A potential therapeutic approach toward mitigating serious general anesthetic complications like intraoperative awareness with recall and delayed emergence is pharmacological manipulation of anesthetic state stability. Anesthetics engage endogenous sleep-wake neurocircuitry and transitions between sleep and wake are largely regulated by orexin peptide-expressing hypothalamic neurons. However, the direct contribution of the orexin system to the stability of entering and exiting the anesthetized state remains unclear. Here, we test the hypothesis that selective orexin activation stabilizes, while antagonism destabilizes, the anesthetic state. We exposed wildtype mice to equipotent, sub-hynotic concentrations of volatile anesthesia, where at the pharmacokinetic steady-state individuals fluctuate stochastically between responsiveness and unresponsiveness as measured by righting reflex. These spontaneous state fluctuations exhibit resistance to state transitions (RST) such that when responsiveness is assessed serially, there is an increased probability of the individual remaining in the same state as previously observed. Larger RST values reflect increased state stability. We measured RST on trials where mice received systemic injections of the orexin receptor-2 agonist YNT185 (40mg/kg), dual orexin receptor antagonist suvorexant (20mg/kg), or corresponding vehicle. Compared to vehicle, YNT185 treatment significantly increased, while suvorexant treatment significantly decreased, RST. We replicated this latter finding using selective genetic ablation of orexin neurons in a separate cohort. While orexin promotes wakefulness, these RST changes under anesthesia were not associated with significant changes in individual response probability nor asymmetric changes in the probability of remaining in the responsive state. Rather, YNT185 stabilized, and suvorexant destabilized, the probabilities of remaining responsive or unresponsive on consecutive trials equally. These results are the first to show that the propensity to exhibit state transitions under steady-state anesthesia can be manipulated bidirectionally by targeting the orexin system. While orexin receptor-selective drugs are in development for sleep disorders, future studies should explore their role as therapeutic adjuncts in the peri-anesthetic period.

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41. DO FEMALE RATS DEMONSTRATE HEIGHTENED ANXIETY, FEAR, AND SENSORY SENSITIVITY POST-TBI COMPARED TO MALES?

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Mild traumatic brain injury (mTBI) affects millions of people per year. mTBI has recently gained more attention as it is highly comorbid with post-traumatic stress disorder (PTSD), however their relationship is still not well understood. Research investigating the effects of TBI in both humans and animals has largely only included male subjects up to this point, therefore there is a need to investigate sex differences when studying the effects of TBI. In this study, female and male Long-Evans rats underwent a 5mm left lateral craniotomy followed by a fluid percussion injury (FPI) to model mTBI, and control rats underwent a sham procedure. Anxiety behaviors were assessed both pre- and post-injury in an open field and elevated plus maze. To test for sex differences in sensitivity to sensory stimuli we evaluated the freezing response to seven 30 second presentations of novel white noise 48 hours post-injury. Finally, to understand how TBI alters fear memory, rats underwent a single trial of fear acquisition one day before surgery; rats received a series of 10 0.7 mA foot shocks. On days 15 and 16 post-injury rats underwent four trials of fear extinction. On day 35 post-injury one trial of reinstatement was conducted in which rats received a single 0.7 mA foot shock; this was followed by an additional three trials of fear extinction. We hypothesize that FPI females will demonstrate more anxious behaviors, freeze more in response to a novel sensory stimulus, and take longer to extinguish learned fear when compared to all other study animals. Understanding how TBI alters anxiety levels, fear memory and sensory processing, and how sex influences those responses, may contribute to our understanding of TBI-PTSD comorbidity.

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42. PATHWAY ANALYSIS IDENTIFIES ADRENERGIC RECEPTOR SIGNALING AS POTENTIAL MECHANISM OF CMVD-ASSOCIATED FOG2^{S657G}.

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Coronary microvascular disease (CMVD), defined as disease of the coronary pre-arterioles, arterioles, and capillaries, is a significant cause of ischemic heart disease. Increased sympathetic tone, which is mediated by adrenergic (Adr) receptors, has been implicated in the pathogenesis of CMVD. Cardiomyocyte Fog2 is a transcriptional co-regulator crucial for the development and maintenance of the coronary microvasculature. In prior work, we identified a variant of FOG2 (A1969G, S657G) which is associated with CMVD. The mechanism of how FOG2^{S657G} contributes to CMVD is not known. We hypothesize that FOG2^{S657G} promotes CMVD via dysregulation of cardiac adrenergic receptor expression. RNAseq gene expression data from 8-week-old mouse hearts showed that Fog2^{S657G} increased adrenergic gene expression. Gene set enrichment analysis of the top differentially regulated genes identified Adr receptor activity as the top gene ontology (GO) molecular function (Enrichment score (ES) 7.92, nom_p=3.64E-4). This was driven by increased expression of *Adrb1, Adrab1, Adrab2,* and *Adrad1*. We confirmed that *Adrb1* and *Adra1b* were increased in FOG2^{S657G}

mouse hearts and iPSCs through qRT-PCR, Western blot, and immunofluorescence. To determine whether these changes resulted in systemic alterations of heartrate (HR) and blood pressure (BP), we analyzed electronic health record data from 11,105 patients: 8,206 AA, 2,665 AG and 234 GG, and found no differences. Therefore, $FOG2^{S657G}$ may affect the coronary microvasculature directly through the cardiomyocytes independent of HR and BP. Future studies will further define the mechanism by which $FOG2^{S657G}$ interacts with transcription factors to regulate expression of Adr.

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43. THE MOLECULAR ROLE OF TYPE III COLLAGEN DEFICIENCY IN CARTILAGE MATRIX ASSEMBLY.

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Osteoarthritis (OA) is a widespread and debilitating joint disease characterized by the irreversible breakdown of the cartilage extracellular matrix (ECM). However, successful regenerative strategies are challenged by our limited understanding of the molecular activities that regulate the proper assembly of cartilage matrix. Recent data from the Volk laboratory support that type III collagen (Col3) is an essential regulator of cartilage ECM structural integrity and cartilage health. We hypothesized that Col3 could serve as a target for recapitulating assembly of native cartilage matrix during cartilage repair.

To elucidate the role of Col3 loss in cartilage neo-matrix assembly and integrin-dependent mechanotransduction, we compared expression of pro-chondrogenic genes and integrins in control and Col3-deficient ATDC5 cells, a chondroprogenitor cell line, cultured under chondrogenic conditions. SiCol3a1 treatment reduced Col3 gene expression > 90% at two days post-transfection compared to controls (SiCntrl). Surprisingly, RT-qPCR revealed the upregulation of pro-chondrogenic genes Col2 and ACAN in the Col3-knockdown cells compared to controls. Pro-chondrogenic integrin α_{10} (*ITGA10*) and pro-fibrogenic integrin α_{11} (*ITGA11*) were similarly upregulated. To query whether inflammatory conditions (to model of cartilage degradation) would reconcile our results with previous *in vivo* data, we added exogeneous IL-1β (10 and 50 ng/μL) two days post-transfection for 24- and 48-hours. Based on induction of matrix metalloproteinases, 24 hours of 10 ng/μL IL-1β treatment was sufficient to activate an inflammatory cascade. While Col2, ACAN, ITGA10, and ITGA11 were upregulated in chondrogenic conditions, this response was not evident or was dampened under inflammatory conditions.

While our findings do not support the hypothesis that Col3 knockdown is detrimental to chondrogenesis, future studies will examine the role of Col3 deficiency in 3D culture and cartilage explants. This will help rectify our understanding of translating from *in vitro* to *in vivo* studies, a longstanding challenge in cartilage research.

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44. EVALUATION OF AAV9-PGUSB-NPC1 FOR TREATING NPC1 DISEASE.

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Niemann-Pick disease type C1 (NPC1 disease) is an inherited disorder caused by loss-of-function mutations in the *NPC1* gene, leading to lysosomal accumulation of cholesterol and sphingolipids. Disease onset is typically in childhood and presents as clumsiness or learning disability. Symptoms include cerebellar ataxia, cognitive impairment, dystonia, supranuclear gaze palsy and dysphagia. Disease in the feline model follows an orthologous mutation to that in humans and recapitulates symptoms observed in patients. In previous works, biweekly administration of 2-hydroxy-propyl-beta-cyclodextrin (HPβCD) in the cisterna magna prevented ataxia and Purkinje cell storage and death but was ototoxic and did not resolve all symptoms. In prior gene therapy studies, affected cats treated with 1E14 vg of AAV9-pGUSB-NPC1 administered at the cisterna magna showed increased Purkinje cell survivability and diminished ataxia. We hypothesized that optimizing IC administration of AAV9-pGUSB-NPC1 would optimally transduce cells in the cerebellum and result in a more uniform outcome. Using immunological markers for Purkinje cells and NPC1, we identified improved viral transduction of Purkinje cells and concomitant increases in cell survivability and axonal preservation in cats treated optimally. Treated cats also displayed normal gaits and saccadic eye movements, without the ototoxicity seen following treatment with cyclodextrin. These data suggest that treating NPC1 disease with a viral vector is effective in minimizing Purkinje cell loss and cerebellar disease. This study highlights the potential of AAV9-mediated gene therapy to successfully treat inherited cerebellar neurodegenerative disorders.

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