

BioMed Insider

Research Newsletter

cvmresearch@oregonstate.edu

Fall 2018



Artwork by Sally Byrd, M.D.



Oregon State
University

Carlson College of Veterinary Medicine
Department of Biomedical Sciences



Editorial:

Letter from Luiz E. Bermudez, M.D., Department Head, Biomedical Sciences

Research is an arduous process. It takes a long time for your findings to be incorporated in the common knowledge, and even longer to be assimilated into the clinics. It requires technical and intellectual skills and persistence.

Although the Carlson College of Veterinary Medicine is a small institution, we have a vibrant research component. Many faculty are recognized to be associated with advances in medicine (human, veterinary) epidemiology, public health, and other fields. Appropriately for an educational institution, students, are part of the research, and many times are part of the inspiration.

That said, “basic” research has sometimes been valued over work that is applied, or “clinical”, as if it requires a greater degree of intellectual rigor. Of course, there is no question that insights in genetics, molecular biology and other basic sciences, have opened doors for healing and preventing diseases. Currently, good evidence is found for the adoption of individualized medicine. It is clear, however, that basic and clinical science exist on a continuum, and the individuals carrying out the work ultimately need to be connected to optimize their ability to improve animal, human and environmental health of our communities.

For the past two decades, pressure has been mounting to translate basic science discoveries into clinical advances. The result is higher esteem for applied research, while still recognizing the importance of basic science as the foundation of medical progress.

The Carlson College of Veterinary Medicine faculty hold funding from federal institutions (NIH, USDA, NSF) and from a large number of foundations. Our goal for the future is to emphasize research areas like immuno-nutrition, inflammation, metabolism, infectious disease, neuroscience, regenerative medicine, cardiovascular diseases and oncology. The basic knowledge accumulated will allow us to improve the development of a robust preventive medicine approach to be applied for animals and humans.

This current issue of Biomed Insider acknowledges our efforts to expand opportunities for basic scientists to explore the world of clinicians and vice-versa. We feel encouraged about the success of the pathway for the improvement of the well-being of patients.

Luiz E. Bermudez, M.D., Department Head
Department of Biomedical Sciences
Carlson College of Veterinary Medicine
Oregon State University

TABLE OF CONTENTS



Editorial:

Letter from Luiz E. Bermudez, M.D., Department Head, Department of Biomedical Sciences, Carlson College of Veterinary Medicine, Oregon State University -----Page 1

The Scope: “When Fed Foods with Similar Palatability, Healthy Adult Dogs and Cats Choose Different Macronutrient Compositions” -----Page 2

The Scope: “*Lactobacillus gasseri* Ameliorates Diet-Induced Diabetes in Mice via Changing Lipid Metabolism Gene Expression in the Gut” ----- Page 2

The Scope: “Circadian Dysregulation via Exposure to Light at Night Alters DNA Methylation of Cancer-Associated Genes in Murine Mammary Tissue” ----- Page 3-4

The Scope: “Translator” ----- Page 5-6

The Scope: “Creating New and Innovative Therapies for the Treatment of Leishmaniasis”-----Page 7

The Scope: “Host Immune Response and Antigen Presentation” ----- Page 8

Paws for the News: “Cancer: Challenges of Surgical Removal” -----Page 9-10

Paws for the News: “The Use of Vesicles (Exosomes) to Treat Diseases” -----Page 10

Paws for the News: “Respiratory Syncytial Virus” -----Page 11-12

Paws for the News: “Can we Control Bovine TB by Using a Novel Vaccine?”-----Page 12

Paws for the News: “The Future of Orthopedic Medicine” ----- Page 13-14

Paws for the News: “A Retrospective Study in the Hospital to Identify Potential Causes of Infection”-----Page 15

Paws for the News: “Clinical Experience: Improving Care Via 3D Modeling”----- Page 16

CVM Cutting Edge News: “ONE HEALTH Program” ----- Page 17

The Scope

“When Fed Foods with Similar Palatability, Healthy Adult Dogs and Cats Choose Different Macronutrient Compositions”

Dogs and cats make short-term food choices based on palatability. Dr. Hall's group hypothesized that if palatability were masked, long-term food choices would be based on physiologic requirements, and circulating metabolite concentrations would reflect those choices. Four experimental foods with similar palatability, but varying in macronutrient composition, were prepared for healthy adult dogs (n=17) and cats (n=27). Food 1 was high protein; Food 2 was high fat; Food 3 was high carbohydrates; and Food 4 was balanced for macronutrients. By choosing any combination of foods, dogs and cats could individually set their macronutrient intake. Plasma metabolomic profiles were determined at baseline and after animals had consumed their food intake of choice for 28 days. Based on food intake calculations over 28 days, dogs on average chose to consume most of their calories from fat ($41.1 \pm 4.3\%$) and then carbohydrate ($35.8 \pm 3.7\%$), whereas cats on average chose to consume most of their calories from carbohydrate ($43.1 \pm 4.0\%$) and then protein ($30.3 \pm 3.9\%$; all $P < 0.001$). Age and lean or fat body mass also influenced protein intake.



Younger, leaner cats consumed more protein compared with older cats, whereas younger leaner dogs consumed less protein compared with dogs having more fat body mass. Older cats with moderate protein intake had lower circulating docosahexaenoic acid (DHA) concentrations as well as higher concentrations of sulfated microbial catabolic products compared with younger, leaner cats. **In summary, when fed foods with similar palatability, dogs and cats consume different macronutrient compositions, and concentrations of circulating metabolites in cats reflect food choices.**

“Lactobacillus gasseri Ameliorates Diet-Induced Diabetes in Mice via Changing Lipid Metabolism Gene Expression in the Gut”



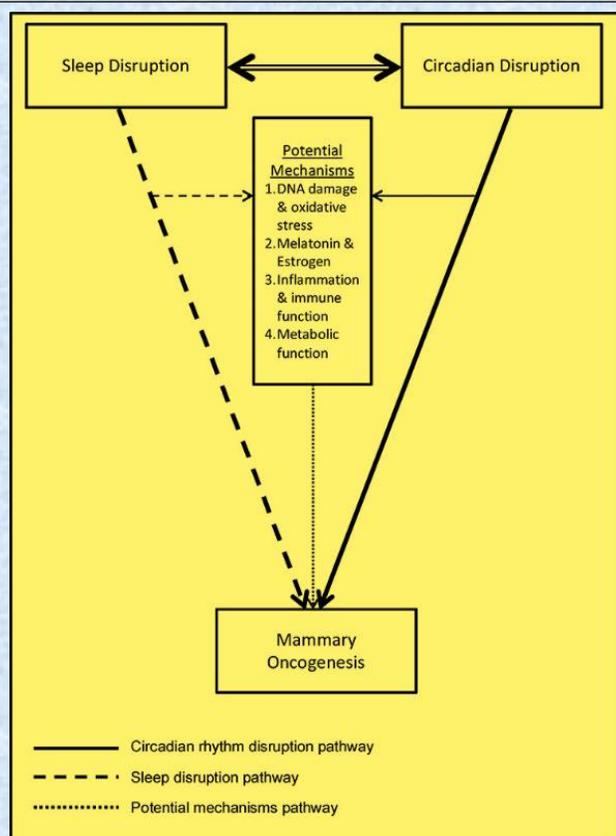
Lactobacillus gasseri could prevent low-grade inflammation associated with obesity and glucose metabolism disorders

Gut and gut microbiota plays an important role in metabolic diseases like such as type 2 diabetes (T2D) and has potential to be used to manage the disease. For this, identification of specific microbes is necessary to explore their role in prevention or treatment of T2D. Dr. Shulzhenko's research team used High Fat High Sucrose (HFHS) diet to induce glucose intolerance in mice and determined the phenotypes altered by HFHS diet. Using 16S rRNA gene sequencing of ileum, we evaluated microbial perturbations in the gut. To identify microbial candidates that can affect glucose homeostasis, we used casual inference analysis and transkingdom networks for the changed metabolic parameters and perturbed microbes. We predicted several candidate microbes with potential beneficial effects on glucose homeostasis. *Lactobacillus gasseri* (Lg) was identified as a top candidate and was tested *in vivo* by gavaging it into HFHS-fed mice. Supplementation with Lg ameliorated glucose intolerance development and reduced adiposity in HFHS fed mice. To evaluate the mechanisms of action of Lg in the gut, we sequenced ileum gene transcripts. We found that Lg regulated expression of about 100 genes in the ileum and about 90% of them were restored to normal levels. Genes involved in fatty acid and steroid biosynthesis that had been downregulated by HFHS diet were upregulated by administration of Lg. **Thus, these findings suggest that Lg promotes beneficial effects in diet-induced glucose intolerance by modulating gene expression in the gut. Further analyses are in progress to identify key genes modulated by Lg to restore metabolic homeostasis in T2D.**

The Scope

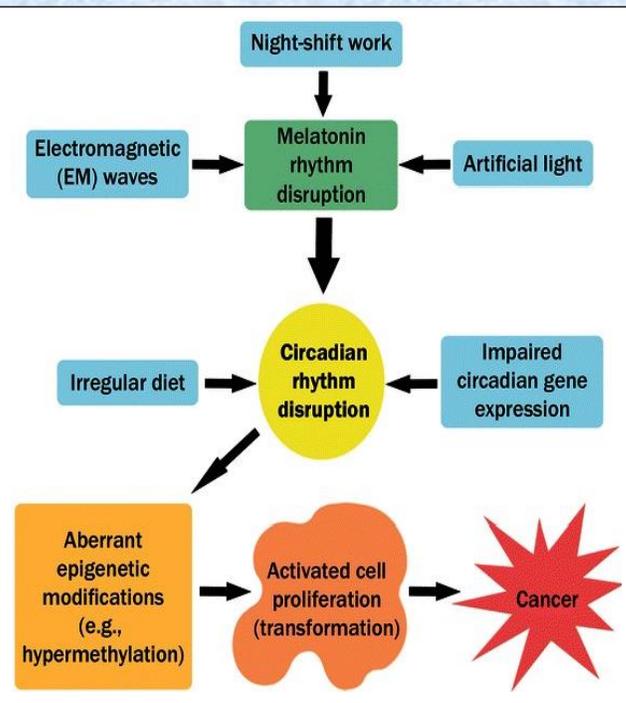
“Circadian Dysregulation via Exposure to Light at Night Alters DNA Methylation of Cancer-Associated Genes in Murine Mammary Tissue”

Breast cancer is a leading cause of cancer deaths among women, with an estimated 12.4% of American women diagnosed with this tumor type. Evidence suggests that exogenous disruptors can exert changes leading to aberrant gene expression, putatively shifting the balance toward oncogenesis. While chemical pollutant exposure is well studied, light exposure may exert diverse effects that are often overlooked. Mounting epidemiological evidence suggests that chronic circadian dysregulation is associated with increased breast cancer risk.



Exposure to light at night (LAN) via ambient nighttime lighting, digital technology, shiftwork, and trans-meridian travel can alter cell cycle regulation and tissue proliferation.

Previous studies demonstrated that animals exposed to altered light cycles exhibited marked DNA methylation pattern changes, and aberrant DNA methylation is noted across multiple loci in the cancer genome. LAN exposure may thus contribute to the etiology of breast cancer via alteration of the epigenome and subsequent temporal disruption of typical gene expression patterns.

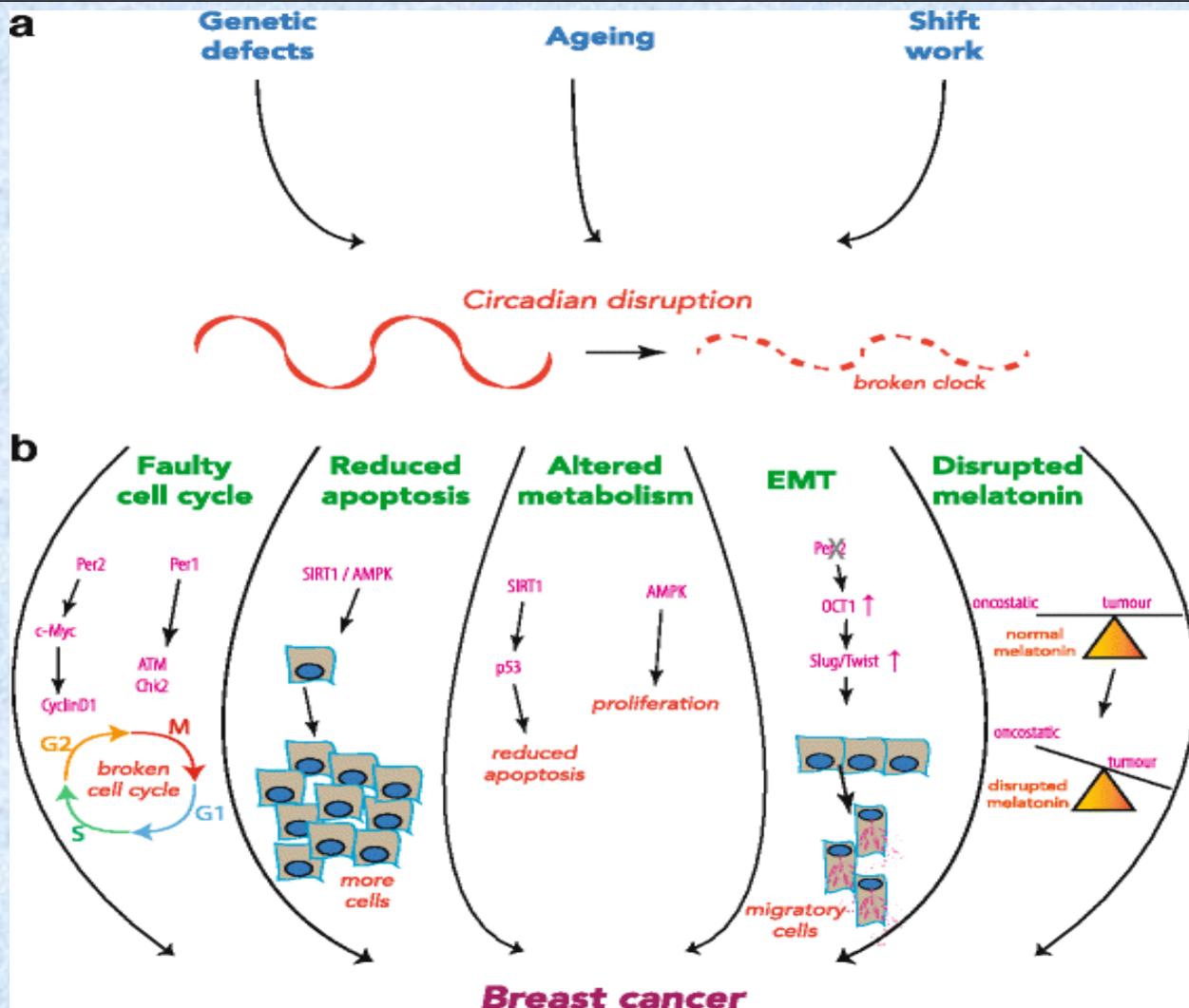


Dr. Chappell's group has previously found (unpublished data) that *mPer2::luc* females exposed to 21 days of LAN (18:6 LD) exhibited profound circadian disruption particularly in mammary chain, in comparison to control-exposed (12:12 LD) mice, and that LAN exposure significantly decreased mammary ER α and ER β expression. We are continuing to explore LAN-induced molecular alterations in the mammary via whole-genome bisulfite sequencing, to determine if inappropriate light exposure is an etiological risk factor of hormone-dependent mammary cancer. Post-pubertal female mice were exposed to LAN or control light cycles for 21d prior to mammary tissue harvest as described above for *mPer2::luc* knock-in mice. Genomic DNA samples were bisulfite-converted and sequenced, aligned to

The Scope

Continued from Page 3.

a bisulfite-converted genome, and analyzed to identify differential DNA methylation, using probes for regulatory regions (-500 to +2000 bp), exons, introns, and CpG islands. LAN exposure induced changes in mean methylation of probes across 6.4% of regulatory regions (2044 of 32025), 5.5% of exons (1752 of 32025), 0.03% of introns (577 of 190791), and 1.9% of CpG islands (269 of 13840) individually analyzed and annotated with surrounding or downstream genes (adjusted $p < 0.05$, abs. min. difference of means > 5). Significant methylation changes resulting from LAN were noted in multiple genetic loci implicated in cancer, including directional methylation changes intermediate between control and 4T1 breast cancer models across various *Hox* genes, *Hic1*, *Cdkn1c*, and other genes associated with proliferation and oncogenesis, with commensurate changes in gene expression also observed. We are currently working to confirm relative expression of commensurate genes via qRT-PCR. Therefore, a potential mechanism by which night light exposure may initiate cellular dysregulation within mammary tissue also observed. We are currently working to confirm relative expression of commensurate genes via qRT-PCR, and a potential mechanism by which night light exposure may initiate cellular dysregulation within mammary tissue.



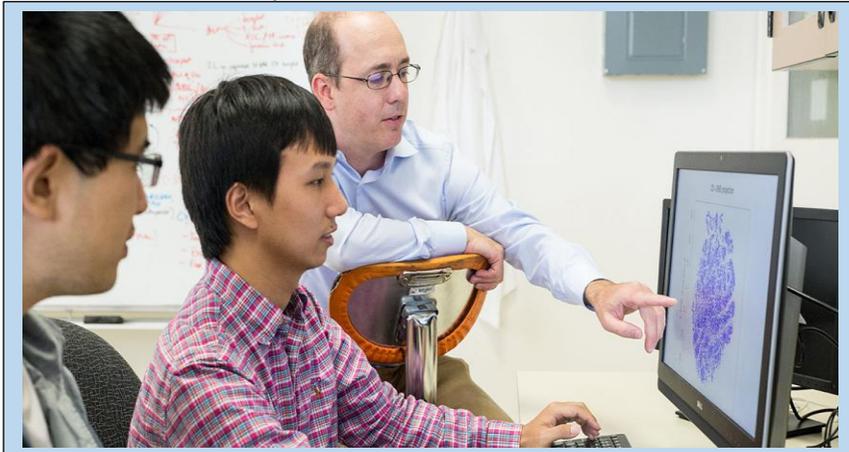
Breast Cancer Research - BioMed Central

Circadian disruption can drive breast cancer. a. Intrinsic factors such as genetic defects or ageing, and extrinsic factors such as irregular shift work, can severely disrupt the body's internal timing system. b. In turn these factors, either singularly or collectively, link a faulty circadian clock to an increased risk of breast cancer. A faulty clock can directly disrupt the gating of the cell cycle and reduce apoptosis, These effects can also be brought on indirectly through altered metabolism in response to a broken circadian clock. They can also lead to elevated EMT, driving the formation.

The Scope

“TRANSLATOR”

Since the emergence of the Internet, improvements have consistently been made to better connect users to content. It’s difficult to fathom the level of progress achieved in such a relatively short time. Competition among technology developers continues to make information more ubiquitous.



Now, a tremendous amount and diversity of information is available and searchable in the palm of your hand.

Search engines have been a pivotal to this worldwide transformation, allowing semantics to guide the vast field of information. There are still a few caveats though. A key obstacle that remains is searching for *correlative* information. For example, if someone were to enter the question “how does Malaria incidence in West Africa affect migration patterns in North America” into a search engine, the results of the search would likely be insufficient to answer the question. Answering complex questions such as this today requires a bespoke search strategy. This is especially common in the field of biomedical science, which generally requires multiple lines of evidence and supporting information to reach even a speculative conclusion.

The National Center for Advancing Translational Sciences (NCATS), a subdivision of The National Institutes of Health (NIH), is developing a reasoning system that will allow users to find correlative scientific information pertaining to biomedicine and human diseases. The project, called “Translator,” has one team internal to NCATS and nine external teams that were selected via a unique competition. Candidate teams were challenged to solve a series of computational tasks and puzzles in order to demonstrate their technical proficiency. Of hundreds of applicants, a team from Oregon State University, headed by Dr. Stephen Ramsey of the Department of Biomedical Sciences and Dr. David Koslicki of the mathematics department was selected.

As one of five reasoning tool teams selected in 2018, Dr. Ramsey and his group (which NCATS has dubbed “Team X-ray”) are responsible for developing a software program called RTX. RTX enables searching across—and connecting and analyzing

information from—22 databases of biomedical knowledge, including information about diseases, phenotypes, genetic variants, drugs, molecular pathways, and anatomy. The team and RTX work in concert with (and interoperate with) the other reasoning tool teams in the Translator consortium, whose tools enable access to complementary knowledge domains.

“The challenge is unifying the information”, says Dr. Ramsey. Biomedical knowledge databases have widely varying methods and standards for knowledge acquisition, distillation, and representation, typically involving either human curation or computer-based synthesis from various sources. Computer-based synthesis is faster, but computers are generally limited to natural language processing of biomedical scientific texts or making inferences from structured knowledge representations that must be meticulously constructed. In contrast, with human curation, experts individually review and distill information connections. The two contrasting styles each has advantages and disadvantages, and unifying them is difficult because of a lack of standardization in the identifiers that computer databases use to refer to concepts such as diseases, drugs, or phenotypes. To overcome this obstacle and get a leg up on developing new methods for reasoning on biomedical data, Team X-ray is focusing in depth on a few particular diseases including diabetes and Fanconi anemia, and working with subject-matter experts in these diseases to assess the quality of RTX’s responses to questions.

After only a few months, the reasoning tool teams have developed prototype systems and have made progress toward federating them into a ‘Translator’ system that will be used by a select group of early adopters.

The Scope

Continued from page 5.

The project has the potential to improve understanding and lead to discoveries in a variety of areas of biomedicine, such as genetic factors that influence diabetes, lifestyle modifications that correlate with decreased mortality rates, environmental modifiers of disease risk, and new indications for FDA-approved drugs (known as "drug repositioning"). NCATS's team coordinates the work of the Translator consortium and is already using the system to study rare diseases and neurodegenerative diseases.



The OSU team at the NIH Hackathon in Bethesda, MD



They and the other five internal teams believe that the next step is to further integrate the program and improve upon its user friendliness; making it faster, more compatible and capable of managing users on a large scale. Dr. Ramsey believes that the Translator's unique and highly collaborative consortium organization is a key advantage in tackling such a difficult problem, and he and his team are thrilled for the opportunity to contribute to a project that could revolutionize the way we search for information.



Stephen Ramsey (OSU), Mark Williams (NIH), Steve Cox (UNC Chapel Hill)

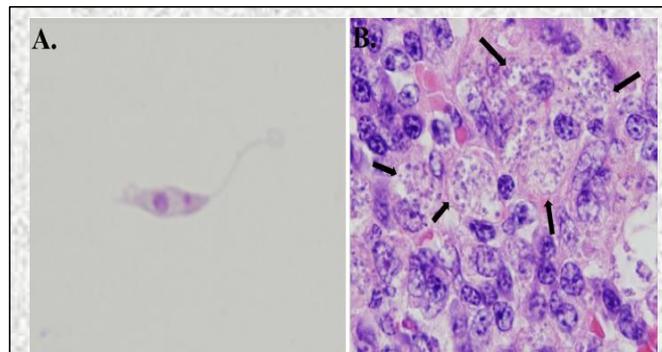
The Scope

"Creating New and Innovative Therapies for the Treatment of Leishmaniasis"

Dr. Deidre Johns, a chemist in the Biomedical Sciences Department, has taken a special interest in the fight against leishmaniasis. Leishmaniasis is a spectrum of diseases that affect over 12 million people worldwide, predominantly people and animals in the poorest regions of the world. It is caused by *Leishmania* parasites, which are protozoan parasites transmitted by the sand fly. Significant numbers of wild and domestic canines are also infected and serve as a reservoir for the spread of disease. Dr. Johns' goal is to make new molecules that are safer and more effective for treating infectious diseases, including leishmaniasis. After working in the pharmaceutical industry to discover new therapies for cancer, Dr. Johns realized the treatment options available for many infectious diseases are severely inadequate. Resistance is rapidly decreasing their efficacy and, in the case of leishmaniasis, the available drugs are highly toxic. Using her medicinal chemistry experience, she aims to alleviate this burden. She is one of very few people world-wide who is working to create new medicines for leishmaniasis.

The majority of infected individuals do not develop the disease, but can unwittingly spread the infection. The disease presents in two main forms: 1) cutaneous leishmaniasis, which is the more mild form, and 2) visceral leishmaniasis, which is typically fatal when untreated. The symptoms of cutaneous leishmaniasis are disfiguring skin and mucosal lesions that can become life-threatening. Visceral leishmaniasis has been recognized by scholars for centuries, often referred to as 'Black Fever' or Kala-azar. Leishmaniasis treatment relies on old drugs that are toxic and resistance has rendered several drugs ineffective. The mainstay treatments include pentavalent antimony compounds, amphotericin B, and miltefosine.

Thus, there is an urgent need for safer and more effective treatment for leishmaniasis. The chemical series Dr. Johns is working to optimize has a chemical structure that is related to the FDA approved oral drug raloxifene. She was particularly attracted to this scaffold since raloxifene has been safely used for many years and it can be administered orally.



Leishmania parasites in culture and in a tissue section.
(A) Leishmania amazonensis promastigote

Raloxifene does have one problem though; its primary action is to modulate estrogen receptors. This would not be well-tolerated in the broad population. Fortunately, the chemical group required for estrogen receptor activity is not required for anti-*Leishmania* activity. The analogs Dr. Johns is making do not affect estrogen receptors and are more potent against *Leishmania* parasites than raloxifene, however they need further improvement to be used as drugs. Dr. Johns describes her research as basic science – trying to understand what is required for activity and safety. Her lab must first determine how chemical modifications affect biological activity, as a first step toward a new therapeutic for leishmaniasis.

These new molecules are prepared in Dr. Johns's lab using cutting-edge synthetic organic chemistry techniques. Each new molecule is constructed by performing multiple chemical reactions. The reactions are often performed under an inert environment, using argon or nitrogen gas. Dr. Johns has a microwave reactor specific for chemical synthesis, that her lab uses to shorten reaction times, similar to how a household microwave can heat more quickly than the stovetop. The new molecules she prepares are characterized using OSU's state-of-the-art Nuclear Magnetic Resonance (NMR) imaging facility. Dr. Johns collaborates with researchers at the Walter Reed Army Institute of Research, where compounds are evaluated in multiple models of the disease.

Dr. Johns' work is driven by consistent progression of iteratively learning from their biological activity to gradually improve her compounds and eventually discover a better treatment for leishmaniasis.

The Scope

“Host Immune Response and Antigen Presentation”

Brian Dolan is a Professor of Immunology at OSU who focuses on studying the vertebrate adaptive immune system. His most recent studies involve various processes that impact antigen presentation and its effect on the immune system. His goal is to discover information about the mechanisms of antigen presentation, assisting the development of treatments for infectious disease.

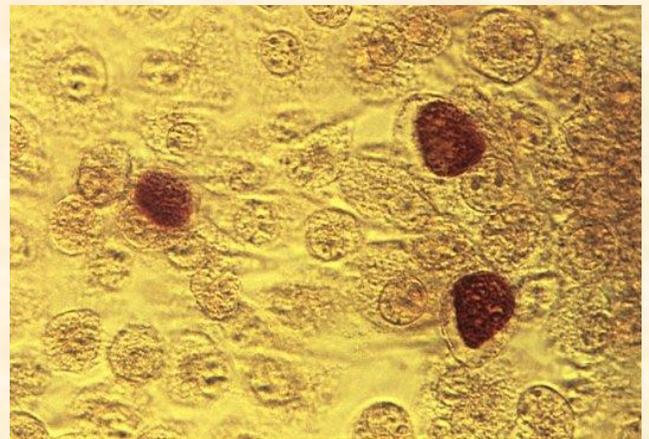
An essential function of the adaptive immune system is the production of antigens by diseased cells of the body which need to be eliminated for the good of the body. Prior to being recognized by the immune system, antigens must be present on the cell's surface. Proteins produced in the cell are degraded into peptides, and the proteins which are responsible for diseases (i.e., viral proteins or oncogenic proteins) yield specific disease-associated peptides upon degradation. These peptides are loaded onto Major Histocompatibility Complex (MHC) class I molecules which present the peptides to the T cells of the body a process referred to as antigen presentation.

In collaboration with Dr. Rockey, Professor Dolan has been working to understand the influence *Chlamydia spp* has on the adaptive immune response. *Chlamydia spp* bacteria are intracellular pathogens and have multiple mechanisms to hide from the host immune system. Once in a cell, *Chlamydia spp* increase the host's peptide presentation to prevent their own antigen presentation. This makes recognition by cytotoxic T-cells challenging. *Chlamydia spp*, like all organisms, has a cellular membrane containing a specific lipooligosaccharide (LOS) that is vital in the process of reproduction. This is an opportune target for Dr. Rockey and Professor Dolan. By inhibiting LOS production, *Chlamydia*

spp is unable to complete its reproductive cycle. Restricting reproduction can have important implications in the development of anti-chlamydial antibiotics.

Professor Dolan's newest study in relation to antigen presentation is the impact of the 'unfolded protein response' (UPR).

For various reasons, peptide chains and subsequent proteins improperly fold during translation. Improper protein folding has been associated with multiple neurological diseases including Alzheimer's, Parkinson's disease and amyotrophic lateral sclerosis (ALS). The UPR's primary function is to maintain protein homeostasis (proteostasis) within the cell, by regulating misfolded proteins. The UPR has two mechanisms of maintaining proteostasis, downregulating the synthesis of proteins and upregulating the endoplasmic reticulum's ability to fold or degrade misfolded proteins. Downregulation of peptide formation has been found to impact antigen production and simultaneous presentation, potentially reducing the effectiveness of the immune response.



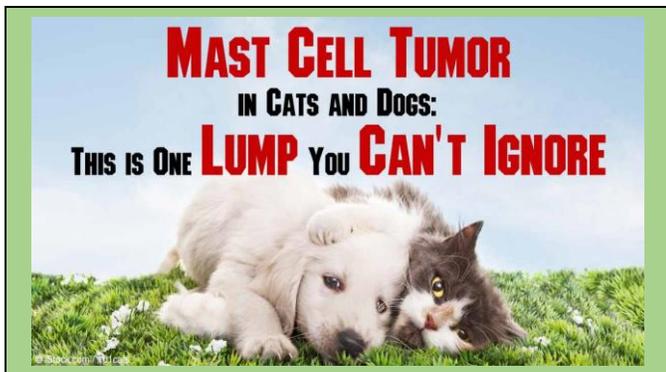
Chlamydia trachomatis bacteria magnified 200X.

Professor Dolan believes that modulating the UPR and understanding the totality of its repercussions on the immune system can lead to improvements to potential immunotherapies.

Professor Dolan has extended his studies to the impact of ubiquitin signaling, as well as studying non-model organisms such as bighorn sheep, and Pacific salmon. Refining our knowledge of mechanisms involved in antigen production and presentation will help develop future immunotherapies.

Paws for the News

“Cancer: Challenges of Surgical Removal”



Many know that cancer is one of the most life-threatening diseases. It is estimated that 1.6 million new cases are diagnosed annually in the United States alone, resulting in nearly 600,000 deaths (NCI). The effect extends beyond just people. [Animals are also susceptible to cancer and the numbers affected is high for household pets.](#) Nearly 6 million dogs and 6 million cats will be diagnosed with cancer this year. This is a major problem for pet owners and veterinarians. Veterinarians with large numbers of cancer patients have constraints in terms of treatment options. A common protocol is to surgically remove the malignant tissue and/or treat with chemotherapeutics, but ridding a patient of cancer is not that simple. According to small animal surgeon Dr. Milan Milovancev, of Oregon State University's Carlson College of Veterinary Medicine, there are two basic questions that must be answered when surgically removing a tumor: 1) Where to cut in order to maximize chances of removing the entire tumor while sparing as much patient tissue as possible 2) How to know if a tumor has been completely removed. Answering these questions is much more complex than one might think. There is no consensus among surgeons on how to best surgically remove a tumor. Therefore, Dr. Milovancev has sought an empirically validated answer to these questions when treating patients. "Where to cut?" Answering this question is often difficult due to the complexity of tumor growth. Tumors are highly invasive. Once uncontrolled proliferation occurs, cancerous cells need large amounts of nutrients to maintain metabolism and

division. This need is often achieved by the attraction of blood vessels to the growing tumor. Radiographic imaging is a common technique to locate the tumor, but vessels growing close to the tumor make it challenging to differentiate between malignancies and potentially healthy tissue. There can also be complications with location, depth, and cancer type which is why there is no standard protocol for removal. To try and overcome these obstacles, Dr. Milovancev is studying specific cancer types to provide an evidence-based rationale for determining surgical protocols. By analyzing tissue samples from certain cancers, Dr. Milovancev has been able to quantify surgical margins for injection site sarcomas in cats and both soft tissue sarcomas and mast cell tumors in dogs. As the most common cancers affecting dogs, being able to better determine the ideal location of "where to cut" these tumors, makes an immense difference in the health of the patient.

Once the tumor is excised, the team of veterinary specialists treating a dog or cat with cancer must turn their focus toward another question, "did we get it all out?" Evaluation of surgical excision includes histopathologic examination of the removed tissue by a pathologist followed by the clinician's interpretation of the pathologist's report. A common problem with excised tumors is the distortion of tissues (length changes, folding, etc.) that occurs post-excision and during formalin fixation. From the operating table to the pathology lab, tumors acquire artifactual changes to their dimensions and relationship to surrounding tissues, making it difficult to correctly evaluate the location of malignant tissue. If the pathologist cannot properly analyze the tissue, then the interpretation of whether the cancer was sufficiently removed may be flawed. Dr. Milovancev collaborates with pathologists and medical oncologists at OSU to evaluate the post-excision tissue changes for certain cancers, but he believes that there is still more that can be done. To improve the accuracy of evaluation of completeness of excision, Dr. Milovancev has studied variables using surgical margin inks, alternative and complementary methods of margin evaluation such as shaved margins and imprint cytology, and the effect of sectioning technique and/or presence of microscopic artifacts on the pathologist's report. Many factors come into play when surgically removing a tumor.

Continued from Page 9.

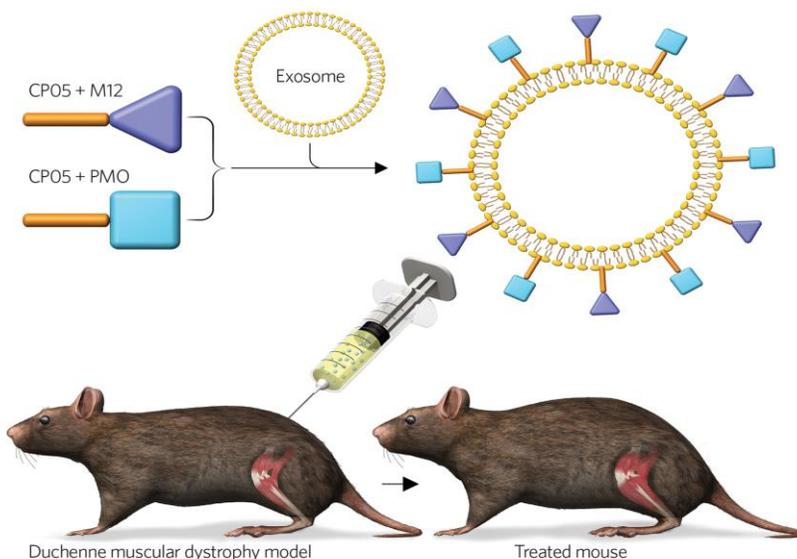
Tumor type, grade, and molecular characteristics all play a role in the complexity of cancer. Limited answers to these questions are currently available, and Dr. Milovancev and his collaborators have set out to try and answer some them. His contributions to the quantification of surgical margins and evaluation of excision have made a pivotal difference in the health of patients at OSU.

Dr. Milovancev's future plans include evaluating real-time intra-operative near-infrared fluorescence imaging as a method of determining surgical margins. While striving for the best care for his patients, Dr. Milovancev hopes that his work will help answer questions for other surgeons, creating a more informed consensus on how to best treat cancer.



"The Use of Vesicles (Exosomes) to Treat Diseases"

Exosomes are circulating nanovesicular lipid carriers of macromolecules, increasingly used for diagnostics and therapeutics. Engineering of these particles for therapy of disease would be useful. The ability to load and target patient-derived exosomes without altering exosomal surfaces is potentially important for unlocking their therapeutic potential. We demonstrate that a small protein (peptide) (CP05) capture of exosomes from diverse origins, including patient-derived exosomes, through binding to CD63 molecules—an exosomal surface protein. Systemic administration of exosomes loaded with CP05-modified, dystrophin splice-correcting phosphorodiamidate morpholino oligomer (EXOPMO) increased the dystrophin protein 18-fold in quadriceps of dystrophin-deficient mdx mice compared to CP05-PMO. Loading CP05-muscle-targeting peptide on EXOPMO further increased dystrophin expression in muscle with functional improvement without any detectable toxicity. Our study demonstrates that an exosomal anchor peptide enables direct, effective functionalization and capture of exosomes, thus providing a tool for exosome engineering, probing gene function *in vivo*, and targeted therapeutic drug delivery.



TISSUE-TARGETED THERAPEUTIC EXOSOMES:

To treat a mouse model of Duchenne muscular dystrophy, molecules of the exosome-binding peptide CP05 are linked either to the muscle-targeting peptide M12 or an oligomer (PMO) that corrects a splicing error in the gene for dystrophin. These two conjugate peptides paint the surface of exosomes from cultured mouse cells. When injected into the mice, the exosomes home to muscle, where they deliver the oligomer, boost functional dystrophin levels, and improve muscle function.

Paws for the News

"RESPIRATORY SYNCYTIAL VIRUS"



Respiratory Syncytial Virus (RSV) is one of the leading causes of lower respiratory infection in infants and elderly and many of us have had this infection as a common cold at one time or another. RSV is a highly contagious virus that can be contracted via inhalation or direct contact with an open orifice. The CDC reports that RSV patients usually remain contagious for the first 3-8 days after infection. For non-infant or elderly patients, RSV typically causes mild congestive symptoms such as coughing, runny nose and wheezing. These symptoms can be managed with over-the-counter medications and commonly subside after one to two weeks. In immunocompromised patients (preterm infants, elderly), RSV can lead to more severe secondary infections and is the primary cause of bronchiolitis. In parts of the U.S. and other economically developed countries, severe bronchiolitis and RSV can usually be managed through hospitalization, but in impoverished areas, RSV can lead to death and predispose those that survive to asthma later in life. The mechanistic basis by which RSV infects the airways of infants with such severity is not completely understood. This has been the area of study for one of The College of Veterinary Medicine's newest professors, Dr. Mark Ackermann. His work has been focused on creating a model that emulates infant RSV patients with the intent of testing new and innovative drug therapies, helping further understand the pathological basis behind premature lung infection.

Dr. Ackermann has worked with several pharmaceutical and biotechnology companies to assess newly developed, and often novel therapeutic compounds. After a potential drug has

been engineered, the goal is to test it in a live host. Prior to human trials, promising new therapeutic compounds are tested in an animal model. This has been difficult historically for RSV because a successful animal model needs to be analogous in RSV pathogenicity, airway structure and tissue maturation of an infant patient. Also, the animal model must be susceptible to human strains of RSV. Over the years, Dr. Ackermann has been able to establish a sufficient model using newborn lambs. What makes lambs so compatible? Well for starters, lambs are still in an immature stage of growth, meaning their lungs closely match the construct and tissue maturation of an infant lung. The other curious discovery is that lambs manifest the infection extraordinarily similarly to humans. In infants with RSV induced bronchiolitis, lesions emerge in the small airways along with inflammation and damage to the cells lining the small airways (bronchioles). When a lamb is infected with human RSV, lesions in bronchioles identical those in infants develop as do similar inflammatory and immune responses. This unusually close relationship allows Dr. Ackermann to minimize any deviation between human and animal trials.

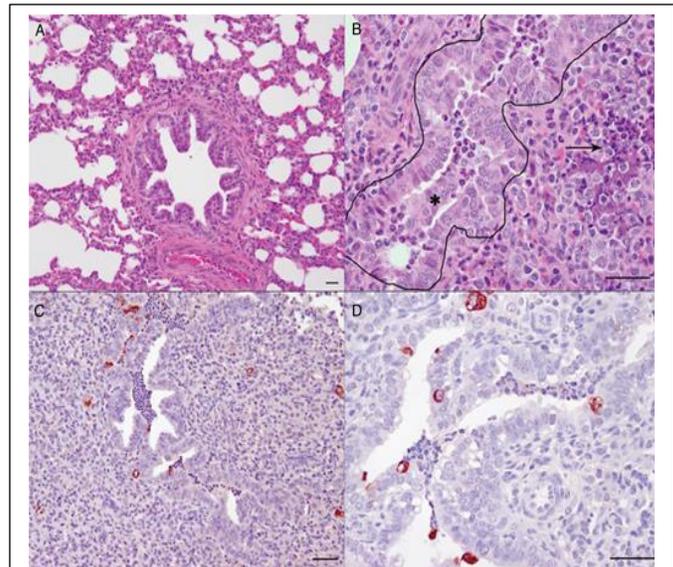
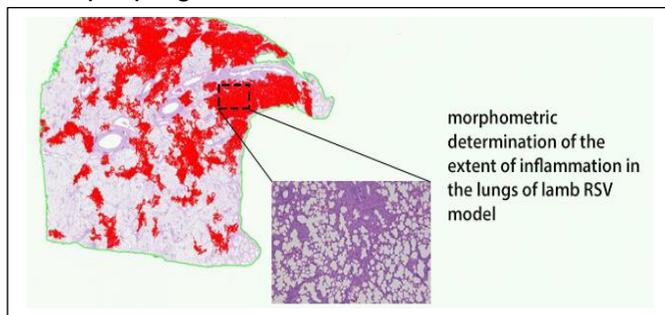


Figure 1. Respiratory syncytial virus (RSV) strain A2 causes bronchiolitis in lambs (Olivier et al. 2009). (A) Lung from a control lamb not infected with RSV that contains a bronchiole and alveoli. (B) Lung from a lamb 6 days after inoculation with RSV with a bronchiole (outlined) containing epithelial cells admixed with neutrophils (*). Alveoli around the bronchiole are collapsed with accumulation of degenerate neutrophils and areas of necrosis (arrow). (C and D) Immunohistochemical detection of RSV antigen in lung from a lamb 6 days after inoculation with RSV, in which RSV viral antigen is present within the bronchi, epithelial cells lining the bronchi, and the syncytial cells in areas of alveolar consolidation. Bars = 25 μ m.

Continued from Page 11.

Dr. Ackermann works with several major companies that develop therapies for RSV, including Janssen (part of Johnson and Johnson (JNJ)) and 'Ablynx', a biotechnology company in Belgium. The therapeutic compounds reduce RSV infection by either binding and inhibiting RSV's attachment to cells, or reducing viral replication. The science behind this incorporates some complicated biochemistry, but the basics typically involve targeting the viruses' surface with antibody to prevent adherence to cells. This is effective because it not only inhibits the virus, but also allows the immune system to progress and assist in the patient's recovery; reducing viral replication impairs the virus from forming new virions. The studies have also given some insight into the pathological basis behind RSV virulence factors, determining the extent to which these therapies can work in lungs of newborns where immune system and airway lining cells are not fully developed. Much more is still to be uncovered, but there is significant and rapid progress.



Pathogenesis and vaccine development for respiratory syncytial virus and parainfluenzavirus-3

The primary goal is to return patients to a clean bill of health at a low cost and Dr. Ackermann may have played a major role in contributing to this accomplishment. In the lamb model, Dr. Ackermann looks for several factors to assess the effects of a treatment. These factors include microscopy to analyze the reduction of lesions and mRNA sequencing to quantify replication rates of RSV. If each test can be repeatedly satisfied, then a treatment compound has a chance to progress to human trials. Due to the collaborative work between Dr. Ackermann and companies like Janssen (JNJ) and Ablynx, new therapeutic drugs may be headed to the market and available for qualified patients. After promising results in the lamb studies, both Janssen (JNJ) and Ablynx have therapeutic compounds that have progressed onto human trials. If regulatory proceedings can be met, then the drug could reach the market perhaps in a few years. **The impact of this therapy could affect countless lives; not only if made available in underdeveloped areas, but also if used worldwide. This is no small achievement and should be something of great pride for Dr. Ackermann and his colleagues.**

"Can we Control Bovine TB by Using a Novel Vaccine?"



TB in cattle is caused by the bacterium *Mycobacterium bovis* (*M. bovis*). Cattle, buffalo and bison are the natural hosts of *M. bovis*, but nearly all mammals are susceptible to the infection to a variable degree. The organism also has the capacity to infect and cause TB in humans.

Bovine tuberculosis (bTB) is a highly transmissible infection and remains of great concern as a zoonosis. The worldwide incidence of bTB is rising, creating a potential reservoir and increased infection risk for humans and animals. In attempts to identify novel surface antigens of *M. bovis* as a proof-of-concept for potential inducers of protective immunity, Yongyui in Dr. Bermudez's laboratory investigated surface proteomics of *M. bovis* bacterium cultured under a granuloma-like condition and also demonstrated that bacteria exposed to the biologically-relevant environment have greater binding and invasion ability to the host cells than bacteria incubated under regular laboratory conditions.

The group showed that 957 surface-exposed proteins were identified on *M. bovis* cultured under laboratory conditions, whereas 1097 proteins were expressed under the granuloma-like condition. The overexpression of some of the proteins (Mb1524, Mb01_03198 and Mb1595_p0530 (HbHa)) surface proteins on non-pathogens *M. smegmatis* lead to increased binding and invasion of respiratory mucosal cells. The group also examined the ability of purified recombinant proteins as well as *M. smegmatis* overexpressing these surface antigens in mice to stimulate antibody production. High levels of specific IgA and IgG antibodies were observed in the recombinant protein-immunized groups by both inhalation and intraperitoneal (IP) routes but only IP delivery induced high total IgA and IgG levels. The group did not detect significant differences in antibody levels in a *M. smegmatis* group that overexpressed surface antigens. The significant reduction of the bacterial load in lungs was observed only in mice immunized with the combination of inhaled recombinant proteins. In conclusion, these findings suggest that the activation of the mucosal immunity can lead to increased ability to confer protection upon *M. bovis* infection.

Paws for the News



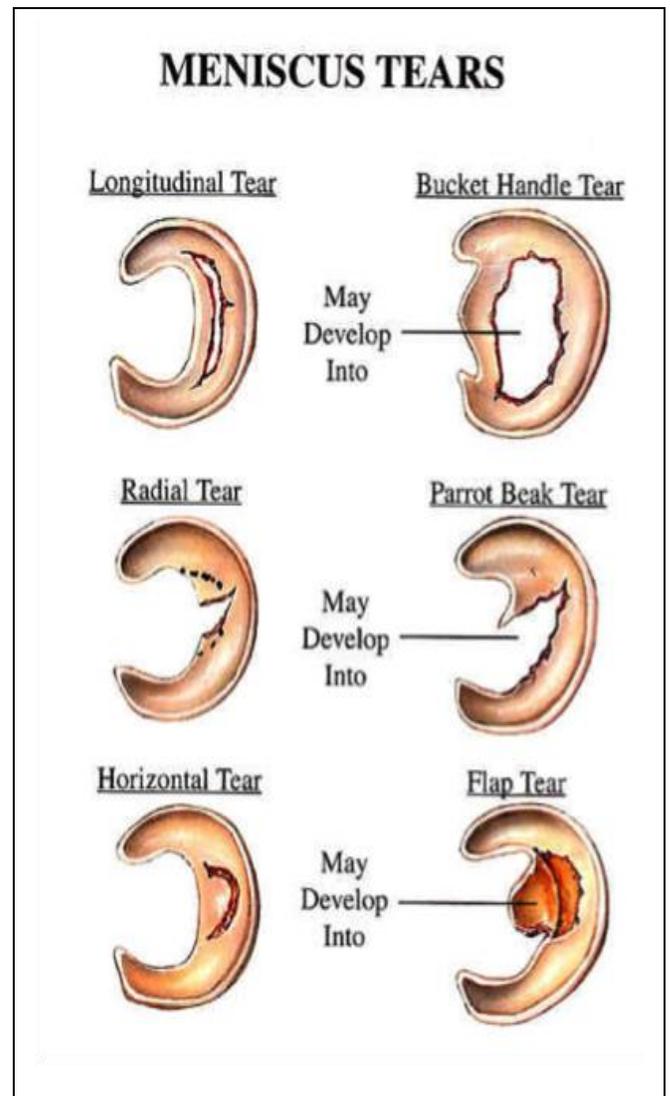
“The Future of Orthopedic Medicine”

Orthopedic injuries are among the most frequently encountered in emergency departments. Though the body can withstand extensive amounts of mechanical stress, it is not impervious to injury. Anterior cruciate ligament tears have become somewhat of an ‘epidemic’ in the past decade for athletics and meniscal injuries are even more common. Those who have experienced an orthopedic injury know that they are immediately debilitating and often result in a lifetime of challenges.

Though it is not often considered, animals are just as susceptible to these types of orthopedic injuries. Small animal surgeon Dr. Jennifer Warnock is an orthopedist at the Carlson College of Veterinary Medicine who treats a high number of patients that come in with major joint damage. It has been her experience that household pets such as cats and dogs can be more likely to experience personal injury than most people. Animals are fun loving and typically enjoy playful exercise, but they do not always know their limits. The type of activity that they partake in can be strenuous and often leads to injuries of the lower limbs. Advancements in orthopedic medicine have thankfully produced groundbreaking techniques for treating patients with musculoskeletal damage, but Dr. Warnock believes it can be taken a step further and may have an answer as to how. Dr. Warnock has begun a project that focuses on an orthopedic therapy that engineers a patient’s own cells to create usable tissue.

As in humans, small animal meniscal tears are the most commonly-treated injury in veterinary orthopedics. The cartilage cushion between the proximal and distal portions of the lower extremities,

the meniscus is under hundreds of pounds of pressure at any given time. Managing the constant stress is an astounding feat in itself, but physical stress eventually accumulates. The slightest pressure to a weakened area can result in a tear. Comprised of fibrocartilage, the meniscus has a low blood supply, making healing a challenge. Instead, the knee responds by filling the joint with synovial fluid to alleviate pressure and prevent friction. Studying this biological response has led researchers to discover that the synovium contains a relatively high concentration of stem cells. It is theorized that some of these cells will differentiate into new cartilage, but this repair method is minimally effective, as the meniscus seldom heals well without



surgery. Rather than allowing these stem cells to go underutilized, Dr. Warnock salvages them for study. She conditions the cells to differentiate into collagen, facilitating the growth of healthy and usable cartilage.

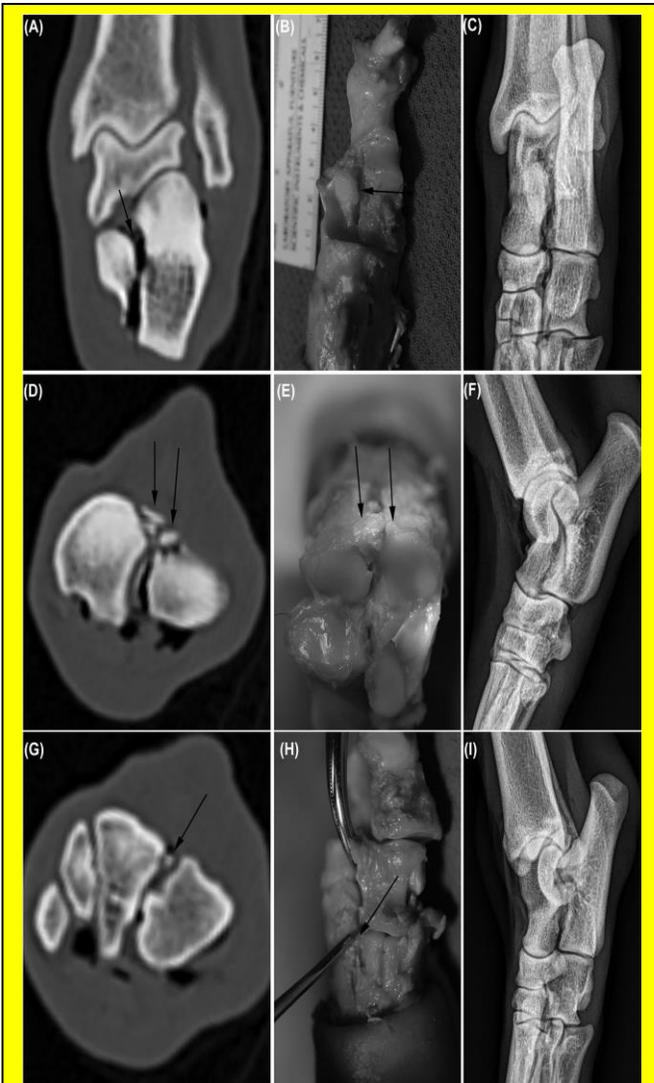


FIGURE 1. Post fracture images of limb 10. Based on dissection, this limb sustained a fracture of the calcaneus separating the sustentaculum tali from the body of the calcaneus (B, arrow) as well as chip fractures of the proximal 4th and proximal central tarsal bones (E, arrow) and distal 3rd tarsal bone (H, arrow). The fracture of the calcaneus was seen on the dorsal plane computed tomography (CT) images (A, arrow) and on the two-view (C), but not the ten-view radiographs (F, I). The chip fractures of the proximal 4th and central tarsal bones were seen on transverse CT images (D, arrow), but not on radiographs (C, F, I). The chip fracture of the distal third tarsal bone was seen on transverse CT images (G, arrow) but not on radiographs (C, F, I). Bone algorithm CT images are displayed in a bone window and level (W: 2000, L: 400). Of the ten-view radiographic study, the dorsoplantar (C), lateromedial (F), dorsolateral-plantaromedial oblique (I) projections are shown.

The differentiation process may be the most difficult and time-consuming part of the study. For cells to differentiate, they need specific growth and environmental factors according to their cell type. These factors induce the expression of genes that code for responses that lead to individual cell types. Trying to replicate this process *in vitro* requires an immense amount of finesse. After some experimentation, Dr. Warnock was able to develop a method using an unusual technique. Along with a list of growth factors, Dr. Warnock uses pressure to induce differentiation. It seems that these cells have developed a need for environmental pressure to activate expression. It makes sense that this genetic pre-disposition arose, since fibrocartilage is usually under such high levels of pressure.

At first, Dr. Warnock uses a simple technique, placing a small glass slide on the cells and then progressively building the pressure as the cells begin to develop. Once the cells mature into collagen fibers, they undergo a process that induces cell-cell tension, facilitating the formation of tissue. In an ideal scenario, Dr. Warnock can use this tissue to replenish or even replace a patient's damaged meniscus. The benefits of this could revolutionize orthopedic care. Not only will it be new and healthy tissue, but it will come directly from the patient. This individualization of treatment will minimize the immune response to foreign tissues, allowing for quicker recovery times. Generating a meniscus may be just the start. Imagine the possibilities; new ligaments, muscular tissue, even bones. Dr. Warnock recently encountered sibling kittens that suffer from a rare congenital disease, resulting in the patients being born without both of their tibias. Thankfully, the veterinary orthopedists at OSU successfully operated on both and have begun rehabilitation with the intent of helping these kittens live a fulfilled life. Imagine though, being able to surgically implant a new tibial bone synthesized directly from the patient. This kind of progress takes time and is not something people should necessarily expect in the near future. It is though, a very real possibility and Dr. Warnock is pushing the envelope to make it happen.

Paws for the News

"A Retrospective Study in the Hospital to Identify Potential Causes of Infection"



Records of all VDL submissions from August 2012 - May 2015 were examined and subjected to analysis according to species, location of infection, species of bacteria, and antibiotic resistance/susceptibility. As described by Hanna Shoen, a student of Dr. Bermudez's, a total of 23.8% of all culture isolates were *Staphylococcus spp.* Of those *Staphylococcus spp.*, 43% were isolated from surgical site infections.

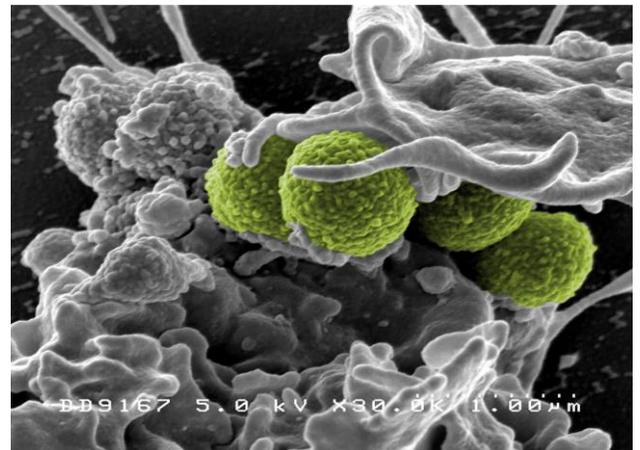
Staphylococcus pseudintermedius accounted for approximately 28% of all *Staphylococcus spp.* cultures, while methicillin-resistant (MR) *S. pseudintermedius* accounted for 8% of all staphylococcus cultures. Environmental samples were also collected by swabbing surfaces in the intensive care unit (ICU) and anesthesia prep room at the OSU VTH. Swabs were streaked onto agar plates and resulting colonies were subjected to PCR for species identification and for the presence of the *mecA* gene associated with methicillin resistance. Ability of horizontal transfer *in vitro* of the *mecA* gene was evaluated by incubating the *mecA* positive bacterium, with the *mecA* negative bacterium in Luria-Bertani (LB) broth at multiple time points, and then plating onto agar plates infused with concentrations of oxacillin previously established to be able to inhibit MR staphylococcal growth. Colonies were then subjected to PCR for species and *mecA*

identification. Horizontal transfer of the *mecA* gene was demonstrated and confirmed via PCR from MR *S. epidermidis* to MS *S. pseudintermedius* in an *in vitro* model that mimicked the veterinary hospital environment. Biofilms were established using four *Staphylococcus* species (two were *mecA* positive) isolated from swabbing the Intensive Care Unit (ICU) and anesthesia prep room. The biofilms were then exposed to the current cleaning agent used (VEDCO-D256) and then plated onto agar media.



Antibiotic resistance has two causes: healthcare and agriculture. Now the two worlds are converging as animal-based organisms are being seen in hospitals.

Staphylococcus species make up nearly 1/4 of all infections submitted for culture at OSU VDL during the four years of the study. MS *S. pseudintermedius* was shown to acquire the *mecA* gene in an *in vitro* environment from a coagulase-negative *Staphylococci* (CoNS) donor.



Hospital-associated Methicillin-resistant *Staphylococcus aureus* MRSA Bacteria

Paws for the News

“Clinical Experience: Improving Care Via 3D Modeling”



We spoke with veterinary radiologist Dr. Susanne Stieger-Vanegas. Dr. Stieger-Vanegas received her veterinary degree in Austria at the University of Veterinary Medicine, followed by a diagnostic imaging residency program and completed her studies at UC Davis with a PhD in comparative pathology. She now specializes in diagnostic imaging; primarily focusing on gastrointestinal disorders, complex cardiac diseases and soft tissue injuries in sporting animals. The image findings she presents assist clinicians in their assessment and plan of care.

In the field of radiology, producing precise images is essential. Standard image techniques such as an X-ray or CT scan typically produce a 2-dimensional representation of the localized anatomy. For complicated cases, it can be challenging to interpret the images, given the complexity of anatomical structures. Dr. Stieger-Vanegas has been using 3-dimensional modeling and printing to recreate the patient's own anatomy. A 3D printer works similarly to a normal printer. Rather than pressing ink into the page, a standard printer essentially stamps the surface with a stable layer of material. Now imagine repetitively layering material into defined locations over and over again; eventually, the layers would accumulate and create a 3-dimensional structure. This is the basic idea of a 3D printer. Using a malleable material and an accurate design pattern, the printer is able to create nearly any lifelike structure.

Dr. Stieger-Vanegas typically uses the patient's images from a CT scan as the template for the printer. With some effort, the 3D structures it develops are as precise as the patient's own anatomy. An orthopedic surgeon at the veterinary hospital recently encountered a canine who needed surgical repair of its lower limb due to an angular limb deformity. However, the procedure was not routine, and any mistake could affect the patient's long-term health and ability to move without pain. Rather than risking the procedure on the patient, Dr. Stieger-Vanegas created a mold of the patient's limb for the surgeon to practice on. After a few

trials with the model anatomy, the surgeon was able to perform the procedure without complication and the patient recovered successfully.

Assisting clinical surgeons is just one example of the benefits of Dr. Stieger-Vanegas' project. As a DVM working in a school of veterinary medicine, Dr. Stieger-Vanegas is involved in teaching young up-and-coming veterinary students about diagnostic imaging and veterinary anatomy. Using the 3D printer, she has been able to recreate complex anatomical abnormalities for her students to study. She recently conducted a more personalized study on the benefits of 3D modeling in relation to learning and understanding complex cardiac anatomy. She found that students who studied using 3D anatomical structures typically felt more comfortable understanding the complex anatomy than those who used traditional 2D methods.

Maybe the most important function of the printing project is the peace of mind it brings to patients. Dr. Stieger-Vanegas is able to provide a simplified representation directly to patients and their owners. It reassures people knowing that Dr. Stieger-Vanegas and the veterinarians at OSU are well equipped to take care of those they care about. The project has already assisted veterinarians with treating several anatomically complex cases, including congenital abnormalities, heart diseases, and cancer. As she continues to implement this creative innovation, she hopes that others will follow; adding stability to the field of veterinary radiology, one layer at a time.

CVM CUTTING EDGE NEWS

ONE HEALTH Program



The ONE HEALTH program has been launched. It is directed by Dr. Beechler and aims to introduce the concept of one medicine to students. During a field trip to Costa Rica, Dr. Beechler and students from the Carlson College of Veterinary Medicine and the Microbiology Department at OSU participated in two field projects related to antibiotic resistance in the microbiome of cows as well as tick and mosquito-borne disease. The students collected samples, performed tests in the laboratory and had the opportunity to interact with local farms and see firsthand the connections between human, animals and environment that are associated with disease and health.

The program will be expanded next year to include a seminar series.



Editor: Janice S. Blouse
Assistant: Jordan K. Rice

CARLSON COLLEGE OF VETERINARY MEDICINE
Department of Biomedical Sciences
Oregon State University, 106 Dryden Hall
Corvallis, Oregon 97331-4802
T 541-737-6532 | F 541-737-2730
oregonstate.edu/vetmed
Copyright ©2018 Oregon State University