Proceedings From the 2024 Noorda College of Osteopathic Medicine Research Symposium

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Intermountain Journal of Translational Medicine

Volume 1 Issue 2 Supplement 2

DOI: 10.5281/zenodo.13629627

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September 07, 2024

Preface

We are pleased to present this conference program containing select abstracts from the 2024 Noorda College of Osteopathic Research Symposium, which took place on May 16th, 2024. This symposium showcased student and faculty research at NoordaCOM and surrounding institutions and has been growing rapidly over the past several years. Out of several hundred abstracts in total, these thirty-four have been selected as outstanding contributions by a team of symposium judges as well as editors at *Intermountain Journal of Translational Medicine*.

Alfred Amendolara, MS *Co-Editor in Chief*

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Contents

1. Suggested Citation Structure

The following citation structure has been provided in AMA format and is the recommended way to cite individual abstracts.

Authors. Article Title [Abstract Number]. *Journal Title*. Year;Volume(issue): page numbers.

- Author names should be listed as Last Name First Initial.
- Abstract numbers can be found before titles in this document (**ex**. BMS 01) . Please note that these numbers are specific to this program are are not related to any number you may have received previously i.e., for submission or presenting at the symposium.
- For *journal title* we suggest using the shortened version of *Intermountain Journal of Translational Medicine* which is: *IMJ Translational Med.*
- This program is Volume 1 Issue 2 Supplement 2.

Ex: Paxton Z, Huynh M, Ramana K. Benfotiamine and fursultiamine prevents endotoxin-induced cytotoxicity in human dermal fibroblasts [BMS 02]. *IMJ Translational Med.* 2024;1(2 Supplement 2):7.

2. Biomedical Science

2.1. BMS 01: Exercise Ameliorates Ethanol-Induced Changes of Sensitization and Expression of Kappa and Delta Opioid Receptors in Nucleus Accumbens and Ventral Tegmental Area

Brandon Burger[1], Bridger Gunter[1], Kyle Bills[1], Alexander Kim[1], Laura Minor[1], Jude Emego[1], James Brundage[2], Benjamin Williams[3], Christina Small[1], Jordan Yorgasen[4], Scott Steffenson[4]

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2. University of Utah
3. Wake Forest University
4. Brigham Young University

Purpose: Exercise has been increasingly used as an adjunctive therapy in the treatment of alcohol use disorder (AUD). Despite this, the mechanism by which it influences the mesolimbic circuitry changes underlying alcohol addiction is not well understood. Previous studies have shown alcohol dependence to lead to upregulation of the Dynorphin-Kappa Opioid Receptor (KOR) system, making it a potential target for therapeutics. The Delta Opioid Receptor (DOR) system is also influenced by alcohol dependence. Thus, gaining a better understanding of these pathways will help develop evidence-based guidelines for integrating exercise into therapies for the treatment of AUD.

Methods: Mice were divided into four cohorts: Ethanol injections with access to a running wheel, ethanol injections without access to a running wheel, saline injections with access to a running wheel, and saline injections without access to a running wheel. Following 14 days of the respective protocols, mice were anesthetized, and slices of the striatum were prepared for either fast scan cyclic voltammetry (FSCV) or immunohistochemical (IHC) analysis of KOR/DOR expression. FSCV was performed at baseline, followed by bath application of either U-50488 (a KOR agonist) at 0.3 uM or 1 uM, followed by a reversal dose of 1 uM nor-BNI (a KOR antagonist). The same protocol was performed using selective DOR agonist DPDPE at 1 mM, followed by a reversal dose of 1 mM naltrindol, a selective DOR antagonis on separate slices.t. IHC was performed to evaluate the expression of KORs/DORs in both the nucleus accumbens (NAc) and the ventral tegmental area (VTA). A continuous two bottle choice compared EtOH consumption before and after EtOH dependence was established in an exercise and non-exercise group.

Results: IHC revealed that voluntary exercise decreased the expression of KORs in the NAc and the VTA in both ethanol-dependent and non-dependent mice while increasing expression of DORs in the VTA. In addition, in ethanol-dependent mice, voluntary exercise blunted the hypersensitization of KORs and altered evoked dopamine release in the nucleus accumbens. Exercise was also found to desensitize KORs in ethanol non-dependent mice and alter evoked dopamine release in the nucleus accumbens. In the two-bottle choice experiment, mice who exercised during the dependency period were found to have a much smaller increase in EtOH consumption after dependence was established.

Conclusions: A regimen of voluntary exercise competitively altered the changes in KOR/DOR expression seen in ethanol dependent mice and influenced ethanol seeking behavior.

These findings provide insight into a potential mechanism by which exercise contributes to the treatment of AUD. However, further research is needed to fully understand the role of exercise in altering the expression and sensitivity of KORs/DORs and other opioid receptors that influence the mesolimbic circuitry.

2.2. BMS 02: Benfotiamine and fursultiamine prevents endotoxin-induced cytotoxicity in human dermal fibroblasts.

Zackery Paxton[1], Mindy Huynh[1], Kota Ramana[1]

1. Noorda College of Osteopathic Medicine

Wound healing is a fundamental physiological process crucial for survival, involving a complex cascade of cellular events aimed at repairing damaged tissue. Impairments in wound healing mechanisms, often exacerbated by conditions such as diabetes, infections, and oxidative stress, present significant clinical challenges, necessitating novel therapeutic approaches. Oxidative stress, marked by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, is a key factor that can impede the wound healing process. This study investigates the therapeutic potential of benfotiamine and fursultiamine, two lipophilic derivatives of thiamine (vitamin B1), in preventing lipopolysaccharides (LPS) induced human dermal fibroblast apoptosis. Benfotiamine and fursultiamine are known for their ability to increase thiamine levels in tissues and exert antioxidative effects, thereby potentially countering oxidative stress and its detrimental effects on wound healing. Our research utilizes LPS to induce oxidative stress in human dermal fibroblasts and how vitamin B1 derivatives improves it. The treatment effects of benfotiamine and fursultiamine will be assessed in terms of cell viability, proliferation, migration (key processes in wound healing), and their capacity to modulate oxidative stress markers. Human dermal fibroblasts were treated with LPS in the absence and presence of various concentrations of vitamin B1 derivatives and cell viability was determined. Our results suggest that both benfotiamine and fursultiamine prevents LPS-induced decrease in cell viability. We next planned to investigate how thiamine derivatives prevents LPS-induced apoptotic signals in fibroblasts. We will measure activation of caspase 3, cleavage of PARP, expression of apoptotic markers. Further, we will use an animal model of wound healing to examine how thiamine derivatives improves the wound healing.

2.3. BMS 03: Examining Limbic Sexual Dimorphism in Schizophrenia

Kennedy Madrid[1]

1. Noorda College of Osteopathic Medicine

Schizophrenia is a mental disorder that affects a significant number of individuals in the United States and can have numerous different symptoms. Interest in the differences between the neuroanatomy of individuals with schizophrenia and individuals without schizophrenia has emerged, specifically the sexual dimorphism in individuals with schizophrenia. The overarching aim of this study was to characterize the shape features of the hippocampus and amygdala and determine whether they are different between men and women with schizophrenia. Understanding the differences in brain anatomy between men and women diagnosed with schizophrenia, specifically the hippocampus and amygdala due to their involvement with the pathology of schizophrenia, can open a discussion on how sex-related neuroanatomical

differences can affect the manifestation of symptoms and, eventually, lead to better disease management and effectively treat unique symptom profiles. This study utilized archival data collected from the Conte Center for the Neuroscience of Mental Disorders (CCNM) at Washington University of St. Louis and the Center for Biomedical Research Excellence (COBRE) through the Mind Research Network and the University of New Mexico. The raw T1-scans of the magnetic resonance images were first processed using the neuroimaging toolkit FreeSurfer v6.0 and editing included fixing skull stripping errors, fixing intensity normalization errors to increase white matter surface, fixing topological errors, fixing white matter errors, and fixing pial errors. Characterization of the shape feature of the amygdala and hippocampus was accomplished using a high-dimensional brain mapping procedure known as Large Deformation Diffeomorphic Metric Mapping. Imaging data from both COBRE and CCNM cohorts were harmonized using neuroComBat procedures to eliminate or account for the effects of the use of different scanners in both datasets and its potential effects on further analysis of the regions of interest. Differences between groups in the surface shape of both the amygdala and hippocampus were accomplished by constructing vertex-wise t-maps for each group comparison. The data demonstrated that women with schizophrenia exhibited an inward deformation in both the hippocampus and amygdala, whereas men with schizophrenia exhibited an outward deformation in the hippocampus. These results suggest that men and women with schizophrenia do possess differences between the hippocampus and amygdala and that these differences may impact symptom manifestation and potential treatments.

2.4. BMS 04: Semaglutide prevents hyperglycemia-induced endothelial cell dysfunction

Jesse Kupfer[1], Kenyon Mitchell[1], Kota Ramana[1]

1. Noorda College of Osteopathic Medicine

Endothelial cell dysfunction is a significant risk factor for cardiovascular complications. Hyperglycemia is well known to increase intracellular reactive oxygen species (ROS), subsequently inducing apoptotic cell death, inflammation, and injury in endothelial cells. Although several anti-diabetic drugs have been shown to prevent endothelial dysfunction, the role of glucagon-like peptide-1 analogue (GLP-1A), semaglutide, on hyperglycemia-induced endothelial dysfunction is not known. We hypothesize that semaglutide induces cytoprotective effects by preventing G-protein coupled receptor signaling pathways, thereby mitigating hyperglycemia-induced endothelial dysfunction. Human umbilical vein endothelial cells (HUVECs) were treated with high glucose in the absence and presence of various concentrations of semaglutide for 24 and 48 hours. Cell viability was determined by MTT assay. Expression of various anti- and pro-apoptotic factors was determined by Proteome Profiler Array Human Apoptosis kit. Caspase-3 activity was measured with a specific caspase-3 activity assay kit. The degree of monocyte adhesion to HUVECs treated with high glucose in the presence and absence of semaglutide was determined via monocyte adhesion assay. Our results indicate that treatment of HUVECs with high glucose induces endothelial cell death, and semaglutide prevents hyperglycemia-induced cell death in a time and dose-dependent manner. Additionally, semaglutide regulated high glucose-induced expression of various pro-apoptotic and anti-apoptotic proteins, prevented high glucose-induced caspase-3 activity in endothelial cells, and decreased the degree of monocyte adhesion to HUVECs in the setting of high glu-

cose. Further experiments are being undertaken to examine the effect of semaglutide on eNOS expression and nitric oxide production. Our studies demonstrate that semaglutide ameliorates hyperglycemia-induced endothelial dysfunction and suggests its potential use in preventing cardiovascular complications in the setting of hyperglycemia.

2.5. BMS 05: Exploring the Impact of Wrestling Experience on Shoulder Range of Motion: A Correlational Analysis

Isaac Parrish[1], Justin Guffey[1], Ethan Hampton[1], Branson Fonnesbeck[1], Sara Beaudry-Wiltse[1], Michael Cosgrave[1], John A Kriak[1]

1. Noorda College of Osteopathic Medicine

Wrestling is a physically demanding sport known for its risk of shoulder injuries, particularly dislocations resulting from forced external rotation. It is believed that wrestlers have more internally rotated shoulders due to the nature of the sport. This study aimed to investigate whether years of wrestling correlate with compensatory changes in shoulder internal rotation range of motion (ROM). High school wrestlers (n=51) from three schools participated, undergoing passive and active ROM tests using a goniometer for both shoulders. Statistical analyses revealed no significant correlation between years of wrestling and passive internal rotation ROM for the left (p=0.120) or right (p=0.145) shoulder, nor for active internal rotation ROM for the left (p=0.126) or right (p=0.397) shoulder, at a 95% confidence level. These findings suggest that wrestling experience may not directly impact shoulder internal rotation ROM. Further research is needed to explore other factors contributing to shoulder injuries in wrestlers and potential strategies for injury prevention.

2.6. BMS 06: N-acetylcysteine can be used as a potentiator of oxidative stress in Staphylococcus

Mark Stoll[1], Kaden Bentley[1]

1. Noorda College of Osteopathic Medicine

Purpose: Antibiotics have arguably been among the greatest medical discoveries; however, bacteria have continued to evolve ways to survive and evade their destructive effects. Strains of bacteria which were once easily treated have again become dangerous. Currently Medicare's non-reimbursement policy penalizes hospitals approximately $350 million per year for not preventing hospital acquired infection, resulting in increased patient costs and mortality. Antibiotic resistance is well described and the possibility of using either reactive oxygen species (ROS) or antioxidants as adjuvants to boost the log kill of an antibiotic is an active area of research. The purpose of the current study is to test how bacterial growth is affected by N-acetylcysteine (NAC) in the context of an ROS attack simulated by H2O2.

Methods: Two baselines were set up using approximately 1x10^9 CFU/mL of S. epidermidis treated with 0.1% of H2O2, or 10mg/mL of NAC for varying lengths of time. A third set of S. epidermidis was pretreated with 10mg/mL NAC for 20 minutes followed by a wash and then varying lengths of time of 0.1% H2O2. Final growth was measured using OD600 and biofilms were measured using a solubilization assay read at OD530.

Results: Bacterial growth was stunted by H2O2, whereas NAC had minimal effect at the given concentration. It is noteworthy to say NAC at higher concentrations also stunted bacterial growth. Interestingly, when NAC was used as a 20 minute pretreatment the stunting effect of H2O2 was magnified between 10 to 100 times. Biofilm was reduced by both NAC and H2O2 but biofilm per growth at OD600 was reduced by NAC to a much greater effect.

Conclusions: These results support previous literature that state NAC may act as a biofilm inhibitor and adds that ROS attacks can be potentiated by disrupting the biofilm. Together, they can act synergistically to stunt prokaryotic bacterial growth. Especially of interest is that additional literature supports NAC as a cytoprotective antioxidant for eukaryotes. The differential effect between prokaryotes and eukaryotes, alongside its synergist effect, suggests that NAC could be a useful antibiotic adjuvant.

2.7. BMS 07: Sulforaphane Pre-treatment Improves Alveolar Macrophage Killing after Alcohol-Induced Dysfunction in Human and Murine Cells in vitro

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1. Noorda College of Osteopathic Medicine
2. Northern Arizona University

Purpose: Alcohol is associated with increased mortality and morbidity globally. Pulmonary infections with opportunistic pathogens can occur in healthy humans; however, binge alcohol intoxication (≥ 0.08% BAC) is a major risk factor. We have previously shown that a single dose of alcohol comparable to binge alcohol intoxication increases infection by reducing alveolar macrophage function in vivo. The aim of this study was to 1) test the therapeutic potential of the phytonutrient sulforaphane (SFN) given as a pre-treatment, and 2) test the alcohol-induced effects on phagocytic function in murine and human macrophages in vitro.

Methods: The primary readout was intracellular phagocytic killing via colony forming units (CFU), and a secondary outcome was cytokine expression via ELISA.

Results: Dose response curves indicated that SFN concentrations less than 20 μM were not cytotoxic in both MH-S (murine) and THP-1 (human) cells and that the TD50 in THP-1 cells was 90 μM. Live infection assay results showed MH-S and THP-1 cells pre-treated with SFN (5 μM) and challenged with 0.2% (v/v) alcohol for 3 or 8 hours prior to live B. thailandensis or S. epidermidis infection improved intracellular pathogen killing approximately 15- and 10-fold respectively, compared to macrophages treated with alcohol alone. ELISA analysis indicated that SFN significantly reduced levels of TNF-α expression at 3 and 8 hours compared to controls and alcohol-treated THP-1 cells.

Conclusions: Taken together, SFN-induced cytoprotection was extended beyond murine cells to include human cells, and different opportunistic pathogens that include gram-negative and positive organisms were tested. These data demonstrate that SFN may be an effective pre-treatment option to prevent alcohol-mediated innate immune dysfunction and restore alveolar macrophage phagocytic killing during opportunistic pulmonary infections.

2.8. BMS 08: DNA Extraction Method Development for Ocular Tissues

Mike Trapnell[1], Conrad Ashby[1], Walker Kay[1], Jonathon Reynolds[1], Noah Schultz[1], Brandon Burger[1], Christina Small[1], John Kriak[1], Kyle Bills[1], David Sant[1]

1. Noorda College of Osteopathic Medicine

Purpose: DNA extraction kits are traditionally developed to work with liquid tissues such as blood, saliva, and swabs, but some have been proposed to work with solid tissues. Somatic variation in cancers can be important for tumor subtyping and treatment guidance, including ocular tumors. Additionally, epigenetic marks such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are tissue-specific and change in disease states, particularly evident in diabetic retinopathy and age-related macular degeneration. Commercial DNA extraction kits are available from several vendors, but the various kits have different strengths and weaknesses, and the removal of PCR inhibitors will vary with each kit. This project investigates the yield and purity of DNA from ocular tissues using commercial DNA extraction kits.

Methods: Cornea, neural retina, RPE/choroid layer, optic nerve, and capsular bag were collected and aliquoted into 15 mg aliquots. Extractions were performed using the following kits: DNEasy Blood and Tissue Kit (Qiagen;), GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific), Monarch HMW DNA Extraction Kit for Tissue (New England Biosciences), and genomicPrep Mini Spin Kit (Cytiva). DNA was quantified using the Qubit Fluorometer and molecular weight was checked by agarose gel. Several more kits are currently being tested.

Results: All four kits yielded high molecular weight DNA (above 20 kbp). The Monarch HMW kit yielded DNA with significantly higher molecular weights. The DNA yields per milligram of tissue were highest using the DNEasy Blood and Tissue Kit for optic nerve, neural retina, and RPE/choroid. The yield was highest for the cornea using the genomicPrep Mini Spin Kit. Only the genomicPrep Mini Spin Kit yielded sufficient DNA for quantification from the capsular bag, and total yields were minimal (600 ng or less). Additional kits are currently being tested, but initial results indicate that several commercial kits will be sufficient for DNA extraction of ocular tissues. Further work is needed to purify epithelial cells and stem cells from the intraocular lens.

Conclusions: Of the kits tested, all are sufficient to obtain significant amounts of DNA from all ocular tissues aside from the capsular bag. The Monarch HMW yielded the highest molecular weight, but significantly lower quantities of DNA than the other kits, indicating that it may not be ideal for most purposes. Protocol development for the capsular bag is still underway.

2.9. BMS 9: Deciphering psilocybin: Cytotoxicity, anti-inflammatory effects, and mechanistic insights

Salma Laabi[1], Claire LeMmon[1], Callie Vogel[1], Mariana Chacon[1], Victor M. Jimenez Jr.[1]

1. Noorda College of Osteopathic Medicine

A decade of clinical research has indicated psilocybin's effectiveness in treating various neuropsychiatric disorders, such as depression and substance abuse. The correlation between increased pro-inflammatory cytokines and the severity of neuropsychiatric symptoms, along

with the known anti-inflammatory potential of some psychedelics, suggests an immunomodulatory role for psilocybin. This study aims to understand the mechanism of action of psilocybin by investigating the cytotoxic and immunomodulatory effects of psilocybin and psilocin on both resting and LPS-activated RAW 264.7 murine macrophages. The study evaluated the cytotoxicity of psilocybin and psilocin using an LDH assay across various doses and assessed their impact on cytokine production in RAW 264.7 cells, measuring cytokine expression via ELISA. Different doses, including those above and below the LC50, were used in both pre-treatment and post-treatment approaches. The LDH assay revealed that psilocybin is almost twice as cytotoxic as psilocin, with an LC50 of 12 ng/ml and 28 ng/ml, respectively. In resting macrophages, both psilocybin and psilocin triggered significant release of TNF- α after 4 h, with the lowest doses inducing higher levels of the cytokine than the highest doses. IL-10 expression in resting cells was only triggered by the highest dose of psilocin in the 4-hour incubation group. In LPS-stimulated cells, psilocin reduced TNF- α levels more than psilocybin in pre-treatment and post-treatment, with no significant effects on IL-10 in pretreatment. Psilocin, but not psilocybin, induced a significant increase of IL-10 in post-treatment, leading to the conclusion that psilocin, but not psilocybin, exerts anti-inflammatory effects on classically activated macrophages.

2.10. BMS 10: Anthracycline -induced vascular endothelial cell death is prevented by Semaglutide

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Chemotherapeutic agents like doxorubicin (Dox), daunorubicin, and epirubicin belong to the anthracycline drug family and have proven efficacy in treating a spectrum of cancer types. However, the utilization of these drugs in advanced cancer cases is hampered by the unwanted cardiotoxic effects. To address this challenge, there is a critical need for novel approaches that not only enhance the therapeutic potential of these drugs but also alleviate undesirable side effects, particularly those affecting the heart. In this context, Semaglutide, a well-known glucagon-like peptide-1 (GLP-1) receptor agonist commonly used in managing type-2 diabetes, was the focus of our investigation. We sought to explore its potential in preventing Dox-induced vascular endothelial cell toxicity. Our research uncovered compelling evidence supporting the protective role of Semaglutide against Dox-induced cell death in human umbilical vein endothelial cells (HUVEC). Furthermore, our study demonstrated that Semaglutide exhibits the capacity to block Dox-induced apoptosis in HUVECs, concurrently mitigating reactive oxygen species generation and suppressing the activation of caspase-3. In addition, Semaglutide displayed a regulatory influence over the expression of various anti- and pro-apoptotic, as well as inflammatory factors induced by Dox. In conclusion, the findings from our present study strongly suggest that this anti-diabetic drug as the potential to inhibit Dox-induced vascular endothelial cell death and this protective effect is attributed to the modulation of various pro-apoptotic, anti-apoptotic, and inflammatory markers. These results position Semaglutide as a potential adjuvant therapy, offering the prospect of mitigating the adverse effects associated with anthracycline chemotherapeutic drugs.

2.11. BMS 11: DNA Extraction Method Development from Solid Tissues

John Dougherty Jr, Ezenna Obilor, Alexander Ruiz, Ryan Powers, Noah Schultz, Brandon Burger, Corwin Frey, Nathaniel Hill, Lara Laughrey, P. Tanner Brain, Sara McMahon, Parker Feltner, Steven Tung, Daoud Sajady, Elden Jenkins, Julian Jarquin, Serin Baker, Aaron Andrews, Christina Small, John A. Kriak, Kyle B. Bills, David Sant

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Note: Individual author affiliations not provided.

Hypothesis/Purpose: Although germline variation testing is traditionally performed using DNA obtained from blood or other liquid samples, determining somatic variation in cancer samples requires DNA extraction directly from tissues. Additionally, epigenetic markers, such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are tissue-specific and change in selected disease states. However, several substances present in tissues are known to inhibit downstream reactions, including polymerase chain reaction PCR). For this project, we are assessing the quantity and quality of DNA obtained from extractions of various vital organs using 30 different commercially available DNA extraction kits to determine optimal kits for each tissue.

Methods: Samples from several vital organs have been collected and homogenized using a hand-held homogenizer. Samples were aliquoted into tubes at the maximum recommended sample size for each DNA extraction kit. Extractions were performed using the following kits: DNEasy Blood and Tissue Kit (Qiagen), GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific). Quantity has been tested using a Qubit Fluorometer (Thermo Fisher Scientific) and average molecular weight has been checked by agarose gel electrophoresis. Several more kits are currently being tested. Extracted DNA will be tested for inhibitors using quantitative polymerase chain reaction (qPCR).

Results: Extractions have been performed for several tissues across a few kits. Using fresh tissue rather than frozen tissue greatly improved both yield and average molecular weight of DNA extracted. Although all kits yielded high molecular weight DNA (>20,000 bp) from fresh tissue, the Monarch HMW kit yielded DNA with significantly higher molecular weights. On average, the DNA yields per milligram of tissue were highest using the DNEasy Blood and Tissue Kit and the genomicPrep Mini Spin Kit. The GeneJET Genomic DNA Purification Kit was predicted to have minimal yields, but actual yields were greater than 75% that of competing kits, and the average molecular weight appears to be higher by agarose gel electrophoresis.

Conclusions: Only a few extraction kits have been tested to date, but all gave high yields and high molecular weight DNA. The results of this study will allow us to identify the most suitable kits for DNA extraction from various tissues.

2.12. BMS 12: Potential Benefits of Vialinin-A on Doxorubicin-induced Cardiac Toxicity

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2. Independent Researcher

Doxorubicin (DOX), an anthracycline, is used as an antineoplastic agent for multiple cancers but has established dose-dependent, cumulative, and progressive cardiotoxicity. DOX is a known inducer of reactive oxygen species (ROS) leading to increased oxidative stress. The oxidative stress generated by DOX can alter cellular redox balance by modulating various oxidative and anti-oxidative pathways causing cellular toxicity. Clinically DOX, and other anthracyclines, have historically been correlated with reduced left ventricular ejection fraction and symptomatic heart failure in approximately 5% of patients. Efforts to reduce the cumulative dose to 400mg/m2 have been successful in curbing the heart failure rate to 3.5%. Due to its efficacy Doxorubicin continues to be a chemotherapeutic of choice but its success comes at a heavy cost to a patient's quality of life. Vialinin A (VA) is an antioxidant extracted from Chinese edible mushroom T. terrestris and T. vialis. VA targets Ubiquitin-specific-peptidase 5, which prevents cell ubiquitination and is considered a core molecule in TNFα production. TNFα is a known pro-apoptotic factor and a common target in cancer therapeutics. Additionally, VA is an antioxidant that reduces reactive oxygen species (ROS) within a cell, therefore, we predict that it will restore the damage caused by DOX. Using human vascular endothelial cells (HUVEC), we have assessed how VA regulates the cytotoxicity induced by DOX. Our results indicate that VA prevented the DOX-induced decrease in the cell viability. Further, VA also prevented the apoptosis of endothelial cells and activation of caspase 3. Furthermore, VA prevented the DOX-induced ROS generation, and restored the activities of antioxidant enzymes such as catalase and glutathione peroxidase. VA also regulated the expression of various apoptotic markers in HUVECs. Thus, our results suggest that VA could be used to prevent the cardiotoxic side effects of DOX. Further, experiments are in progress to identify its beneficial effects in animals as well as in cardiac myocytes.

2.13. BMS 13: Antioxidant Isolated from Chinese Edible Mushroom Prevents Colorectal Cancer Growth.

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Background: Colorectal Cancer (CRC), ranking as the third leading cause of cancer-related mortalities in the United States, has traditionally been subject to screening initiation around the age of 50. However, an emerging trend in recent years underscores a rise in CRC incidence among younger demographics. The etiological underpinnings of this disease are posited to involve genetic predisposition, oxidative stress, and inflammatory processes. Conventional therapeutic modalities, including chemotherapy and bowel resection, are associated with undesired side effects and risks such as cardiotoxicity, notably observed in chemotherapeutic agents like anthracyclines. In this context, Vialinin-A, an isolated compound sourced from the Chinese mushroom Thelephora Vialis, presents a promising avenue for exploration due to its purported antioxidant and anti-inflammatory properties. This study investigates the potential of Vialinin-A in impeding CRC cell proliferation through anti-carcinogenic mechanisms, specifically by inducing apoptosis in colon cancer cells.

Methods: In order to study the effect of Vialinin-A on colon cancer cells, SW480 and CaCO-2 CRC cancer cell lines were treated with varying concentrations of the compound. Cell viability

was assessed at 24 and 48 hours for both cell lines through the execution of an MTT Assay. The activation of caspase-3 and the induction of apoptosis were measured using specialized assay kits. A Human Apoptosis array was employed to quantify the expression of diverse apoptosis markers in CRC cells treated with Vialinin-A. Additionally, a multiplex of arrays will be conducted to identify markers associated with oncogenesis, inflammation, and apoptosis. In vivo investigations into the chemopreventive potential of Vialinin-A will be conducted utilizing nude mice xenografts.

Results: Preliminary findings indicate a dose-dependent inhibition of CRC cell growth by Vialinin-A. The compound effectively curtails the formation of reactive oxygen species, activates caspase-3, and induces apoptosis in cancer cells. Moreover, Vialinin-A exerts regulatory control over the expression of various apoptotic markers in CRC cells. Ongoing experiments seek to delve deeper into the intricate mechanisms by which Vialinin-A prevents CRC growth.

2.14. BMS 14: In silico identification of small molecule agonist binding sites on KCC2

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1. Noorda College of Osteopathic Medicine

Purpose: Potassium-Chloride Cotransporter 2 (KCC2) is a neuronal membrane protein specific to the central nervous system. It is responsible for removing Cl- ions from the intracellular space, maintaining a normal Cl- gradient essential for proper function at inhibitory synapses. Dysregulation causes an upward shift in the Cl- reversal potential resulting in a hyperexcitable state of the postsynaptic neuron. Existing literature indicates that KCC2 may be involved in the addiction pathway of a variety of drugs of abuse, including opioids and alcohol. This makes KCC2 an attractive potential drug target when treating substance use disorders. A novel direct KCC2 agonist, VU0500469, was recently identified experimentally; however, no binding sites were identified or characterized. The goal of this project is to identify likely binding sites of this protein-ligand pair via computer simulation.

Methods: A 3D model of human KCC2 was obtained from RCSB Protein Databank. VU0500469 was reconstructed manually. Using the PrankWeb interface, a structural binding pocket identification was performed with P2Rank. Blind, semi-flexible docking of the ligand, VU0500469, and KCC2 was performed using QVina-W and GNINA. Results from P2Rank, GNINA, and QVINA-W were manually overlayed using PyMol to visualize overlapping conformations and/or pockets. Sites with at least two overlapping results were selected as probable binding sites for further investigation. Inputs for the molecular dynamics simulation were generated using CHARMM-GUI and passed to CHARMM.

Results: Results between simulations were then compared, and several possible VU0500469 binding pocket sites were successfully identified. Geometric, template-free binding site prediction with P2Rank revealed 27 potential binding sites. GNINA was set to produce nine outputs, resulting in 18 total conformations. QVINA-W was set to produce 20 outputs. To aggregate these simulation results and determine likely binding sites, outputs from all three simulations were overlayed in PyMol. Only one site was identified by all three simulations. This was identified as the most promising binding site.

Conclusions: The binding sites identified may represent targets for the development of additional KCC2 agonists. Future plans are focused on drug discovery, screening, and potential therapeutic applications.

2.15. BMS 15: Xylazine's Cytotoxic Effects on Central and Peripheral Tissues—Deciphering a Biphasic Expression

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1. Noorda College of Osteopathic Medicine

Purpose: Xylazine, an α2-adrenoceptor agonist, is a compound conventionally used as an anesthetic in veterinary medicine. Sharp exacerbations of the U.S. opioid overdose crisis are linked to the polysubstance use of synthetic xylazine. Although xylazine is well-documented in veterinary medicine, the pharmacological features associated with substance abuse are less understood. The purpose of the current study was to 1) test the cytotoxic effects of xylazine in a dose-specific manner and 2) test the effects of xylazine in brain and peripheral tissues.

Methods: The primary readout was lactate dehydrogenase (LDH) in RAW264.7 macrophages and SIM-A9 microglial cells. Xylazine was serial diluted with PBS from 2 mg/mL to 1 pg in saline.

Results: Contrary to conventional linear dose-response relationships, our findings revealed that xylazine's cytotoxicity exhibited a biphasic pattern, with heightened toxicity observed at both the extremes of a U-shaped curve. The LC30 is greater in microglial cells compared to RAW264.7 macrophages, suggesting an increase in potency in the brain compared to the periphery. Xylazine presents in a biphasic, U dose-response curve in both tissue types. The shared biphasic response suggests a common underlying mechanism and signaling pathways in xylazine-induced cytotoxicity. These data provide a framework to better understand the cytotoxic effects of xylazine in two different tissue types.

Conclusion: Understanding the pharmacology of xylazine is crucial for refining dosage recommendations and enhancing the safety profile. To better understand xylazine's signaling pathways, our future directions include 1) evaluating the cytokine inflammatory responses at cytotoxic and noncytotoxic doses, and 2) extending the study beyond xylazine to include a morphine base compound to test interaction in the context of infection and inflammation. Harm reduction-informed public health guidelines and programs are urgently needed to prevent and respond to xylazine-involved overdoses more effectively.

2.16. BMS 16: Edible mushroom-derived compound, vialinin-A, prevents ocular inflammation.

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Uveitis, an ocular inflammatory complication, is one of the major causes of visual impairment worldwide with unknown etiology. Infections, autoimmune diseases, and other factors could lead to this complication that damages the uveal tract and adjacent ocular structures. Corticosteroids are commonly used for the therapy of uveitis, but their prolonged use has unwanted

side effects. Therefore, the development of potential therapeutic approaches is required to treat ocular inflammatory complications with better safety and efficacy. Vialinin-A, isolated from the edible Chinese mushroom, has been shown to be a potent antioxidant with anti-inflammatory actions. However, its role in preventing uveitis is not known. We hypothesize that vialinin-A could prevent ocular inflammation in normal and hyperglycemic conditions. Since uveitis is a systematically originated complication, we examined the effect of vialinin-A first on macrophages then locally on HNPECs. Human non-pigmented ciliary epithelial cells (HNPECs) and Thp-1 monocytes were used to determine the anti-inflammatory effects of vialinin-A. The cells were treated with LPS and/or high glucose in the absence/presence of vialinin-A, and cell viability was determined by MTT assay. The expression of various inflammatory cytokines, chemokines, and growth factors was determined by antibody arrays. Endotoxin-induced uveitis will be developed in normal and diabetic rats, and the effects of vialinin-A treatment in preventing ocular inflammation will be examined in various ocular tissues. Treatment of Thp-1 cells with LPS caused cell death in time-dependent manner and vialinin-A prevented the LPS-induced cell death dose-dependently. Similarly, in HNPECs LPS-induced decrease in cell viability was reversed by vialinin-A. Further, hyperglycemia-increases LPS-induced decrease in cell viability and vialinin-A prevented it. Vialinin-A prevented expression of various cytokines and chemokines and activation of NF-kB in the Thp-1 cells. Further studies are in progress to examine how vialinin-A prevents ocular inflammation including animal studies using endotoxin-induced uveitis in normal and diabetic conditions. Our results indicate that vialinin-A could prevent endotoxin-induced ocular inflammation by regulating the NF-kB mediated expression of cytokines and chemokines, suggesting it could be developed to treat uveitis.

2.17. BMS 17: Gas Station Heroin: Tianeptine's Cytotoxic Effects on Central and Peripheral Tissues in vitro

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Purpose: Tianeptine is a tricyclic antidepressant, mainly acting on mu-opioid receptors, approved for the treatment of major depressive disorders in many countries, not including the United States. Also known as 'gas station heroin', tianeptine has the potential for abuse in both inpatient and illicit settings due to its euphoric effects and rapid tolerance. Currently, tianeptine's immunomodulatory effects are relatively under-researched. The purpose of the current study was to 1) test the cytotoxic effects of tianeptine in a dose-specific manner and 2) test the effects of tianeptine in brain and peripheral tissues.

Methods: The primary readout was lactate dehydrogenase (LDH) in RAW264.7 macrophages and SIM-A9 microglial cells. Tianeptine was administered in media supplemented with doses titering (10 mcg/ml –0.01953 mcg/ml) and then incubated for 2 hours. Cytotoxicity of tianeptine was then determined based on levels of Formazan in solution.

Results: Unlike conventional linear dose-response relationships, our findings revealed that tianeptine's cytotoxicity exhibited a biphasic pattern, with heightened toxicity observed on the left arm of a U-shaped curve. The findings indicate a U-shaped dose-dependent increase in cytotoxicity for both peripheral and brain derived macrophages. At doses greater than 0.3125

mcg/ml, the cytotoxic effect is approximately 3-fold greater in RAW264.7 macrophages compared to microglial cells. At doses greater than 0.3125 mcg/ml, cytotoxicity decreases for both cells; the lowest cytotoxicity was observed in microglial cells.

Conclusions: Understanding the pharmacology of tianeptine is crucial for combating the opioid epidemic and enhancing the safety profile. Misuse of tianeptine can lead to euphoric, opioid-like highs with the potential for chronic users to develop dependence and tolerance. Overdose and use in suicide attempts have also been documented. To better understand tianeptine's signaling pathways, future directions include 1) evaluating the cytokine inflammatory responses at cytotoxic and noncytotoxic doses, and 2) testing the effects of poly-use that include tianeptine and other opioid agonists.

2.18. BMS 18: Role of NLRP3-Inflammasome-Mediated Inflammatory Response Induced by Amyloid Precursor Protein (APP695) and its Swedish Mutant in the Development of Alzheimer's Disease.

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1. Noorda College of Osteopathic Medicine

Purpose: Alzheimer's disease (AD) is a progressive neurodegenerative condition that has become an increasing burden to the healthcare industry among the aging population. With advanced age being the greatest risk factor for developing the disease, an estimated 6.7 million individuals over the age of 65 are living with AD in the United States. The pathology is associated with the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular Tau tangles in the brain which is exacerbated by neuroinflammation. We hypothesize that the NLRP3 inflammasome pathway induced by excessive Aβ-production plays a significant role in the development of AD pathology, especially the Swedish mutant APP695 isoform. The inflammasome is a multiprotein complex that promotes activation of caspase-1 and secretion of interleukin 1β (IL-1β) and IL-18, which leads to neuronal apoptosis and in turn neurocognitive impairments.

Methods: To test our hypothesis, SH-SY5Y neuroblastoma cells, APP695 isoform overexpressing SH-SY5Y cells (SH-APP695), and SH-SY5Y cells overexpressing Swedish Mutant of APP-695 isoform (SH-APP695SW) were co-cultured with primary microglial cells. The Swedish mutation of amyloid precursor protein (APP695SW) is known to increase aberrant cleavage of amyloid precursor protein which can cause elevated levels of Aβ and Tau tangles. The cells and supernatants were collected and analyzed for the expression of APP, Caspase-1, mature IL-1β, and IL-18 proteins by Western Blot assay and ELISA.

Results: The Western Blot results suggest that APP expression is significantly higher in APP695 cells when compared to the APP695SW mutant. Concurrently, we have observed significantly increased cleaved IL-1β expression in APP695 cells than in APP695SW cells. No change in IL-18 expression was observed in these cells when compared to the control SH-SY5Y cell group.

Conclusion: In contrast to our hypothesis, APP695-expressing neuronal cells significantly expressed APP and induced cleaved IL-1β expression when compared to the APP695SW mutant-expressing neuronal cells.

2.19. BMS 19: Vitamin B1 derivatives benfotiamine and fursultiamine prevents glioblastoma cell growth in vitro and in vivo.

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Background: prevent glioblastoma Brain tumors are responsible for over 15,000 deaths per year with 49% of those tumors defined as glioblastomas, the most malignant and aggressive forms of CNS tumors. Five-year survival rates currently only stand at 36% with treatment options limited to supportive care, tumor resection and intensive chemotherapy regimens. Some immunotherapies have demonstrated marginal effectiveness but present considerable risks to patients. Thiamine, more commonly known as vitamin B1, has been shown to play a crucial role in the regulation of cellular metabolism and the nervous system. Benfotiamine and fursultiamine are the lipid soluble derivatives of Vitamin B1 with better absorption and retention rate when compared to water soluble thiamine. Benfotiamine and fursultiamine with their potent antioxidative and anti-inflammatory actions shown to prevent several complications including diabetic neuropathy, neurodegenerative diseases, alcoholic polyneuropathy. However, their anti-carcinogenic effects are not well known. Hypothesis: We hypothesize that with their potent antioxidative and anti- inflammatory actions, lipid soluble derivatives of vitamin B1 could prevent glioblastoma

Methods: Human glioblastoma (U87) cell lines grown in Eagle's Minimum Essential Media (EMEM) were treated with various concentrations of benfotiamine and fursultiamine. Cell viability was determined by MTT assay in a time- and dose-dependent manner. We then examined how vitamin B1 derivatives prevent the growth of U87 cells by examining apoptosis using Annexin-V staining and live-dead cell assay kits. Expression of various pro-apoptotic and anti-apoptotic factors, along with other inflammatory factors, including Bad, Bax, Bcl-2, cIAP-1, cIAP-2, and procaspase 3, was determined using a human apoptosis array. Finally, we planned to inject glioblastoma cells into the athymic nude mice xenograft model and examine the growth of glioblastoma in the mice treated with vitamin B1 derivatives.

Results: Our initial results indicate that the vitamin B1 derivatives, benfotiamine androwth of glioblastoma cells at optimal concentrations of 50μM benfotiamin fursultiamine, decrease the ge and 75μM fursultiamine. Lipid- soluble derivatives also increased caspase-3-mediated apoptosis. Furthermore, these derivatives regulated the expression of various pro- apoptotic, anti-apoptotic, and survival factors in glioblastoma cells. Further research is in progress to understand the molecular mechanisms. So far, our studies indicate that lipid-soluble derivatives of thiamine could prevent the growth of glioblastoma cells in culture.

Conclusion: So far, our studies indicate that lipid soluble derivatives of thiamine could prevent the growth of glioblastoma cells in culture.

2.20. BMS 20: Development of a protocol for obtaining biological samples for genetic testing from remote individuals

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Hypothesis/Purpose: Pharmacogenomic sequencing allows individuals to learn more about how they will respond to certain medications but requires shipping of a biological sample. One complication of sending biological samples to remote laboratories is stability. Blood generally yields sufficient quantities of high-quality DNA but requires a clinic visit. Saliva and buccal swabs are routinely used for DNA extractions, but the DNA quality is notoriously low due to the presence of bacteria in the mouth. Additionally, elderly individuals have difficulty producing enough saliva for testing, and the tubes contain several milliliters of liquid and shipping requires special considerations. Dried blood spot cards, which serve as an alternative to saliva and buccal swabs, yield high-quality DNA and ship easily, but may produce a lower yield. This project aims to determine which biological sample methods can reasonably be obtained from remote individuals.

Methods: Swab and saliva kits from Mauwi, Zymo, Gentueri, and DNA Genotek have been purchased for testing. Filter paper for DBS collections has been purchased from Qiagen and Cytiva. Forty different DNA extraction kits from various companies have been obtained. Extractions up to this point were performed using the Beckman Coulter GenFind V3 kit.

Results: Completion of dried blood spot cards required more lancet punctures than anticipated, with a median of 3 punctures (range 2-11) per card. Out of 17 completed cards, only 3 of them required more than 4 punctures. Yields per tissue were as follows: 400 µl of buffy coat - 5.6-24 µg; 200 µl from Mawi swab kits – 2.5-3.7 µg; 400 µl from DNA Genotek swab kits – 2.4-4.4 µg; 400 µl from DNA Genotek saliva kits – 1.9-2.1 µg; One square inch Qiagen FTA transfer card – 350-400 ng; One square inch Cytiva Whatman filter paper – 600 ng.

Conclusions: Preliminary results indicate that the yield from swabs are greater than from saliva, but not as high as buffy coat. Yields from dried blood spots were significantly lower than from other tissues. Sample size and purity have not yet been evaluated.

3. Case Studies

3.1. CS 01: Case Report: Pars Defect resulting in 29- year History of Painful Anterolisthesis Resolved with Anterior Lumbar Interbody Fusion with Minimally Invasive Posterior Fixation

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Pars defects typically develop as a stress fracture in the teenage years. This additional lumbar disc stress accelerates degenerative changes such as disc height loss, anterolisthesis, and neuroforaminal stenosis, requiring surgical intervention. One treatment strategy is an Anterior Lumbar Interbody Fusion (ALIF) with posterior minimally invasive fixation. We present a 50-year-old male with lumbar pain radiating posterolateral, bilaterally with right worse than left. Onset was 29 years prior with recent worsening pain described as 8-9/10 most days, experiencing frequent falls due to pain and lower extremity weakness and numbness. He reported inability to work for the past year, limited walking, and difficulty eating. Imaging showed a grade II anterolisthesis of L5 to S1, and bilateral L5-S1 pars defects. Preoperative conservative care included physical therapy, pain management and smoking cessation. Despite conservative care, his symptoms persisted. An L5-S1 ALIF with posterior fixation was performed. During the anterior surgery, we removed the entire L5-S1 disc and the posterior longitudinal ligament down to the dura and out both neuroforamina. An anterior interbody cage, filled with allograft bone, was placed at L5-S1 and anchored to S1. The patient was repositioned prone. Neuronavigational was utilized to place bilateral percutaneous screws at L5-S1. Spondylolisthesis was reduced, and set screws were tightened with rods in place. The result was restoration of disc height, reduced spondylolisthesis, and complete indirect neuroforaminal decompression. Postoperatively, the patient reported continued improvement of symptoms. The combined approach is beneficial because it is technically very difficult to fully restore disc height from a posterior approach and to fully reduce the spondylolistheses without fully mobilizing the segment. It allows for complete restoration of the neuroforaminal cross section and full nerve root decompression. This approach increases the likelihood of fusion given better alignment and large surface area. Epidural scar formation and nerve root injury are prevented.

3.2. CS 02: De Novo Microdeletion Spanning YWHAE and CRK in an Individual with Intellectual Disability and Stunted Growth

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Purpose: In this report, we present a case of a 20-year-old female with congenital intellectual disability, stunted growth, and hypothyroidism. Competitive genetic hybridization (CHG) revealed a loss of a portion of 17p13.3 at least 195 Kb in size, not present in either parent. This area of chromosome 17 is associated with Miller-Dieker Syndrome (MDS) and Isolated Lissencephaly Sequence (ILS), but these conditions are related predominantly to PAFAH1B1, which is not included in the patient's deletion.

Methods: Peripheral mononuclear cells (PBMCs) were used for karyotyping and competitive genetic hybridization (CGH) at Baylor College of Medicine. Further bioinformatic analysis was carried out using the Genome Data Viewer (ncbi.nlm.nih.gov/genome/gdv). Further confirmation of endpoints is planned using qPCR and long-range PCR. Assent was obtained from the patient and consent was obtained from the patient's parents prior to beginning the study.

Results: Symptoms included congenital intellectual disability, stunted growth, and hypothyroidism. Karyotype was found to be normal, but CGH revealed a deletion toward the tail end of the p-arm of chromosome 17, 17p13.3. At least 134 genes are present in this genomic location, 35 of which are uncharacterized. Both MDS and ILS are characterized by a smooth cerebral cortex, which was not found in this patient. Notably, PAFAH1B1, which is thought to cause the majority of the symptoms of MDS and ILS, was not deleted. YWHAE and CRK were both deleted and may contribute to this unique phenotype. Deletion of CRK is associated with growth abnormalities, including stunted growth. Although not traditionally treated with growth hormone, the patient grew more than 12-inches in height with treatment, suggesting that growth hormone therapy may be effective for treating growth retardation, at least partially. Several reports have suggested that deletion of YWHAE without deletion of PAFAH1B1 is associated with intellectual disability similar to MDS, but without lissencephaly, and deletion of YWHAE is believed to contribute to a more severe phenotype in individuals with MDS.

Conclusions: Here we present a patient with intellectual disability and a previously uncharacterized deletion on chromosome 17. Analysis of the literature indicates that CRK and YWHAE are likely responsible for the phenotype. Further directions include confirming the endpoints of the deletion.

3.3. CS 03: Non-Traditional Presenting Grade II Brain Meningioma: A Case Study

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1. Noorda College of Osteopathic Medicine

Introduction: Meningioma is a relatively common form of cancer, occurring in approximately 97 out of 100,000 individuals. Although it arises from the meninges surrounding the central nervous system (CNS) rather than from neurons, it is classified with CNS tumors due to overlapping symptoms caused by compression of nerves and vessels in the head. Extracranial metastases are rare (liver, femur, and vertebrae L5 in this case), at less than 1%, and correlate with reduced survival rates.

Methods: Magnetic resonance imaging (MRI), whole exome sequencing (WES), and immunohistochemistry (IHC)

Case: This case study examines the presentation, progression, and treatment of an atypical case of meningioma. A 49-year-old male presented with chronic migraines which did not respond to traditional treatments or procedures. By MRI, a unilateral mass was found in the right parietal region measuring 6x4x2 cm. The mass was removed surgically, and the patient was diagnosed with atypical grade II meningioma. Immunohistochemistry results indicated positive staining for epithelial membrane antigen (EMA), S100 (focally positive), progesterone

receptor (PR), and a Ki-67 of 20%. Somatic mutations were detected in NF2 (p.Y144fs) and SUFU (p.N374fs). Over the next eight years, the patient had several (12+) recurrences of meningiomas in various locations non-adjacent to the original tumor. The patient was treated with various modalities including 7 additional craniotomy surgeries, chemotherapy, gamma knife radiation, and immunotherapy. Immunohistochemical analysis on a meningioma removed in the latest surgery indicated that Ki-67 levels had risen to 70%. Further analysis of sequencing data revealed several somatic copy number variants, including the deletion of the unmutated copies of both NF2 and SUFU, which are suspected to have been deleted in the original tumor. Eight years after the resection of the original tumor, the patient was found to have masses in the liver, vertebrae L5, and right femur. The mass from the liver was removed and stained positively for EMA, suggesting that it originated from the meninges. The tumor grade was raised to level III following the discovery of a metastasis. Femur and vertebrae masses have not yet been evaluated, but are believed to also be metastases from the meningioma. Further chemotherapy is planned to shrink metastases and prevent further spread.

3.4. CS 04: Cancer, Lipoma, Fibroma, Oh My!

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Case Presentation: We present a newly described benign, soft tissue tumor found within the back muscles of a 66-year-old male with a past medical history of HTN, HLD, OSA. After a diagnosis of malignant spindle cell neoplasm was made, the case was referred to the Sarcoma Department and the mass was excised. Intraoperatively, it appeared to be well encapsulated and was sitting in between the muscle bellies. On gross examination, the tumor was a well-circumscribed, heterogeneous mass, measuring 5.8 x 3.5 x 2 cm, tan-brown to white-yellow cut surfaces with focal areas of hemorrhage and without definitive necrosis.

Lab Results: H&E sections show a well-circumscribed mass composed of relatively uniform, bland spindle cells within a variably myxoid-to-collagenous stroma, scattered lymphoplasmacytic infiltrates, and prominent and complex vascular pattern, some with hyalinized walls and fibrin depositions. Immunohistochemical stains were performed; the neoplastic cells showed EMA immunoreactivity with retained Rb nuclear staining, but negative for CD34, Pan-cytokeratin, MUC4, SMA, Desmin, and S100 protein. UCSF500 cancer gene panel test was performed, which revealed a pathogenic translocation involving AHRR, the gene encoding the aryl hydrocarbon receptor repressor, and NCOA2, the gene encoding the nuclear receptor coactivator 2. Overall, the clinical, histological, and immunohistochemical findings are most consistent with angiofibroma of soft tissue (AFST).

Differential Diagnosis: Presumed diagnosis was malignant neoplasm until biopsy and pathology was obtained. Based on vascular pattern and stroma, the possibility of a myxoid liposarcoma was considered but lack of lipogenic differentiation argues against it. Therefore, to further characterize the lesion and confirm the diagnosis UCSF500 cancer gene panel was performed as noted above.

Discussion & Conclusion: Genetic testing is an essential component of accurate diagnosis as the appearance of these lesions can mimic other, more harmful neoplasms. AFSTs often contain a unique NCOA2 fusion gene as well as other identifiable positive markers which include desmin, CD34, α-SMA, and epithelial membrane antigen. Immunohistochemical analysis frequently shows the AHRR-NCOA2 driver mutation which is thought to be associated with t(5;8)(p15;q13), also commonly found in AFSTs. In combination, these immunohistochemical findings can help result in accurate diagnosis of angiofibroma of soft tissue in all the locations it can be found in. The accurate diagnosis of this tumor is essential to prevent additional unnecessary treatments and aggressive surgeries, and as mentioned above, this can be achieved by proper genetic testing for the presence of markers including the NCOA2 fusion gene, CD34, α-SMA, and epithelial membrane antigen.

3.5. CS 05: An Adolescent with Skin in the Game

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Initial Presentation: A 13-year-old male presented to the emergency room with 4 days of fever and worsening left inner thigh pain and redness. There was no history of trauma to the area. Associated signs and symptoms included cough, shortness of breath, nausea, vomiting, and diarrhea. Physical Exam: Physical exam in the emergency center was notable for an area of mild erythema and tenderness on the left inner thigh measuring approximately 15 x 20 cm. Vital signs were significant for fever (39.7 C), tachycardia (151 bpm).

Diagnostic Evaluation: Lab results included a white blood cell count of 15.5 K/mcL with 87% neutrophils and a CRP of 28.8. A computed tomography scan was notable for moderate surrounding inflammation/edema in the subcutaneous fat and superficial fascia without abscess. He was diagnosed with cellulitis and admitted on cefazolin and IV clindamycin. A full body, erythematous rash erupted shortly after beginning cefazolin and it was temporarily discontinued and clindamycin was started. Scarlett Fever was suspected, so cefazolin was restarted in conjunction with clindamycin. However, the margins of erythema slowly expanded, and fevers continued despite nearly 48 hours of treatment (Figure), though repeat CRP decreased to 22.4. Considering his continued worsening, osteomyelitis and pyomyositis were considered. An MRI on hospital day 3 showed cellulitis with extensive myositis and fascial involvement without evidence of gas, likely due to myositis. Despite slow progression and inconclusive imaging, orthopedics was consulted due to concern for possible necrotizing fasciitis; he was taken to the operating room for incision and drainage.

Diagnosis: Operative findings were consistent with necrotizing fasciitis (NF). Operative cultures grew group A streptococcus, and he was diagnosed with type II monomicrobial NF. He required four separate operations and received a prolonged course of IV penicillin G.

Discussion & Conclusion: NF is a life-threating, rapidly progressing infection that rarely occurs in children. It typically involves superficial and/or deep fascia muscles of an extremity and presents with fever, marked tenderness, and toxic appearance. Treatment involves surgi-

cal exploration, which is both diagnostic and therapeutic, with adjunctive antibiotic treatment until resolution. This case is interesting given the slow progression and equivocal imaging and laboratory findings, highlighting the importance of maintaining a high index of suspicion for the disease. Providers should familiarize themselves with signs and symptoms of NF, including indications for further workup in cases of apparent cellulitis that do not resolve as expected.

3.6. CS 06: Successful Management of Pregnancy Complicated by Bilateral Cystadenomas through Surgical Intervention: A Case Report

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Background: Cystadenomas, benign ovarian neoplasms characterized by mucinous or serous fluid-filled cysts, pose unique management challenges during pregnancy. Their potential to cause significant morbidity due to size, location, and the risk of malignant transformation necessitates a nuanced approach, especially when balancing maternal and fetal health concerns. This case report elucidates the complexities and successful surgical management of bilateral cystadenomas in a pregnant patient, underscoring the criticality of multidisciplinary care.

Methods: A 25-year-old female at 9 weeks of gestation presented with symptoms of lower abdominal pain, hyperemesis, fatigue, and insomnia. An MRI revealed multilocular bilateral ovarian cysts measuring 16 cm and 20 cm, consistent with cystadenomas. A comprehensive multidisciplinary evaluation involving obstetrics, gynecology, oncology, and surgical teams was conducted to assess the potential risks and devise a management plan.

Results: Considering the significant size of the cystadenomas and the associated symptoms, a decision was made to proceed with a laparotomy at 15 weeks of gestation. The surgery aimed to alleviate the patient's discomfort and mitigate any potential risks to the fetus. The procedure was executed without complications, and the patient was discharged on the fifth day post-operation, with ongoing pregnancy confirmed to be safe.

Conclusion: The intricate management of cystadenomas during pregnancy demands a balanced and individualized approach, emphasizing the importance of multidisciplinary collaboration. In this case, surgical intervention via laparotomy provided a safe and effective resolution for both the mother and fetus, highlighting the viability of surgical options in managing significant ovarian cystadenomas during pregnancy. This case contributes valuable insights into the surgical strategies that can be employed to address complex gynecological conditions in pregnant patients, advocating for early intervention and careful monitoring to ensure optimal outcomes for both mother and child.

3.7. CS 07: Multiple Gastrointestinal Stromal Tumors in a 73-Year-Old Woman: An Uncommon Presentation of a Rare Neoplasm

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Gastrointestinal stromal tumors (GISTs) are rare neoplasms of the gastrointestinal tract. They usually present as a solitary lesion with a significant potential for malignant trans-

formation. This case report details the clinical presentation, diagnostic features, and therapeutic management of a 73-year-old female diagnosed with multiple GISTs and an adrenal nodule. Notably, the presence of multiple GISTs is rare, in all already rare neoplasm. The instances where there have been multiple GISTs are often associated with specific syndromes such as familial GIST syndrome, neurofibromatosis type 1, and the Carney triad. However, this patient's presentation was unusual due to the absence of these syndromes, highlighting the rarity of her condition. The diagnosis was established based on CT, revealing two GIST tumors alongside an adrenal nodule. Despite the potential for aggressive behavior, the tumors were classified as low risk based on their small size and low mitotic index. Genetic testing was prioritized to assess for mutations in the CKIT and PDGFRA genes, which are known to influence responsiveness to imatinib, a tyrosine kinase inhibitor. The therapeutic strategy for this patient included a 3-year course of imatinib, complemented by genomic testing to monitor the efficacy and guide potential adjustments in treatment. Additionally, regular monitoring of iron and vitamin B12 levels was recommended due to the known risk of deficiencies in GIST patients on long-term imatinib therapy. This case underscores the importance of comprehensive diagnostic evaluation and personalized treatment plans in managing GISTs, particularly when presenting in a rare multiplicity. It also highlights the crucial role of genetic testing in identifying mutations that may guide the use of targeted therapies, offering insights into the genetic landscape and treatment responsiveness of multiple GISTs.

3.8. CS 08: Urogenital Vascular Anomaly: A Cadaveric Case Study

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Introduction: The kidneys are bilateral filtration organs that are perfused by the right and left renal artery. The presence of multiple renal arteries are anatomical variations, with varying health implications and outcomes. The current literature varies in classification methods, with no classification method fully encompassing all variations. This makes it necessary to utilize multiple classification systems. This report utilizes the Cases et al. classification system to classify the renal artery variations. To classify the testicular artery variations, the Machnicki and Notkovitch classifications were used.

Materials and Methods: A cadaveric dissection at Noorda College of Osteopathic Medicine presented with abnormal urogenital vascular findings. A standard ruler was used to measure the length and width of each artery in situ. All measurements were recorded in centimeters by three observers. A camera was utilized to document findings. The renal artery data was classified by the Cases system, which separates renal arteries by origin and insertion points, and the number of renal accessory arteries (RAA) present. The testicular artery data was classified by the Machnicki and Notkovitch systems which identifies variations based on origination and course.

Results: Upon dissection, a 67-year-old male donor presented with five renal arteries, and two testicular artery variations. There was one RAA on the right and two RAAs on the left. The right and left renal arteries originated from the abdominal aorta at the L2 level and inserted at the renal hilum. The right RAA originated 3 cm superior to the abdominal aortic bifurcation

and inserted at the inferior pole of the right kidney. One of the left RAAs originated from the left renal artery and inserted into the superior pole of the left kidney and the other left RAA originated at the abdominal aorta, 4.4 cm superior to the abdominal aortic bifurcation and inserted into the inferior pole of the left kidney. An early bifurcation of the renal arteries was noted bilaterally. The right testicular artery originated from the right renal artery and bifurcated 3.8 cm below the origin. The left testicular artery originated from the aorta, above the renal vein.

Conclusion: Multiple urogenital vascular anomalies were discovered in a cadaver during a dissection lab at Noorda College of Osteopathic Medicine. According to the Cases system, the right kidney had a Type D, Pattern II classification. The left kidney had a Type B and D, Pattern III classification. According to the Machnicki classification system, the right testicular artery is Type B. According to the Notkovitch classification system, the left testicular artery is Type II.

4. Clinical and Translational Research

4.1. CTR 01: Neurocognitive Considerations and Impacts in Chronic Migraines

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Migraine, characterized by moderate-to-severe headache, may arise from neurological, psychological, orthopedic, metabolic, or endocrine origins. Pain associated with migraine, while commonly cited as the primary patient concern, only represents a small portion of short- and long-term effects caused by the condition. Many presenting cases include neuromuscular dysfunction, increased neuronal firing, inflammation, and cortical spreading depression. These effects can induce multiple symptoms such as pain, aura, brain fog, confusion, hangover, multiple hypersensitivities, and decreased memory capacity. These effects and symptoms can lead to neurocognitive and neuropsychological deficiencies in many patients. This study aims to investigate the relationship between migraines and neurocognitive function. Neurocognitive skills were elevated across migraine patients utilizing Creyos for data collection and analysis. Preliminary data (n=173) gathered neuropsychiatric results individually via computer program across 12 neurocognitive metrics. Migraine patients were compared to standardized results for significant variations in cognitive performance. Additionally, following treatments including chiropractic manipulations, diet modifications, posture aids, medications, and injections, several neurocognitive performance areas improved. Preliminary descriptive statistical findings indicate multiple areas with below-average performance among migraine patients. Of the 12 Neurocognitive metrics, 5 demonstrated that migraine patients scored significantly lower than average (p-value/FDR: 6.42E-13/7.71E-12; .00035/7.92E-4; 2.98E-6/8.95E-6; 2.02E-8/8.06E-8; .0004/7.92E-4) with only 2 metrics showing higher than average performance (p-value/FPR: 2.31E-10/1.38E-9; .0061;.01), and 5 within normal limits. Significant below-average metrics include episodic and verbal short-term memory, visuospatial processing and rotation, and reasoning and inhibition. Initial findings indicate neurocognitive performance increases as treatment reduces migraine frequency and regresses with migraine relapse. These findings indicate a significant negative relationship between migraines and neurocognitive performance. As migraine frequency increases, neurocognitive performance decreases. Additionally, as migraine treatments reduce frequency, neurocognitive performance improves. With more data, our goal is to add a new and innovative treatment option for patients who suffer from migraines. Additionally, understanding specific neurocognitive domains affected by migraines may guide more precise treatment methods. With these treatment methods reducing the frequency of migraines, our data suggests that these patients will have increased neurocognitive skills and a decrease in negative symptoms associated with focus, organization, memory, and other neuropsychological functions.

4.2. CTR 02: Significant Relief of Osteoarthritic Knee Pain After Ultrasound-Guided Peripheral Nerve Stimulation (PNS) for 60 Days: A Retrospective Case Series Study

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1. Noorda College of Osteopathic Medicine

Purpose: It is challenging to treat patients with chronic osteoarthritic (OA) knee pain who failed conservative treatments and are not good candidates for total knee replacement. Ultrasound-guided peripheral nerve stimulation (PNS) has become an emerging treatment option. Currently there is no published study to validate the methods and evaluate the efficacy of PNS for the treatment of OA knee pain.

Methods: We evaluated thirty-eight cases of osteoarthritic knee pain which had undergone ultrasound-guided PNS dual-channel implantation with different combination of nerves. The stimulated nerve selections varied depending on the patient's pain location. The PNS systems were removed after 60 days stimulation treatment. The primary outcome measured was the percentage pain relief reported by patients at 2 weeks and 2 months after implantation.

Results: The average age of patients in this study was 75 years old at the time of stimulation procedure (75 +/- 13 years). The body mass index (BMI) ranged from 23.1 to 54.7, and averaged BMI was 36.3. Kellgren-Lawrence (KL) grading scale was used to assess the severity of knee osteoarthritis. The average KL score was 3.3 (moderate to severe). Saphenous nerve and Femoral nerve were selected for anterior and medial knee pain, and Sciatic nerve (Tibial and Common Fibular nerve) was selected for posterior and lateral knee pain. Among the 38 patients, total 31 patients were selected for Saphenous and Femoral nerves treatments, 4 patients were selected for Saphenous and Sciatic nerves treatments, and 3 patients were selected for Sciatic and Tibial nerves treatments. The nerve stimulation leads were implemented under ultrasound guidance. No neurovascular complications were reported during and after procedures. All patients had responded to the 60 days PNS treatment. On average, patient-reported improvement of pain level was 70% at two weeks follow-up and 83% at two months follow-up. Pain relief sustained after removal of PNS systems. Cumulative 4 months and 6 months postoperative pain reduction reports are still pending.

Conclusion: All patients with osteoarthritic knee pain in this study had responded of pain relief after 60-day PNS treatment. The dual channel system allows various combination of nerves stimulation based on the patient's pain location. Most of the patients had significant pain relief at two weeks and two months follow-up. Ultrasound-guided PNS system is a promising treatment option for patients who failed conservative treatments but not good candidates for total knee replacement.

Significance: This study is the first retrospective review of patient outcomes after 60 days of PNS treatment for severe osteoarthritic knee pain. Ultrasound-guided PNS system provides an innovative treatment option with promising pain reduction for chronic knee osteoarthritis.

4.3. CTR 03: Different Clinical Phenotypes of COVID-19? Lessons Learned and Outcomes Achieved from a Post-COVID-19 Outpatient Program

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1. Rocky Mountain University of Health Professions

Background: Up to 60% of individuals infected with COVID-19 may develop Post-COVID-19 conditions (PCC) or Long COVID. Clinically, it presents with fatigue, headache, brain fog, musculoskeletal pain, shortness of breath, and chest pain. The multi-system clinical presentation of PCC can be explained by the pathophysiological changes to the respiratory, autonomic, and cardiovascular systems. Symptom clustering or phenotyping may assist healthcare providers in addressing the multi-modal needs of this population.

Methods: Case studies to demonstrate the role of a physical therapist in the management of three distinct clinical presentations of PCC that were seen in an outpatient physical therapy clinic. Three patients with primary complaints related to the cardiopulmonary, neurological, and musculoskeletal system highlight the need for a thorough physical therapy examination and ideally, collaboration with the patient's primary care provider to provide an optimal rehabilitation program. Possible mechanisms responsible for the symptoms of PCC are clinically correlated and discussed. The case application of evidence-based PCC assessment and treatment strategies including the use of specific outcome measures, targeted exercise programs, breathwork, client education and lifestyle modification, fatigue management, and activity pacing are addressed.

Case 1: Respiratory muscle weakness, dyspnea, and fatigue are common in mild presentations of COVID without evidence of obvious lung pathology. Dysfunctional breathing may be the main reason for these symptoms even when exercise capacity is normal. Patients improve rapidly when breathing is addressed.

Case 2: Dysautonomic presentations require thorough examination and differential diagnosis in PCC. Symptom-titrated autonomic rehabilitation, education and fatigue management can result in functional improvements in patients with dysautonomia.

Case 3. Musculoskeletal implications following PCC can result from chronic inflammation and decreased muscle protein synthesis. Nociceptive, neuropathic, and nociplastic pain presentations require appropriate identification and treatment classifications.

4.4. CTR 04: The Immunomodulating Effects of Delta-9 Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in Human Neuronal SH-SY5Y Cells in vitro

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Introduction: Recreational and medicinal use of the cannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are based on their activity as analgesics, anti-inflammatory agents, antipsychotic and anxiolytic agents. THC and CBD lipophilicity and their neurological

actions makes them candidates as new medicinal approaches. Accumulating evidence suggests that the non-intoxicating cannabinoid compound cannabidiol (CBD) may be a promising new agent in the treatment of psychotic and anxiety disorders. However, the neurobiological substrates underlying the potential therapeutic effects of CBD are still unclear. The purpose of the current study was to 1) test varying doses of CBD and THC in a dose dependent manner, and 2) test the immunomodulating effects of varying doses of CBD and THC in the context of neuronal inflammation.

Methods: The cytotoxic effects of CBD (2, 5, 15, 25, 50, 100 μg/mL) and THC (5, 10, 15, 50, 100, 200 μg/mL) were tested by measuring lactose dehydrogenase (LDH). Human neuronal SH-SY5Y cells were pre-treated with saline, vehicle, or media supplemented with CBD or THC for 2 and 6 hours. The results were read via spectrophotometry.

Results: THC, not CBD, decreased cytotoxicity in human neuronal SH-SY5Y cells compared to control. CBD increased cytotoxicity in a dose-dependent manner; doses 50 and 100 μg/mL significantly increased cytotoxicity compared to control. Whereas THC decreased cytotoxicity in dose-dependent manner; doses 100 and 200 μg/mL significantly decreased cytotoxicity compared to control.

Conclusions: Previous findings in the laboratory indicated that CBD at large doses (> 25 μg/mL) significantly decreased cytotoxicity and inflammation in RAW264.7 macrophage cells. The current study demonstrates that at increased doses of CBD and THC, cytotoxic effects may vary between tissues from the peripheral and central nervous system. The increasing use of "ultrapotent" cannabis that contains deleterious effects of THC in large concentration may be mitigated by CBD; however, the positive effects on cognitive function could be considered through the aspect of cerebrovascular structure and BBB integrity. In addition, delivery of these cannabinoids in the brain following different routes of administration (subcutaneous, oral, and pulmonary) may contribute to differences in cytotoxicity at large doses. Future studies should investigate the mechanism of action from "ultrapotent" THC and CBD as it relates to sex, age, and tissue specific immunomodulation.

5. Education

5.1. ED 01: Discrepancies in the Representation of the Anterior Talofibular Ligament of the Ankle in Educational Resources

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The anterior talofibular ligament (ATFL) of the ankle stands as a frequent casualty in lower extremity injuries, serving alongside the lateral collateral ligament (LCL) complex to provide critical stability against inversion and plantarflexion in both athletes and non-athletes. Despite its critical role in ankle stability, the accurate representation of its true anatomical features in educational resources remains inconsistent. This study attempts to describe the most commonly found characteristics of the ATFL particularly in the number of fibers described in publications and shown via depictions or photographs as educational content. A meta-analysis of relevant literature regarding ATFL fascicles was conducted in September 2023, and it was found that up to 3112 articles mentioned anterior talofibular ligament fascicles. Notably, a significant subset of these publications (n=20) specifically highlighted the number of fascicles observed in cadaveric models, with a consistent finding of two fascicles, designated as superior and inferior. Finally, an assessment of 20 anatomical textbooks used in academia underscored a stark disparity in the consistency of accurate depiction of the ATFL and its fascicles. It was found that only 5 out of 20 textbooks depicted the bi-fascicular nature of the ATFL. In conclusion, the ATFL is an important structure that is crucial to the stability and correct functioning of the LCL complex and the ankle. Published research done on cadaveric models consistently demonstrate the presence of two distinct fascicles. However, the discrepancy between research findings and educational materials underscores the necessity for enhanced accuracy in depicting this crucial anatomical feature, ensuring optimal comprehension, and facilitating learning among practicing healthcare providers and students alike.

6. Public Health

6.1. PH 01: LSTM-based Recurrent Neural Network Predicts Influenza-like-illness in Variable Climate Zones

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Purpose: Influenza virus is responsible for a recurrent, yearly epidemic in most temperate regions of the world. For the 2021-2022 season the CDC reports 5000 deaths and 100,000 hospitalizations, a significant number despite the confounding presence of SARS-CoV-2. The mechanisms behind seasonal variance in flu burden are not well understood. Based on a previously validated model, this study seeks to expand understanding of the impact of variable climate regions on seasonal flu trends. To that end, three climate regions have been selected. Each region represents a different ecological region and provides different weather patterns showing how the climate variables impact flu transmission in different regions.

Methods: An LSTM-Based recurrent neural network was used to predict influenza-like-illness trends for three separate locations: Hawaii, Vermont, and Nevada . Flu data were gathered from the CDC as weekly influenza-like-illness (ILI) percents. Weather data were collected from Visual Crossing and included temperature, UV index, solar radiation, precipitation, and humidity. These weather data sets were chosen based on previous work results and a literature search. Data were prepared and the model trained as described previously.

Results: All three regions showed strong seasonality of flu trends with Hawaii having the largest absolute ILI values. Temperature showed a moderate negative correlation with ILI in all three regions (Vermont = −54, Nevada = −0.56, Hawaii = −0.44). Humidity was moderately correlated in Nevada (0.47) and weakly correlated with ILI in Hawaii (0.22). Vermont ILI did not correlate with humidity. Precipitation and wind speed were weakly correlated in all three regions. Solar radiation and UV index showed moderate correlation in Vermont (-0.33, −0.36) and Nevada (-0.5263, −0.55) however only weak correlation in Hawaii (-0.15, −0.18). When trained on the complete data set model performance at +1 week was comparable to the previously validated model.

Conclusions: Preliminary results indicate that temperature is a moderate predictor of ILI rates. Additionally, humidity, solar radiation, and UV index present promising prediction variables. Initial modeling attempts revealed acceptable performance in all regions. While seasonality appeared similar in each region, differences in correlation with weather variables may reveal variability in the driving forces behind ILI rates.

6.2. PH 02: DO (Under)Representation in Guideline Development

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Research and scholarship are core drivers of medicine in the modern era. Evidence-based practice continues to replace expert opinion and long held practice beliefs. Involvement in the development and writing of these guidelines is critical for DOs to maintain a seat at the academic table. According to the American Osteopathic Association (AOA), 11% of practicing physicians in the US are DOs. This number is growing – nearly 25% of current medical students attend an osteopathic medical school. Without involvement in guideline development, DOs risk giving up control of their own practice of medicine. To assess the relative contribution of DOs to the body of literature guiding practice, author information was extracted from all US-based guidelines published in the year 2023 and listed in the ECRI Guidelines Trust database. This database was chosen based on its rigorous inclusion criteria and comprehensive nature. Authors were counted and categorized into one of three groups based on terminal degree: MD holders, DO holders, and Other-degree holders. Two-hundred and fifteen guidelines were published by US organizations in 2023, with 184 reporting author information. A total of 2883 authors were counted. Of that total, 2186 held an MD (75.8%), 41 held a DO (1.4%), and 548 held another terminal degree (19.0%). Approximately 3.8% of counted authors did not have an identifiable degree. Based on these results, we conclude that DOs are significantly underrepresented in the development of guidelines.