TODD Symposium 2024 Poster Abstracts

Poster Number: 1

Presenting Author: DeWeese CE

Additional Authors: Chaudhary CL, Tidgewell KJ

Chronic pain is a debilitating condition that affects one in every four adults in the United States. This pain can last years or decades for many patients with limited relief. Current therapies are efficacious in treating acute pain, but there are significant adverse effects associated with long-term use. There is a critical need for the discovery and development of a safe and effective chronic pain treatment. This research will use a cyanobacterial-derived molecular scaffold, veraguamide, as the basis for drug design and development. This molecule binds selectively to the sigma-2 receptor, which plays a role in neuronal cell calcium homeostasis. This is a mechanism that can be investigated to discover novel ways to treat chronic neuropathic pain. The Stored-Operated Calcium Entry (SOCE) pathway is a known regulator of cellular calcium homeostasis and when this is inhibited, it causes antinociceptive effects. Inhibiting calcium influx via the SOCE pathway with the natural product-derived analogs will investigate the relationship between sigma-2 receptor ligands and the SOCE pathway. About 5-10 designed veraguamide analogs are being synthesized using a convergent synthesis method. They will be tested for receptor selectivity and assessed for efficacy using a calcium-based fluorescent imaging assay. Future work will include the use of chemical biology tools to probe the sigma-2 receptor as a target for the treatment of neuropathic pain.

Poster Number: 2

Presenting Author: Munugoda Hewage UT

Proteome-wide profiling with bioconjugation reagents has enabled the characterization of cysteine reactivity and ligandability. Yet, expanding cysteine bioconjugation via arylation in endogenous sites to engage difficult-to-target proteins (IDPs, glycan binding proteins, PPIs, etc.) with existing electrophiles is relatively unexplored. Here, we report Au(III)-monophosphine arylation reagents as new Lewis acid chemotypes to expand the depth of ligandable cysteines in human proteins. Priming of Au(III) electrophilicity for site-selective cysteine arylation was achieved through a targeted SAR by optimizing cyclometallation and monodentate phosphines towards fast and site-specific covalent modification of unprotected peptides and proteins under physiological conditions. We used a prioritized Au(III) reagent with desirable kinetic and arylation characteristics to probe ligandable cysteines in human breast epithelial cells. Using an unbiased chemical proteomics approach, galectin 1 (GAL1), an endogenous glycan-binding protein with important roles in physiological and pathophysiology was identified. These findings support the broad potential of covalent metal-mediated arylation chemistry for targeting functional but elusive cysteines in the human proteome.

Poster Number: 3

Presenting Author: Wei H

Purpose: In this study, we aimed to characterize the PK/PD relationship of CocH5-Fc(M6) and its efficacy in inhibiting cocaine interoceptive effects.

Methods: To determine its PK/PD relationship, plots of the obtained cocaine/BE/EME AUCs vs the measured CocH5-Fc(M6) concentration were fitted to Emax model with a baseline effect parameter in GraphPad Prism to calibrate the enzyme effects. The Emax model is based on the equation: $E=E0 \pm EMAX*C/(EC50+C)$. Additionally, long-term effects of CocH5-Fc(M6) on the discriminative stimulus effects of cocaine were determined by injecting different doses of CocH5-Fc(M6) (0.3, 1.0, or 3.0 mg/kg; IV) into three groups of trained rats (n = 6–7/group).

Results: A PK/PD relationship was established, which indicates that CocH5-Fc(M6) at a plasma concentration of ~0.15 mg/L was able to decrease the 5 mg/kg cocaine's presence by 50% in blood; when the enzyme concentration was 0.5 mg/L or higher, cocaine was rapidly eliminated with a negligible area under curve (AUC) compared to the baseline cocaine AUC (when C = 0). The cocaine discrimination experiment showed that the duration of CocH5-Fc(M6) in blocking cocaine discrimination was dependent on cocaine dose and CocH5-Fc(M6) plasma concentration.

Conclusion: CocH5-Fc(M6) represents the currently most efficient long-acting cocaine hydrolase with both the highest catalytic activity against the naturally occurring (-)-cocaine and the longest elimination half-life ($t1/2 = 229 \pm 5$ h) in rats.

Poster Number: 4

Presenting Author: Bulgart HR

Additional Authors: Lopez Perez MA, Miller O, Giarrano GN, Wold LE, Weisleder N

Alzheimer's Disease (AD) is a neurodegenerative disease that is associated with amyloid beta (Aβ) that has been shown to localize with the plasma membrane marked by decreased membrane integrity and elevated intracellular calcium. Previously, we have shown a membrane repair defect in A and AD CSF-treated neurons and in APP/PS1 mice. Here, we aimed to assess if recombinant human MG53 (rhMG53) could therapeutically enhance membrane repair. Treated cells/tissue were challenged with an established laser damage assay where a two-photon laser is used to damage the cell membrane in the presence of FM4-64 dye. We treated primary neurons and neuroblastomas with A and CSF in conjunction with 1M rhMG53 and observed a significant increase in membrane repair capacity with rhMG53 treated cells. Additionally, we treated ex vivo 6-month APP/PS1 live brain sections with 1M recombinant human MG53 and observed a significant increase in membrane repair capacity. Lastly, we tested the effect of rhMG53 on decreasing neurotoxicity, and observed a decrease in cell death, intracellular calcium, and oxidative stress in A and rhMG53 treated cells compared to A alone. These results illustrate the therapeutic potential of rhMG53 to enhance membrane repair and reduce neurotoxicity in vitro, and future studies will investigate the therapeutic potential of rhMG53 in APP/PS1 mice.

Poster Number: 5

Presenting Author: Al-Hamaly MA

Additional Authors: Chernyavskaya Y, Haney MG, Jolly JT, Bruntz RC, Blackburn JS

Relapsed T-ALL patients have a poor prognosis, with 5-year survival rate of 10%. Relapse is due to chemotherapy's inability to target Leukemia Stem Cells (LSCs) that have the ability to re-populate the cancer. Therapies that target specific vulnerabilities of LSC holds promise in complete disease eradication. However, in T-ALL there is no specific surface markers for LSCs, complicating the process of drug discovery and target identification. We capitalize on a transgenic zebrafish model of T-ALL with a high frequency of LSCs to screen >770 FDA-approved drugs in vivo, using >2,500 syngeneic CG1 zebrafish. Secondary screening studies confirmed Amiloride as the top hit drug, that is an inhibitor of the Sodium Hydrogen Exachanger-1 (NHE1). Notably, NHE1 has not been previously linked to self-renewal in hematologic malignancies, presenting a novel therapeutic strategy to inhibit LSCs. Knockdown (KD) of NHE1 using shRNA resulted in more than 70% decrease in in vitro self-renewal, a decrease in expression of stemness genes and G1 cell cycle arrest. Bulk mRNA sequencing revealed a down-regulation in the Myc Target genes, KRAS, Hedgehog and Notch signaling in KD cells, all critical pathways in leukemia self-renewal. Inhibiting the NHE1 reduced mitochondrial energy metabolism, decreased mitochondrial mass, and decreased mitochondrial membrane potential. Our data indicate that the NHE1 is a critical target of self-renewal in T-ALL, through regulating the cellular energy homeostasis.

Poster Number: 6

Presenting Author: Mofidi Tabatabaei S

Additional Authors: Tidgewell KJ

Our lab focuses on the Nervous System (NS) activity of the natural products from marine cyanobacteria. Fifty different cyanobacterial collections were extracted by a mixture of methanol and dichloromethane, fractionated into nine fractions based on polarity, and examined by an in vitro radioligand binding assay against a panel of NS targets. 32% of the fractions showed binding affinity to the sigma receptors, and 126 fractions selectively bound to the sigma-2 receptor, which has been recently shown to have a role in neuropathic pain and substance use disorders. We used molecular networking to prioritize our fractions by dereplication of the known compounds based on a library search of MS/MS in GNPS. DUQ0048 was the first collection we studied since it was one of our bigger collections so we will have more of the extract to purify the ligands for further testing. The fractions F2, F3, and F4 of DUQ0048 exhibited selective binding affinity to the sigma-2 receptor, exceeding 50% at a concentration of 10 μM. The chemical composition of this cyanobacteria was investigated in detail using molecular networking. The results led to the identification of ten compounds. We have isolated one of these compounds from the extract and confirmed its 1H-NMR of the structure as well as its MS to prove the presence of this compound in this extract. We will use molecular networking to analyze the other selective sigma2 fractions and prioritize them based on the type of compounds present in them.

Poster Number: 7

Presenting Author: Saryazdi S

Additional Authors: Martin AK, Saghaeiannejad-Esfahani H, Van Lanen SG

In a tryptophan-deficient environments, pathogenic microorganisms like bacteria can produce tryptophan biosynthetically, allowing them to evade the host immune response and promote survival. A key enzyme in this process is anthranilate phosphoribosyltransferase (AnPRT), a homodimeric Mg-dependent enzyme that catalyzes the second step in tryptophan biosynthesis and has been identified as potential target for antibacterial drug development. The enzyme catalyzes a nucleophilic substitution reaction, where it replaces inorganic pyrophosphate from the anomeric carbon of 5-phospho-D-ribose 1-diphosphate (PRPP) with anthranilate, forming N-(5-phosphoribosyl)-anthranilate (PRA). It is important to highlight that PRA, is relatively unstable, which complicates the use of existing biochemical assays. In this study, we expressed and purified the wild-type AnPRT enzyme from the pathogenic bacteria Serratia Marcescens (Sm-AnRPT) and Mycobacterium tuberculosis (Mtb-AnRPT). Instead of anthranilate, we used salicylic acid as a substrate to produce a stable N-(5-phosphoribosyl)- salicylate (PRS) product when reacted with PRPP. We determined the kinetic parameters for Sm-AnPRT and Mtb-AnPRT using a real-time absorbance-based assay in the presence of salicylic acid. Additionally, we developed an HPLC-based assay to evaluate the potential inhibitory effect of the previously reported Mtb-AnPRT inhibitor, ACS172 (anthranilate-like moiety), on Sm-AnPRT and calculated the IC50 and Ki values.

Poster Number: 8

Presenting Author: Adkins JF

Additional Authors: Tidgewell KJ

Store-operated calcium entry (SOCE) is a cellular mechanism critical to maintaining calcium homeostasis within the cell. Alteration of SOCE is thought to be a therapeutic target for several different disease states including pain and neurodegenerative disorders. There is interest in discovering molecules with the ability to modulate SOCE activity to better understand the system and develop new therapeutics. We are investigating marine cyanobacterial natural products for SOCE activity. An in vitro assay using HEK-293 cells is being developed to screen molecules for SOCE activity and understand their impact on SOCE modulation by visualizing calcium levels in response to cell treatment with the compounds. Results and findings to date will be presented, with a focus on SOCE assay development.

Poster Number: 9

Presenting Author: Woloshin EJ

Additional Authors: Chaudhary CL, Luo D, Prisinzano, TE

Extracts of the opium poppy, Papaver somniferum, have long been utilized to manage pain via interactions with μ-opioid receptors (μOR). μOR agonists like morphine (1) and fentanyl (2) comprise the most widely prescribed class of analgesics and are also the most widely abused. The age-adjusted death rate from synthetic opioids increased by 1,040% between 2013 and 2019, primarily driven by 2 and its analogues. More recently, novel potent opioids (NPOs) structurally distinct from 2 have been identified on the illicit drug market, including etonitazene (3) and other 2-benzylbenzimidazole analogs. Nitazenes were first developed by Swiss chemists at CIBA in the late 1950's searching for new analgesics with morphine-like potency. Somewhat unexpectedly, several nitazenes were found to have antinociceptive activity exceeding that of 1, with 3 reported to be ~1000-fold more potent. Based on the high potency of 3 and the obvious structural similarity between benzimidazole and indole, bioisosteric replacement of the benzimidazole core of 3 was explored, leading to discovery of 4 (EC50 = 1.31 nM) which has potency between that of 1 (EC50 = 7.64 nM) and 2 (EC50 = 0.21 nM). This compound represents a new, distinct class of μOR ligands that will aid in understanding mechanisms underlying activation and signaling at the receptor level.

Poster Number: 10

Presenting Author: Kolpek DJ

Additional Authors: Kim J, Kalashnikova I, Park J

Spinal cord injury (SCI) causes paralysis below the injury site and allows innate immune cells to infiltrate through the damaged blood-spinal cord barrier. These cells release cytokines that promote inflammation, inhibiting spinal cord regrowth and recovery. Our previous study showed that 500 nm poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs) upregulated anti-inflammatory gene expression in circulating immune cells after SCI in mice. However, it is crucial to determine whether this altered gene expression affects functional recovery. In this study, we explored the effects of NP size on recovery through an in vivo analysis. PLGA NPs were fabricated at 100 nm and 500 nm sizes using poly(ethylene-alt-maleic anhydride) (PEMA) for negative surface charge. Contusive SCI was induced at the T-10 level via IH impactor (50 kdyne). Mice received daily intravenous injections of PBS, 50 mg/kg of 100 nm NPs, or 50 mg/kg of 500 nm NPs for seven days. Functional recovery of the hindlimb was assessed using the Basso Mouse Scale (BMS) at 1-, 3-, 7-, 14-, 28-, and 42-days post-injury (dpi). Locomotor activity was measured via AnyMaze, and gait analysis was performed at 42 dpi using Catwalk XT. Our data shows that NP size influences immune cell activation and polarization, resulting in differences in functional recovery. Future studies will investigate pro- and anti-inflammatory protein expression in the spinal cord via western blot (WB) and fluorescence-activated cell sorting (FACS).

Poster Number: 11

Presenting Author: Lee KM

Additional Authors: Venditto VJ

Cationic lipids are versatile carriers for vaccine development. Our lab has synthesized a class of triazine lipids for vaccine delivery with evidence of robust and balanced antigen-specific immunity. Encouraged by these data, we sought to examine their utility for subunit vaccine development using ovalbumin (OVA). Although cationic lipids improve immune activation, OVA encapsulation decreases to ~10% with increasing concentration of DOTAP, a commercially available cationic lipid. Conversely, increasing our lipid (TZ3) concentration up to 36 mol% results in improved encapsulation efficiency of ~77%. DOTAP formulations also result in aggregates up to 3.8 μm, while TZ3 formulations maintain diameters <400 nm when encapsulating OVA. Although differences in encapsulation and size are observed, both lipids, individually, are able to induce an antigen-specific reciprocal endpoint titer of 104 in C57BL/6 mice after a subcutaneous injection of a lipid-OVA admixture. Continued efforts to clarify the in vitro and in vivo activity of TZ3 formulations in comparison to DOTAP are underway to understand the versatility of this platform as a subunit vaccine platform.

Poster Number: 12

Presenting Author: Ogidi JO

Additional Authors: Kim J, Kolpek D, Park J

Previous research in our lab have shown that intravenously administered, drug- free poly- (lactide-co-glycolide) (PLGA) nanoparticles promote recovery after spinal cord injury (SCI) by modulating the immune response that characterizes the secondary injury phase. However, key dosing parameters, including dose of nanoparticles, duration of treatment, and therapeutic window are yet unknown. This study aims to determine the optimal dose of PLGA nanoparticles to promote functional and anatomical recovery after SCI. Female C57BL/6c mice (9 weeks old) received a moderate contusion SCI (60kdyn) at T10, and then were intravenously administered PBS (control), or PLGA nanoparticles (500nm) at 25, 50, or 75 mg/kg treatments for 7 days, starting 2 hours after injury. Functional and locomotor recovery were assessed using the Basso Mouse Scale (BMS) and open field tests at 1, 3, 7 days post-injury (dpi) and weekly thereafter, while CatWalk XT and ladder beam tests were used to evaluate gait and motor coordination at 42 dpi. qPCR analysis was also performed to qualify expression of pro and anti-inflammatory markers in the injured spinal cord after nanoparticle treatment. Results indicate that PLGA nanoparticles upregulate the expression of anti-inflammatory markers in a dose-dependent manner. However, no significant differences in functional recovery were observed between NP treatment groups. Future investigations will focus on dose-dependent nanoparticle biodistribution and immune cell infi

Poster Number: 13

Presenting Author: Trivedi R

Additional Authors: Vu L, Luo D, Denehy ED, Songrady JC, Martin J, Chaudhary CL, Woloshin E, Kornberger L, Bhuyian N, Parkin S, Jiang Q, Che T, Alilain W, Turner JR, Bardo MT, Prisinzano TE

Fentanyl, a potent opioid used for pain management and anesthesia, has significantly contributed to the rising overdose deaths in the U.S., largely due to opioid-induced respiratory depression. Naloxone, the current treatment standard, often requires multiple doses to fully reverse respiratory depression, highlighting the need for more effective opioid rescue agents. In our search for novel synthetic agents, we discovered a new chemotype, named atoxifent. This synthetic opioid, created by conformationally constraining a piperazine ring, transforms a MOR antagonist into a potent MOR agonist. Atoxifent displays in vitro agonist activity that surpasses morphine and is comparable to fentanyl. In mouse models, it produced prolonged antinociceptive effects, reversible by naltrexone, and induced tolerance and withdrawal similar to fentanyl. In rat models, atoxifent caused a total loss of locomotor activity like fentanyl but without severe respiratory depression. Brain biodistribution analysis showed rapid brain penetration (Tmax ~0.25 hours). These findings suggest that atoxifent-like molecules offer greater safety than fentanyl, with potential to reduce opioid overdose mortality. Furthermore, systematic exploration of structure-activity relationship of the N-(3-hydroxyphenyl)-3,8-diazabicyclooctane scaffold will identify the key structural features underlying atoxifent's unique pharmacological properties.

Poster Number: 14

Presenting Author: Coenen DM

Additional Authors: Whiteheart SW

Wound healing is a multistep process. Plasma has been used to accelerate wound repair, but functional understanding of how platelets affect wound healing is limited. We studied platelet cargo uptake and release in a full-thickness wound model.

Two dorsal circular excisions were made on mice defective in α-granule biogenesis, endocytosis (platelet-specific), exocytosis, and wildtypes. Wounds were measured daily to assess healing progression. Wound sites were harvested for histology on days 3 and 7. Levels of bioactive molecules were analysed from wound tissue extracts.

Mice with defective α-granule biogenesis and cargo packaging had severely impaired wound healing with distinctive morphology and histology. Mice with improper endocytic trafficking also had remarkably slower wound healing. Exocytosis-defective mice had faster healing, and histologically resembled healing and morphology of wildtypes. During wound resolution, levels of various bioactive molecules changed. Most of the ones examined decreased, except for FGF2 and MMP9, reflecting final remodelling. This was altered in α-granule biogenesis-defective mice, where FGF2 and MMP9 levels decreased as the wounds healed. The correlation between wound resolution and wound IL1β, MMP3, TIMP1, and VEGF levels was less clear or absent in these mice.

Platelets have specific roles in wound healing progression and, potentially via extravascular migration, may impact the presence of bioactive molecules in the skin microenvironment.

Poster Number: 15

Presenting Author: Keady JV

Additional Authors: Shaykin JD, Charnigo RJ, Prantzalos ER, Xia M, Denehy ED, Bumgardner VKC, Miller JB, Delcher C, Moga DC, Ortinski PI, Bardo MT, Turner JR

Opioid use disorder is characterized by compulsion to seek and obtain drug resulting in escalating drug intake, a well-established behavior in rodent self-administration models. Male and female Sprague-Dawley rats (n=72) were trained in a sucrose reinforcement task using a progressive ratio schedule, followed by i.v. fentanyl self-administration (2.5 μg/kg; FR1) across 7 daily 1hr sessions, followed by 21 daily 6hr sessions. The day after the final 6hr session, the PFC was collected for bulk RNA-sequencing. Latent growth curve modeling found sucrose breakpoints were not predictive of changes in fentanyl escalation patterns over time. Weighted gene co-expression network analysis identified a subset of genes correlated to the observed fentanyl escalation phenotypes identified via group-based trajectory modeling. Further, transcription factor analyses of this subset identified EZH2 as a potential transcriptional regulator. In 2020, FDA approved an EZH2 inhibitor, Tazverik, for patients with lymphoma; this provided an opportunity to investigate its use in a population-based sample from private (MarketScan) and public (Medicaid/Medicare) health insurance claims data. Characterization of opioid prescribing patterns in these patients (n<100) compared to alternative interventions are ongoing. While sucrose break points did not predict fentanyl escalation, bulk-sequencing identified a potential transcriptional regulator associated with phenotypic differences in fentanyl escalation.

Poster Number: 16

Presenting Author: Ackerman AM

Additional Authors: Lynn BC, Awuah SG

Lignans are small polyphenolic compounds that play an active role in plant defense against pathogens and predators. Recently, lignan machilin D was reported to be effective as an anti-tumorigenic agent in triple negative breast cancer (TNBC) tumor-bearing mice. Previous studies have relied on plant-extracted material, which limits scalability and diversification of the natural scaffold. Herein, we describe a generalizable one-pot synthesis of machilin D and its derivatives via an iron chloride-induced dimerization of isoeugenol. Employing this synthetic methodology allowed for a robust diversification campaign to access seven (7) new lignan derivatives of the machilin D family with superior anti-proliferative properties in 2D and 3D TNBC models. In addition, the starting material isoeugenol can be derived from lignin, an abundant biopolymer that is wasted byproduct of many industries. This provides a path away from petrochemical-based pharmaceuticals towards “greener” medicine. Overall, this work enables lignan natural product-based drug discovery as a platform to identify new probes to elucidate lignan targets in biology and therapeutics for aggressive cancers such as TNBC, as well as promoting sustainable pharmaceutical discoveries.

Poster Number: 17

Presenting Author: Stonestreet MC

Additional Authors: Shrestha A, Thorson J, Ponomareva L, Shaaban AK, Karim R

Mithramycin A (MTM) is a natural product produced by the bacteria Streptomyces argillaceus that targets EWS-FLI1, a dominant ETS transcription factor in Ewing Sarcoma. Despite the high potency of MTM, it has not proven successful in the clinical setting due to several limitations including low selectivity leading to toxicity, and poor pharmacokinetics. Previously, we synthesized MTM 2'-oximes leading to major improvements in selectivity and pharmacokinetic properties but decreased potency. YL-C09 was identified as one of the potent (IC50 = 21.6 nM in TC32) compounds with a 40-fold improvement in clearance as compared to MTM. We hypothesized that the incorporation of the electron-donating (hydroxyl, methyl, and methoxy) and electron-withdrawing groups (fluorine, chlorine, and bromine) in the indole ring of YL-C09 would improve the overall selectivity, pharmacokinetics, and antitumor efficacy. The synthesized MTM 2'-oximes were evaluated utilizing Ewing Sarcoma cancer cell line (TC32) to determine potency, and Prostate cancer cell line (PC3) to determine selectivity. The chlorine-containing compound displayed improved potency (IC50 = 16.7 nM in TC32), the fluorine-containing compound showed a similar potency (IC50 = 20.9 nM in TC32), and the methoxy-containing compound showed a minor decrease in potency (IC50 = 45.7 nM in TC32) compared to YL-C09. These findings indicate that the compounds containing electron-withdrawing groups (-F and -Cl) maintained or improved potency.

Poster Number: 18

Presenting Author: ROCHA IA

Additional Authors: NORRIS JK, YEARGAN M, KALBFLEISCH T, HOWE D

Equine protozoal myeloencephalitis (EPM) is a common neurologic disease of horses caused by the apicomplexan parasite Sarcocystis neurona. Diclazuril (DICZ) is a coccidiostat drug closely related to triazine herbicides. Although routinely used to treat EPM, its molecular target in the parasite is unclear. To identify the target of DICZ, a chemical mutagenesis scheme was used to generate drug-resistant S. neurona clones. Whole genome sequencing revealed a missense mutation in the gene SN3_01000220 shared by all DICZ-resistant clones which was not present in the parental and control clones. This gene encodes a major facilitator superfamily protein, proposed to be an apicomplexan monocarboxylate transporter (AMT1). To further investigate the involvement of this transporter in DICZ resistance, a CRISPR-based approach was designed to mutate and tag the AMT1 gene in Toxoplasma gondii, a closely related apicomplexan. The mutated T. gondii clones were able to grow in 5 µg/ml DICZ, 1000X the effective concentration, confirming that the missense mutation in TgAMT1 confers resistance. Immunofluorescence microscopy localized TgAMT1 to the apicoplast, an organelle that originated from a photosynthetic endosymbiont. Additionally, DICZ treatment of wild-type S. neurona yielded abnormal apicoplast morphology consistent with disruption of the organelle. Further research should focus on the biochemical characterization of the AMT1 transporter and its role in apicoplast function.

Poster Number: 19

Presenting Author: Mory JA

Additional Authors: Venditto VJ

The recent clinical success of ionizable cationic lipids has driven several advances in recent years, resulting in a significant increase in new architectures with unique physicochemical properties and in vivo transfection efficiencies. Efficient synthetic approaches to achieve compositional diversity are critical to the continued advancement of lipid nanoparticles for drug, gene, and vaccine delivery. Synthetic approaches that achieve large libraries of diverse architectures based on the same platform also generate robust structure-activity relationships for improved in vitro and in vivo properties. To address this, our lab has previously synthesized a small library of triazine-based lipids with unique physicochemical properties and successful in vitro and in vivo transfection efficiency. To build upon this success, we developed a solid-phase synthetic approach to expand this library and achieve increased compositional diversity. Using 4-nitrophenyl carbonate functionalized resin as an acid-labile platform for nucleophilic aromatic substitution with cyanuric chloride, we synthesized a previously identified lead lipid derivative in high yield and generated a characterization strategy to study the reaction kinetics to better understand the versatility of this method. After demonstrating feasibility, further diversification was performed at the third substitution to afford efficient derivatization. Modifying our triazine lipids enables a library for carrier evaluation.

Poster Number: 20

Presenting Author: Elder TR

Additional Authors: Turner JR

Nicotine Use Disorder (NUD) is a major worldwide issue that has had multiple failed treatment plans in the past. NUD causes a high rate of relapse due to the adverse effects of withdrawal phenotypes. This is due to glial cell dysfunction that causes an increase in neuroinflammatory effects within the central nervous system. Targeting glial cells directly, such as astrocytes and microglia, can dampen their activation with a proposed antagonist. A novel drug choice, Fingolimod, could be the answer to these problems. Fingolimod is sphingosine-1-phosphate functional antagonist that is currently on the market and being used for the treatment in Multiple Sclerosis. It has been shown to dampen microglia activation by antagonizing sphingosine-1-phosphate receptors located on astrocytes. This crosstalk between astrocytes and microglia leads to a long-term decrease in glutamate. Fingolimod's target of the ventral hippocampus could potentially have significant efficacy in controlling neuroinflammation.

Poster Number: 21

Presenting Author: Williams KS

Additional Authors: Porter B, Schadler A, Huang H, Alsiraj Y, Ballard HO, Severyn NT, Burgess D, Feola DJ, Bauer JA

Emerging treatments for neonates have increased within the past decade, and a continued rise in medical costs for hospitalization of this population. We seek to utilize Medicaid claims from the state of Kentucky to demonstrate prevalence of neonatal respiratory conditions and associated financial burden, especially in preterm infants. The deidentified ICD-10 codes for pulmonary data analyzed include Respiratory Distress Syndrome, RDS P22.X, Bronchopulmonary Dysplasia, BPD P28.5, and Chronic Respiratory Distress, CRD P27.X. This is inclusive of 18 million individual Medicaid claims, with 180,079 babies billed from 2017 to 2021. Total births average 40,000 annually with over 3,000 preterm. Cost of a term baby averages $8,000, with prematurity driving costs 20-fold in extreme prematurity. Overall incidence of each pulmonary diagnosis is RDS 10.5%, BPD 3.9%, and CRD 1.7%. An overlap of ICD-10 codes occurs with disease progression. Distribution across Medicaid payor regions in Kentucky occur uniformly for preterm birth and RDS. BPD has a 3-fold increase from Western to Eastern KY, and CLD is concentrated in 2 regions with Level IV NICUs. While BPD and CLD occurrence maintained over 5 years, the average cost of these disease states doubled since COVID. Findings indicate Medicaid claims as a valuable clinical research tool. Patterns of diagnosis and financial disposition offer opportunity for improved preventions. Geographical associations provide insight for risk factors in disease

Poster Number: 22

Presenting Author: Kim J

Additional Authors: Kolpek D, Park J

Secondary injury after primary traumatic spinal cord injury (SCI) is accompanied by inflammatory response, caused by the invasion and activation of immune cells. Particularly, males and females have different inflammatory profiles after SCI. In this study, we investigate the immunomodulatory effects of nanoparticles (NPs) on both sexes after SCI by using the IV-administered poly (lactic-co-glycolic acid) (PLGA)-based NPs to reprogram circulating innate immune cells and promote a more permissive environment. Initially, we fabricated 500nm PLGA-based NPs with Poly (ethyl methacrylate) as a surfactant for a negative surface charge and injected the NPs daily for 7 days via the tail vein. Spinal cords were collected 7 days post injury (DPI) and the proportion of immune cells was analyzed using flow cytometry and immunofluorescence imaging. We also confirmed the alteration of Inflammatory-related gene expression through RT-qPCR and NanoString. Functional recovery was investigated through the BMS score up to 56 dpi to confirm the long-term effect. Our data indicates that the decreased extent of the infiltrated immune cells was sex-dependent, and the change in gene expression was also sex-dependent. The BMS sub-score showed differences by sex in the PBS group, but not in the NP treatment group. These findings indicated that the effect of NP treatment is sex-dependent and NP-mediated immunomodulation has the potential to yield sex-specific therapy for inflammation-derived disorders.

Poster Number: 23

Presenting Author: Chaudhary CL

Additional Authors: Hough A, Kolber B, Tidgewell K*

Cyanobacteria are a source of bioactive compounds and have been studied for over 50 years for new antibiotics, antiviral, and anticancer drugs. In our current projects, we are investigating marine cyanobacterial compounds and their synthetic analogs for Ca2+ modulation via interactions with the sigma-2 receptor. We have identified several cyanobacterial natural products including barbamide and several veraguamide which have sigma-2 receptor affinity and alter store operated calcium entry (SOCE). Recent research has shown SOCE and the sigma 2 receptor to be involved in neuropathic pain. Thus, structural modification and optimization of these cyanobacterial compounds using medicinal chemistry are ongoing to develop antinociceptive agents and to better understand the interaction of sigma 2 and SOCE.

Poster Number: 24

Presenting Author: Prantzalos ER

Additional Authors: McLauren KA, Turner JR

Nicotine dependence and schizophrenia are highly comorbid disorders, with a complex interplay of genetic, neurobiological, and behavioral factors. Some antipsychotics used to treat schizophrenia impact smoking habits and cessation ability. Repurposing existing antipsychotics may reduce clinical barriers by addressing both disorders simultaneously. This study investigates the repurposing of the antipsychotic aripiprazole (ARIP) for smoking cessation. We tested concurrent use of ARIP during nicotine exposure or 24-hour withdrawal (24hWD) in adult male and female mice to characterize genomic expression patterns within the medial prefrontal cortex (mPFC) and to determine the efficacy of ARIP in ameliorating WD symptomology. Anxiety-like response was measured via Open Field, immediately followed by tissue harvest for qPCR. Results were analyzed independently for each sex. ARIP treatment exhibited an anxiolytic-like effect during 24hWD in both males and females. Transcriptomic analyses indicate sex-specific changes in genes related to neuregulin signaling (Nrg3, ErbB4) and glutamatergic signaling (Grin1/2A/2B) upon ARIP administration during WD. These alterations correlated with observed behavior changes, providing a molecular basis for ARIP's effects. By targeting shared genetic factors and modulating anxiety-like responses, ARIP may offer a valuable treatment option for both nicotine-dependent individuals and those with comorbid schizophrenia.

Poster Number: 25

Presenting Author: Alsum AR

Additional Authors: Turner JR

In 2020, the United States National Institute on Drug Addiction reported approximately 2.7 million US citizens over the age of 12 had an opioid use disorder (OUD) in the past 12 months. Of this, 2.7 million, 2.3 million individuals had a prescription opioid use disorder. It is imperative for researchers apply new technologies to further unravel the secrets behind OUD and its complex mechanism and bring forth novel therapies to treat patients best in an individualistic manner. In vivo single photon calcium imaging in awake freely behaving mice is an innovative tool that has proven beneficial in examining neuronal activity and behavior in various fields. This technology allows for a more comprehensive understanding of OUD thus providing leads for novel therapeutics. Using in vivo calcium imaging, we observe aberrant firing patterns present with periods of acute to chronic opioid use as well as in different stages of withdrawal.

Poster Number: 26

Presenting Author: Ibnat N

Additional Authors: Masud AA, Mory JA, Marion R, Venditto VJ

Lipid nanoparticles (LNPs) are the most prominent platform for mRNA-based vaccines. Mostly, LNPs contain four components: ionizable lipids, helper lipids, cholesterol, and PEG lipids. Among those, ionizable lipid is the most important element that encapsulates mRNA, however, only ~10% of mRNA is released and transcribed into the cytosol. To increase transfection efficiency, our group have described a library of triazine (TZ)-based cationic lipids. Using a Design of Experiments (DOE) approach, we evaluated seven triazine lipids for eGFP mRNA expression in HEK-293T cells using flow cytometry. Particularly, three triazine lipids surpassed the cell transfection frequency and eGFP protein expression compared to ALC-0315 (Pfizer) as control. We then tested the lipids to deliver firefly luciferase (Fluc) mRNA in vivo, via intramuscular injection. Live animal imaging indicated robust localized expression of Fluc in the muscle without expression in the liver as seen with ALC-0315. Next, we evaluated the potency of TZ lipids for OVA-mRNA expression and the antibody response to OVA-antigen was tested. Ongoing efforts to understand the immune response induced with TZ lipids will help clarify their utility as an mRNA-based vaccine platform.

Poster Number: 27

Presenting Author: Martin JR

Additional Authors: Shaykin JD, Denehy ED, Luo D, Alilain WJ, Turner JR, Bardo MT, & Prisinzano TE

Unlike morphine, high-potency synthetic opioids cause vocal cord closure and rigidity of the chest wall muscles, an effect known as “wooden chest syndrome”. Since this effect may not be fully reversed by pure mu opioid antagonists (MOR) such as naloxone, we are pursuing a drug discovery program in search of an improved MOR antagonist. This study assessed the ability of the novel compound YZ-166 to reverse carfentanil-induced locomotor and respiratory depression.

For locomotion and respiration, male and female Sprague-Dawley rats (n=16) were given a vehicle of 15% methanol in saline or carfentanil (15 µg/kg; s.c.) 15 minutes prior to a second injection of either vehicle, or YZ-166 (0.01-3 mg/kg; s.c.). Rats were immediately placed into a locomotor chamber for 15 minutes, followed by placement into a plethysmography chamber to record ventilatory effort for 30 minutes. After respiration was recorded, rats were immediately placed back into the locomotor chamber for an additional 15 minutes.

YZ-166 reversed the respiratory depressant effects of carfentanil ($F(8, 104) = 13.15$, $p < 0.0001$), and reversed carfentanil-induced locomotor depression ($F(8, 104) = 5.406$, $p < 0.0001$). Most notable, YZ-166 not only reversed carfentanil-induced respiratory depression, it stimulated respiration above baseline control (CI [-0.7758, -0.2332], $p < 0.0001$), suggesting “supra-antagonism”.

This study provides evidence that YZ-166 reverses opioid-induced locomotor and respiratory depression.

Poster Number: 28

Presenting Author: Karim MRU

Additional Authors: Ponomareva LV, Shrestha A, Shaaban KA, Thorson JS

Glycorandomization is a robust platform to enable differential glycosylation of a wide range of complex small molecules and natural products, including drug leads and approved drugs. This technique expands pharmacophore chemical diversity and, in many cases, improves solubility and pharmacokinetics. In the present study, we explored the capabilities of the engineered OleD Loki glycosyltransferase in the context of heterocycles and drugs/leads bearing aromatic-, primary-, secondary- and tertiary-hydroxyls. Representative newly identified heterocyclic OleD Loki substrates from this study included indoles, thiopyrimidines, thiopurines, benzoxazoles, pyridopyrimidine, and imidazopyridines. Loki regio-/stereoselectivity with corresponding substrates was determined via scaled chemoenzymatic production and structure elucidation. Importantly, this study highlights the ability to form novel S-, N-, and O-glycosides and to surprisingly glycosylate sterically constrained acceptor nucleophiles. As first step toward exploiting glycosylation as a potential prodrug strategy, the solubility and plasma stability of representative glycosides were also evaluated.

This work was supported by the COBRE for Translational Chemical Biology (CTCB, NIH P20 GM130456) and the National Center for Advancing Translational Sciences (UL1 TR001998).

Poster Number: 29

Presenting Author: Saghaeiannejad H

Additional Authors: Bailey C, McDonald H, Reagan A, Cavnar M, Kim J

Introduction:

Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive and treatment-resistant cancers. While immunotherapy has transformed the treatment landscape for many cancers, its efficacy in PDAC remains limited. A defining feature of PDAC is its dense fibrotic stroma, which is believed to drive immunosuppression and support tumor progression. This highlights the urgent need to further investigate the immunosuppressive role of the PDAC microenvironment to develop effective therapies.

Methods:

Human PDAC tissue samples were embedded for histology and digested into single cell suspensions. Cancer-associated fibroblasts (CAFs) were isolated, cultured, and their supernatants collected. CD8+ T-cells were bead-isolated from healthy donor PBMCs and exposed to CAF supernatant for 48 hours. Gamma interferon ELISA and flow cytometry were performed to assess immune markers.

Results:

T-cells co-cultured with CAF supernatants from five PDAC patient samples exhibited reduced CD69 expression and elevated PD-1 levels compared to controls (P<0.05). Gamma interferon secretion was also significantly lower (P<0.05), indicating suppressed T-cell activity.

Conclusion:

CAF-derived supernatants significantly suppress CD8+ T-cell function, limiting their immune response. These findings illustrate the potential of targeting the CAF secretome to alleviate immunosuppression and enhance therapeutic outcomes in PDAC.

Poster Number: 30

Presenting Author: Thomason GK

Additional Authors: Green KD, Czuba LC

Microbially-produced secondary bile acids serve a variety of important regulatory and metabolic roles in the gut-liver axis. However, elevated plasma concentrations of secondary bile acids are associated with the progression of non-alcoholic fatty liver disease (NAFLD). These metabolites should be restored to their primary structure upon return to the liver, though it is not entirely known which enzyme(s) provide this function at each step of the process. We hypothesized that two members of the aldo-keto reductase family, isoforms AKR1C1 and AKR1C4, metabolize 3-keto bile acids. These enzymes have high hepatic expression, are involved in de novo bile acid production, and metabolize other steroid hormones. Six bile acids with 3-keto groups were tested as potential substrates. By varying substrate concentrations, kinetic values were calculated by fitting the production of NADP+ over time to a Michaelis-Menten curve. The enzymes differed in their substrate specificity, as 1C1 catalyzed reduction of 3-ketoCDCA, 3-ketoLCA, and 3,7-diketoDCA, while 1C4 catalyzed 3-ketoCA, 3-ketoDCA, and 3,7,12-triketoCA. We next tested six common fatty acids as AKR1C inhibitors with each substrate. Interestingly, we observed isoform-specific patterns of inhibition by fatty acids. Collectively, the data indicates that AKR1C1 and 1C4 serve an important role in the reduction of secondary bile acids and that their metabolic activity may be influenced by disruptions of cellular free fatty acid levels.

Poster Number: 31

Presenting Author: Imam IA

Additional Authors: Al Adawi S, Liu X, Ellingson S, Brainson CF, Moseley HNB, Zinner R, Zhang S, Shao Q

Acquired resistance to first-line treatments in various cancers both promotes cancer recurrence as well as limits effective treatment. This is true for epidermal growth factor receptor (EGFR) mutations, for which secondary EGFR mutations are one of the principal mechanisms conferring resistance to the covalent inhibitor osimertinib. Here, we used all-atom molecular dynamics (MD) simulations to investigate the conformational variation of two reported EGFR mutants (L858R/L718Q and L858R/L792H) that resist osimertinib. The wild-type EGFR kinase domain and the L858R mutant are used as the reference. Our MD simulation results revealed that both the L718Q and L792H secondary mutations induce additional hydrogen bonds between the residues in the active pocket and the residues with the water molecules. These additional hydrogen bonds reduce the exposure area of C797, the covalent binding target of osimertinib. The additional hydrogen bonds also influence the binding affinity of the EGFR kinase domain by altering the secondary structure and flexibility of the amino acid residues in the domain. Our work highlights how the two reported mutations may alter both residue-residue and residue-solvent hydrogen bonds, affecting protein binding properties, which could be helpful for future drug discovery.

Poster Number: 32

Presenting Author: Rahman MM

Additional Authors: Svetlana Zamakhaeva, Jeffrey S. Rush, Catherine T. Chaton, Cameron W. Kenner, Yin Mon Hla, Ho-Ching Tiffany Tsui, Vladimir N Uversky, Malcolm E. Winkler, Konstantin V. Korotkov, and Natalia Korotkova

Background: Despite lacking a tertiary structure, intrinsically disordered regions (IDRs) of proteins play a range of functional roles including cell signaling and protein folding in eukaryotes. However, the functions of bacterial IDRs are poorly understood.

Aims: To understand the function of IDRs of extracytoplasmic proteins in the biology of Streptococcus pyogenes, Streptococcus pneumoniae and Streptococcus mutans.

Methods: In this study, deep learning algorithms were used to predict extracytoplasmic IDRs in the proteome of three human pathogens- Streptococcus pyogenes, Streptococcus pneumoniae and Streptococcus mutans. Proteomics analysis, immunoblotting, and biofilm assays were utilized to identify the functions of IDRs.

Results: We identify that streptococci possess a subset of proteins harboring long-extracytoplasmic IDRs enriched with serine/threonine residues that are O-glycosylated with N-acetylgalactosamine (GalNAc) by pgf operon in S. mutans, and α-glucose by GtrB-glycosyltransferase in S. pyogenes and S. pneumoniae. Peptidyl-prolyl isomerase PrsA and penicillin-binding protein Pbp1A are identified as the major glycoproteins. Furthermore, loss of IDR glycosylation in PrsA resulted in a defect in biofilm formation in S. mutans. Biochemical and functional characterization demonstrates that IDR does not affect PrsA stability and is protected with GalNAc from proteolysis by an unknown protease in S. mutans. Also, PrsA expression and the degree of glycosylation in S.

Poster Number: 33

Presenting Author: Buoncristiani MD

Additional Authors: Bryant LT, Zhu B, Pitts MG, Shedlock CJ, Ribas RA, Gentry MS, Shelman NR, Allison DB, Evers BM, Sun RC, Rellinger EJ

High-risk neuroblastomas (NBs) have poor survival despite aggressive multimodal treatment, highlighting the need to develop new therapeutics. Glycosylation is an underappreciated mediator of cancer progression. Previously we reported enrichment of core fucosylated glycans in MYCN-amplified human NBs using spatial metabolomics. 2-fluorofucose (2-FF) is an orally bioavailable small molecule inhibitor of fucosyltransferases. Herein, we hypothesized that 2-FF would impede MYCN-amplified NB tumor growth.

Kaplan-Meier analysis was performed to determine whether specific fucosyltransferase expression was associated with overall patient survival. Core fucosylated glycan abundance was measured by western blotting, ELISA, and flow cytometry using Aleuria aurantia lectin (AAL). Subcutaneous tumor formation using MYCN-amplified BE(2)-C cells was our measure of in vivo tumorigenesis. Tumor-bearing mice were randomized to receive water supplemented with 2-FF or vehicle control.

High fucosyltransferase 8 (FUT8) expression is associated with poor overall patient survival. 2-FF blocks NB core fucosylation and NB cell growth and adherence in vitro. Oral 2-FF administration blocks core fucosylation in vivo and impedes established tumor progression. Histologic analysis revealed enhanced induction of cell necrosis within treated tumor samples.

These critical findings identify fucosyltransferase blockade as a novel metabolic vulnerability that may be exploited in treatment paradigms for NBs.

Poster Number: 34

Presenting Author: Carrillo AN

Additional Authors:

Phytosterols are compounds that share the common ring structure of many steroids including boldione. Naturally occurring phytosterols in herbal medicines can be converted to steroidal compounds through a process known as biotransformation. As a result of this biotransformation, the use of medicinal plants may be of concern to athletes due to testing for these compounds which are prohibited and discouraged in sports competitions. This project hypothesized that if samples of Rhodiola were analyzed via mass spectrometry it would result in findings of boldione. Various supplements containing Rhodiola as the primary ingredient were obtained to test for the presence of boldione utilizing liquid-liquid extraction, HPLC, and mass spectrometry. One gram of sample was extracted with methanol followed by evaporation of a portion to dryness. The residue was then redissolved in sodium hydroxide and MTBE. The MTBE layer was then dried and redissolved for analysis. A control powder was fortified with known concentrations of boldione and extracted for use to determine the concentration of boldione. Analysis was carried out on a triple quadrupole mass spectrometer in conjunction with an HPLC system. Analysis of the samples resulted in detection of boldione across the Rhodiola test samples ranging from 1.5 ppb to 147 ppb and may be of concern for athletes subjected to anti-doping testing. The data concluded that the hypothesis was supported, as all samples had boldione present.

Poster Number: 35

Presenting Author: Andres MR

Additional Authors: Macheda T, Roberts KN, Lapid MG, Hash HM, Gensel JC, Bachstetter AD

Azithromycin (AZM) is widely known for its antimicrobial properties but has also demonstrated immunomodulatory effects in non-infectious conditions, including spinal cord injury. Given the urgent need for treatments targeting neuroinflammation in traumatic brain injury (TBI), especially in older adults at risk for Alzheimer's disease (AD), we aimed to repurpose AZM as a potential modulator of brain inflammation. Using a closed head injury (CHI) model, we tested AZM in the APPSAA knock-in mouse model of AD. Fourteen-month-old mice, with an equal ratio of males and females, received a 5-day AZM treatment starting 30 minutes post-injury to assess its effects on neuroinflammation and cognitive recovery. Behavioral outcomes, including learning and memory, general health, and nesting behavior, were evaluated six weeks post-injury. In addition, Meso Scale Discovery assays quantified cytokine and chemokine levels to measure inflammation in the brain at the time of sacrifice. While the results showed limited effects of AZM on neuroinflammation and cognitive behavior after mild TBI, except for improved nesting behavior in non-injured females, we are exploring longer dosing regimens of AZM further to assess its potential as a repurposed CNS-targeted immunomodulatory therapy. These ongoing studies will help determine the therapeutic window and optimize dosing to evaluate the full potential of AZM in mitigating the effects of TBI and Alzheimer's disease-related pathology.

Poster Number: 36

Presenting Author: Masud AA

Additional Authors: Fernandez EA, Huang Q, Huang H, Jimring JC, and Wang Q.

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) is a crucial enzyme in the pentose phosphate pathway (PPP), producing NADPH, essential for neutralizing oxidative stress, and pentoses, which contribute to nucleotide synthesis. G6PD deficiency, the most common human enzymopathy, affects 400 million people globally and is linked to hemolysis due to insufficient NADPH in red blood cells (RBCs). Beyond RBCs, emerging evidence suggests G6PD deficiency may impact platelet function, particularly in cardiovascular disease patients over 60. However, its role in platelet activity remains underexplored.

Aim/Objective: This study aims to elucidate the effect of G6PD deficiency on platelet function using a G6PD Mediterranean mutation (Med) conditional knock-in mouse model and G6PD knock-out (KO) mice

Methods: We treated mouse platelets with a G6PD inhibitor and evaluated Ca2+ influx. Additionally, we analyzed platelet functionality in G6PD KO mice, measuring platelet count, morphology, Ca2+ influx and clot contraction thrombin stimulation. Tail bleeding times were also assessed. Similar parameters including clot contraction and tail bleeding were evaluated in G6PD Med-II mutant mice.

Results and Discussion: G6PD inhibition reduced Ca2+ influx in a dose-depend manner while G6PD KO mice showed a reduction in Ca2+ influx, indicating impaired platelet activity. Clot contraction in KO mice was significantly decreased. Tail bleeding time was slightly prolonged in KO mice, indicating