36th Mossberg Honors Symposium - Abstracts

Keynote Presentation Abstract

Ventures into COVID-19 Research: About pigs, cats and hamsters

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The unprecedented current pandemic caused by SARS-CoV-2 has accelerated research on the epidemiology and pathogenesis of this virus. As humans do not have pre-existing immunity to SARS-CoV-2, there is an urgent need to develop therapeutic agents and vaccines to mitigate the current pandemic. The U.S. and the WHO have launched campaigns to develop and test therapeutic agents and vaccines on an unprecedented scale. To test these and other potential medical countermeasures, it is imperative to identify animal models for COVID-19 that provide measurable readouts for potential interventions. In my lecture, I will discuss some of the COVID-19 research conducted in my laboratory at Kansas State University, mainly describing preclinical animal models for COVID-19 and their use to inform public and animal health as well as determine the efficacy of therapeutic agents.

Lecture Presentation Abstracts

L01
Tethered olefin functionalization of allylic and homoallylic alcohols

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Olefin functionalization reactions are often used for the insertion of heteroatoms into organic molecules. Intramolecular olefin functionalization is a known strategy for heterocycle synthesis. However, such reactions generally require multistep syntheses of precursors and often require substrates with pre-existing C-Nuc covalent bonds. Our laboratory is deeply involved in the field of *tethered* olefin functionalization reactions. In such reactions, the nucleophilic auxiliary (the "tether") is appended to pre-existing hydroxyl groups followed by a cyclization reaction. In this talk, I focus on two such reactions: 1. Tethered *aza*-Wacker cyclizations of sulfamate esters 2. Tethered silanoxymercurations of allylic alcohols. Prior to our laboratory's work, there had been no reports of alkene functionalization reactions using *N*-functionalized sulfamate esters and silanol auxiliaries.

L02

The impact of TPGS on solid state stability in a Spray Dried Amorphous Solid Dispersion

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Oral delivery of poorly water-soluble drugs is a major challenge for the pharmaceutical industry. More than half of all newly discovered drug entities are poorly water-soluble and abandoned early on in development since they form a poorly-soluble crystal for which the rate of drug absorption is limited by dissolution or solubility. To increase apparent solubility, formulation approaches such as amorphous solid dispersions (ASD), which are composed of optimized levels of polymer, drug and sometimes surfactant, have been used. The purpose of this research was to examine the effects of TPGS on solid state crystallization of amorphous drug in an ASD. The plasticizing effects of TPGS shown through a glass transition (T_g) reduction and as assessed by modulated differential scanning calorimetry (MDSC) will be correlated with subsequent measurements of crystal growth using powder x-ray diffraction (PXRD) and MDSC, demonstrating the impact of TPGS on the solid-state stability in an ASD formulation. Using spraying drying, TPGS will be incorporated into an ASD containing a the rapidly crystallizing poorly water-soluble model drug, nifedipine. The preliminary studies will focus on the commonly used Hypromellose Acetate Succinate (HPMCAS) polymers. Shelf life stability of the formulation will be analyzed by conducting a modified solid state stability study where each composition of the ASD will be held at temperatures either above or below its midpoint T_g and either in the presence of high or low humidity in order to assess the impact of the surfactant on the long-term stability of the ASD. The impact of TPGS on the solid-state stability of the ASD was than characterized by thermal methods such as MDSC and physical analysis, PXRD, to characterize the impact that TPGS has on the T_g, and crystallization potential. Chemical degradation was also studied by HPLC to examine the influence of TPGS in the ASD. Based on preliminary results, the presence of TPGS caused reduced stability in the presence of high humidity at both temperatures above and below the T_g of the ASD. The rate of crystallization was effectively monitored by a MDSC method and confirmed by PXRD results. Though systems with a lower Tg, whether plasticized by TPGS or by water resulted in greater rates of Nifedipine crystallization. This investigation illuminated the effect of TPGS on recrystallization in the solid state when exposed to stressful conditions not uncommon during manufacturing and shipping. High humidity exacerbates the instability with TPGS when incorporated in the ASD.

L03
Intranasal insulin in Post-Operative Delirium

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Delirium is a confused mental state usually acute in nature, characterized by a fluctuating course of disorganized thinking and disorientation. Patients with delirium are difficult to care for and have and increased morbidity and mortality. Older patients (65 years and older) experience delirium more often, occurring in 29–64% of general medicine inpatients and 11–51% of surgical patients. In mice, intranasal insulin increases mitochondrial ATP production in the cerebrum, hypothalamus, and the hippocampus. Importantly, insulin functions in a significant role maintaining brain mitochondrial homeostasis. The effects of intranasal insulin and occurrence of post-operative delirium (POD) was studied in patients undergoing elective open-heart surgery requiring cardiopulmonary bypass. The incidence of POD in the intranasal insulin group and placebo group was 10% (n=2) vs 30% (n=6) respectively. There was no statistical significance between the groups due to small sample size (p=0.13).

Our intent is to administer intranasal insulin to patients exhibiting post-operative delirium in order to reverse the symptoms. We propose that subjects exposed to one or two doses of intranasal insulin postoperatively will experience more frequent resolution of postoperative delirium when compared to those treated with placebo as defined by the CAM Instrument.

L04

Co-activation of GHSR1α and DRD1 rescues hippocampal lesions in Alzheimer's disease

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Alzheimer's Disease (AD) is a chronic neurodegenerative disorder characterized by insidious onset and progressive cognitive decline. Synaptic injury in the hippocampus constitutes an early and prominent characteristic of AD brains. To date, the molecular mechanisms that underlie hippocampal synaptic injury in AD remain elusive and as a result, effective therapies targeting hippocampal synaptic deficits in AD are as of yet unavailable. Growth hormone secretagogue receptor 1α (GHSR1α) is critical for hippocampal synaptic physiology. The deregulation of GHSR is part of a key mechanism that causes hippocampal synaptic injury in AD-relevant pathological settings. GHSR1α interaction with amyloid beta (Aβ) suppresses GHSR1α activation, leading to compromised GHSR1α regulation of dopamine receptor D1 (DRD1) in the hippocampus from patients with AD. The simultaneous application of a mixture of selected GHSR1α and DRD1 agonists demonstrated a protective effect against Aβ-induced synaptic loss in primary hippocampal neuron cultures. Furthermore, the co-activation of GHSR and DRD1 by a mixture of MK0677 (a specific GHSR agonist) and SKF81297 (a specific DRD1 agonist) restored GHSR1α response to agonist induced activation and protected against Aβ-mediated deficits in hippocampal synaptic function and mouse spatial learning and memory. Collectively, our results reveal a mechanism of hippocampal vulnerability in AD, and suggest a combined activation of GHSR1α and DRD1 may be a promising approach for treating AD.

L05

Modulating Molecular Chaperones: A Potential Therapeutic Approach For X-Linked Charcot-Marie-Tooth (CMT1X) Disease

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CMT1X is an inherited peripheral neuropathy caused by mutations in the GJB-1 gene that encodes for connexin 32 (Cx32). Despite being the second most common form of CMT neuropathies, there are no pharmacologic treatments for CMT1X. We have developed "novologues" as orally bio-available novobiocin analogues that manifest neuroprotective activity by modulating the expression of heat shock protein 70 (Hsp70). The novologue, KU-596, is in clinical trials for treating a metabolic neuropathy and we examined if it may improve neuropathic symptoms in Cx32 deficient (Cx32def) mice, a model of human CMT1X. Cx32def mice develop a significant reduction in metabolic bioenergetics (mtBE), motor nerve conduction velocity (MNCV, ~45 m/sec) and compound muscle action potential (CMAP, ~ 20-25mV) compared to wild-type mice (MNCV, ~60 m/sec; CMAP, ~ 40 mV). Five months of KU-596 treatment significantly improved MNCV (~ 55-60 m/sec) and CMAP (~ 30 mV) but did not improve mtBE. To investigate whether these effects were Hsp70 dependent, Cx32def x Hsp70 knockout mice were treated with KU-596. There was no improvement in MNCV, CMAP and mtBE, therefore therapeutic efficacy of KU-596 is Hsp70 dependent. To determine if KU-596 may be effective in other models of CMT1X, we utilized T55I x Cx32def mice. T55I is a common Cx32 mutation in human CMT1X patients, which leads to accumulation of Cx32 in the endoplasmic reticulum and these mice develop similar deficits in MNCV (~45-50 m/sec), CMAP (~20 mV) and reduced mtBE. Five months of treatment with KU-596 improved MNCV (~ 60 m/sec) but did not significantly improve CMAP and mtBE. Collectively, our data suggest that modulating Hsp70 with KU-596 may be beneficial for treating CMT1X and that efficacy may not be limited by the nature of the underlying genetic mutation in the GJB-1 gene.

L06

Next Generation Approaches for the Discovery and Optimization of Monoclonal Antibodies

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Monoclonal antibodies (mAbs) are considered the most rapidly growing class of human therapeutics, being also used as diagnostic tools. Additionally, by revealing neutralizing mechanisms and immune hot spots, mAbs can be used as guides for efficient vaccine design. In this work, we propose new strategies for the discovery and optimization of monoclonal antibodies. Yellow Fever virus (YFV) is a highly lethal flavivirus, responsible for about 30,000 annual deaths. Even though an efficient vaccine is available against YFV, it presents numerous limitations, and no treatment has yet been discovered for patients that contract the disease. Therefore, monoclonal antibodies could be a promising passive immunization tool, and could also guide the development of effective non-replicating YFV vaccines. Here, we isolated B cells from YFV-immunized donors and captured natively paired heavy-light antibody gene sequences. Libraries were constructed to clone the antibody gene repertoire into yeast display vectors, and antibodies were expressed on the yeast surface in a fragment antigen binding (Fab) format. We then performed multiple rounds of fluorescence-activated cell sorting (FACS) screening to identify anti-YFV Fabs, followed by Next generation sequencing (NGS). Through bioinformatic analysis of the NGS data, a group of antibodies was selected for expression as full IgGs. These expressed antibodies were assayed for neutralization, and a subset of these antibodies displayed highly potent neutralizing activity against five YFV strains, including one extremely potent antibody with a neutralizing IC50 < 5 ng/mL.
The neonatal crystallizable fragment receptor (FcRn) is a membrane-bound fragment crystallizable (Fc) region receptor. The interaction between Fc and FcRn is correlated with recycling of IgGs in the body and consequent half-life of mAbs. There has been a growing interest in antibodies with extended half-life, especially for the treatment of diseases in which an antibody must be administered repetitively. In this project, we used the combination of site-saturation mutagenesis and DNA shuffling to generate Fc libraries that were cloned into yeast. A new strategy was developed using FACS to sort these libraries, selecting variants with increased binding to FcRn at acidic pH and releasing at neutral pH, mimicking what happens in the recycling process of IgGs. Using NGS and bioinformatics analysis, we discovered a set of new Fc sequences that will potentially generate mAbs with increased half-life.

L07

A High-Throughput Approach to Unusual Peptide Variants for Drug Development

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As alternatives to conventional small molecule-based therapeutics, peptides possess many attractive properties; being endogenous, highly selective, and relatively safe. As such, interest in peptides within the pharmaceutical industry has increased dramatically over the past two decades highlighted by the recent approval of more than twenty peptide drugs. While many peptides have already seen great success, skepticism continues to persist due to their intrinsic limitations, mainly related to their ease of proteolysis and poor membrane permeability. Replacing ordinary peptide side chains with unnatural amino acids can overcome the drawbacks posed by traditional peptides. However, limited access to unnatural amino acids continues to hamper their use in modern peptide drug discovery. By combining widely abundant boronic acids as "side chain donors" with the endogenous amino acid dehydroalanine as the "side chain acceptor", a novel method was established to incorporate a diverse pool of unnatural side chains directly into peptides. This method was merged with high-throughput technologies to access a wide array of bioactive peptide analogs for biochemical testing. Using this platform, 96 single-amino acid peptide variants were synthesized, purified, quantified, and evaluated in parallel.

Research Summary Abstracts

Medicinal Chemistry

RS01
Metabolically labelling cancer cells for immune destruction using sialic acid fluorescein isothiocyanate conjugates

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Sialic acids derived from the N-acetyl-neuraminic acid family are common termini of cell surface glycans and are involved in cell-cell signaling between the host cell and a pathogen or an immune cell. The prevalence of sialic acid terminated glycans is significantly increased on the surface of malignant cells, and many components of the biosynthetic pathway that facilitate glycan sialylation are upregulated. The aforementioned biosynthetic pathway can accommodate exogenous C-9 substituted sialic acids. As such, metabolically labelling malignant cell surfaces with exogenous sialic acids in order to modulate interactions with the immune system is an intriguing prospect. One of the desired interactions that we would like to explore is the interaction between malignant cells and natural killer (NK) cells that have been engineered to express chimeric antigen receptors (CAR NKs), which activate upon binding to motifs attached to C-9 of sialic acid. We have chosen to metabolically label target cells with C-9 modified fluorescein isothiocyanate sialic acid (FITC-SA). FITC-SA has minimal cytotoxicity and has been shown to preferentially localize in tumors in animal models.[1] We envision that malignant cells metabolically incorporating FITC-SA will be susceptible to cytolysis by anti-FITC CAR NK cells that we are developing. In this presentation, the premise of this approach and the preliminary data obtained to date will be described.

References

1. Wu, X., Tian, Y., Yu, M., Lin, B., Han, J., and Han, S. Biomater. Sci., 2014, 2, 1120

RS02
SAR of Disulfides: Photocatalytic Cyclization of Peptides

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Peptides are powerful therapeutic agents, characterized by high target specificity and strong binding affinity, yet remain largely underdeveloped due to their poor pharmacokinetic properties. Macrocyclization is an effective strategy to overcome the historic limitations of ordinary peptides as drugs. Cyclization increases the plasma stability and cell permeability of peptides, and markedly improves the affinity of the peptide for its cognate receptor by locking the peptide in its active binding conformation. While macrocyclization is a venerable strategy to develop peptides as drugs, refining the size of the macrocycle and identifying an optimal tethering group is synthetically laborious. Each macrocycle is made as the unique product of a long, tedious, and chemically inefficient solid-phase peptide synthesis (SPPS) procedure. A better approach would be to make one peptide from which any number of cyclic products can be easily accessed in a highly modular way. Our lab has developed a new paradigm for constructing macrocyclic peptides that is synthetically divergent, catalytic, and biocompatible. In our approach, a single peptide is made by conventional SPPS, incorporating two cysteine residues as latent handles for cyclization. The two cysteine residues are then converted into two dehydroalanine residues in one chemical step. Combining diboronic acids – essentially a linker transfer reagent - with a flavin photocatalyst, a wide array of new covalent tethers can be inserted into the peptide, uniting the two pendent Dha fragments. Unique to our approach, disulfide-containing peptides and proteins can also be directly parlayed into our diversification strategy, enabling late-stage editing and structure-activity relationship studies to be performed on pre-existing scaffolds, including many FDA approved biopharmaceuticals.

RS03
Functional probes for γ-secretase in Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes dementia. Pathological markers include neurofibrillary tangles containing tau protein and plaques composed of the amyloid β-peptide (Aβ). γ-Secretase is a membrane-embedded aspartyl protease complex with presenilin as the catalytic component that cleaves within the transmembrane domain (TMD) of the amyloid precursor protein (APP) to produce Aβ. The 42-residue variant (Aβ42) in particular pathologically deposits in the Alzheimer's brain. Dominant missense mutations in APP and presenilin cause early-onset familial Alzheimer's disease (FAD). Complex proteolysis of APP by γ-secretase follows either of two pathways of Aβ production: Aβ49 → Aβ46 → Aβ43 → Aβ40 → Aβ37 and Aβ48 → Aβ45 → Aβ42 → Aβ38.

Understanding how γ-secretase processing of APP is altered in FAD is essential for elucidating pathogenic mechanisms in FAD and developing effective therapeutics. To improve our understanding, we designed synthetic APP-based TMD substrates as convenient functional probes for γ-secretase. Installation of the helix-inducing residue α-aminoisobutyric acid (AIB, U) provided full-TMD helical peptide substrates (HPS) while also facilitating their synthesis and increasing the solubility of these highly hydrophobic peptides. These probes were validated through mass spectrometric (MS) analysis of proteolytic products in purified enzyme assays and were processed in a manner that reproduced physiological processing of APP in many respects. A compound HPS-7 with only two AIBs was found to be trimmed all the way to Aβ37, as seen with APP substrate itself. This functional probe and its analogues will be further used to understand the effects of γ-secretase modulators and FAD-mutant γ-secretase on the processing of APP substrate. We are also synthesizing similar AIB-containing peptide probes with repeating Phe-Ala-Phe (FAF) sequences to better understand the carboxypeptidase activity (trimming function) of γ-secretase independent of the initial endoproteolytic events. Future plans include synthesizing heavy isotope-labelled probes of HPS and FAF peptides to further simplify MS analysis.

RS04

Accelerated Peptide Diversification: Chemo-selective Addition of Amino acid Side-chains into poly-Unsaturated Peptides

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The controlled introduction of amino acid side chains into peptides relies on solid-phase peptide synthesis (SPPS), an iterative process wherein individual amino acids are coupled together in a defined order yielding a single peptide variant. Assembling combinatorial libraries of any one peptide, exchanging both the location and identity of the amino acids, is therefore a laborious prospect, which has hampered the development of peptides, particularly as drugs. The ability to install any number of unique side-chains into a single peptide with complete spatiotemporal control, bypassing repetitive SPPS, would offer a new paradigm for peptide analog synthesis. In this direction, a polyunsaturated peptide containing three dehydroalanine (Dha) residues is prepared via contemporary SPPS. Amino acid side-chains are then selectively introduced, one specific Dha at-a-time, into the peptide by combining boronic acids, i.e., side-chain donors, and a flavin photocatalyst, affording an engineered pool of tri-modified products.

RS05

Photochemical strategies for synthesis of complex bioactive molecules

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Small organic molecules possess unique features that allow them both to probe and to modulate biological systems. Both naturally evolved and synthetically created molecules suggest that the complexity of their structures is important to reliably perturb intricate functions of the biological systems. Diverse chemical origins of molecules (from biosynthetic gene clusters, or combinatorial synthesis schemes) correlate with the uniqueness of their protein-binding profiles. These two notions taken together charge chemists with creating a variety of reaction sequences that produce small molecules with complex structures, resulting in libraries of compounds that can be used to identify new molecules for the treatment of diseases.

In search of such a new molecule, our previous efforts yielded one the active molecule (xk1387) from the collection of a spiroindane series synthesized by photochemistry and assessed by cell painting experiment. Importantly, the corresponding diastereomer of xk1387 was not active in the cell painting experiment, indicating a potential specific interaction with their binding partners. Further, *in-silico* analysis of the compound using a structural protein target prediction algorithm identified β-secretase as its potential target. Indeed, xk1387 was structurally similar to lanabecestat, an oral β-secretase inhibitor under clinical development. Studies are underway to synthesize probe compound of xk1387 for the proteomic identification of their binding partners.

Further, we are working on a strategical method for the synthesis of azetidinol scaffolds by Norrish type II reaction. Azetidinols have elicited interest as attractive scaffolds for biologically active molecules. Such scaffolds can be used as a framework to develop diverse set of libraries of compounds by transition metal mediated site selective C-H functionalization reactions. Synthesized compound library with functionalized diversity can be tested for the bioactivity in cell-based assays to identify new molecules for the treatment of diseases.

Pharmaceutical Chemistry

RS06

Predictive in vitro Dissolution Modeling using Multivariate Analysis for Drug Product Development

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When developing a drug product, it is crucial to understand the correlation between parameter changes in the manufacturing process and product performance. However, developing these relationships becomes difficult as more complex, multi-step manufacturing process are utilized. The current strategy in pharmaceutical development is to run a series of Design of Experiment (DoE) studies to understand the relationship between the CMAs (critical material attributes), CPPs (critical process parameters) and product quality, while one of the product qualities could be considered as dissolution. This study is an exploratory predictive dissolution modeling was developed by multivariate analysis (partial least square regression) using the dissolution data from the current DoE studies, then to direct linkage the parameter changes in the manufacturing process (CMAs)/ CPPs) to dissolution. The multivariate analysis provides a means of correlating results to multiple variables and mathematically describing the impact of changing those variables on the results. This information was used to develop a simple model that can predict the change in each formulation's dissolution curve based on variations in the manufacturing process. With robust product and dissolution understanding/ modeling, a direct linkage between CMAs, CPPs and dissolution can be established to inform the formulation and process development. This study will further enhance predictive modeling capabilities through the use of multivariate analysis

RS07

Platform development for the enrichment of aquaporin-4 autoantibodies in patients with neuromyelitis optica

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Neuromyelitis optica (NMO) is an autoimmune disease that results in damage to the optic nerves and spinal cord, which can lead to blindness and paralysis. NMO patients' immune system develops an antibody (i.e., AQP4-IgG)-mediated immune response against aquaporin 4 (AQP4), a water channel protein expressed on astrocyte cells. Current treatments against NMO are non-specific, such as the use of general immunosuppressants (e.g., corticosteroids), the depletion of B cells (Rituximab), and the removal of inflammatory mediators such as circulating antibodies and cytokines (plasma exchange). These non-specific therapeutics can leave patients immunocompromised and at risk for opportunistic infections. In this study, we developed a method to discover novel AQP4-IgG autoantibodies. This strategy involves the use of a yeast surface display technology to generate a library of antibodies. We created a synthetic library comprised of 1% AQP4-binders and 99% non-binders, and we compared enrichment methods using whole-cell (AQP4-expressing HEK293 cells) or solubilized antigen (DDM detergent-purified AQP4). Next-generation sequencing was performed after each round of selection to monitor the enrichment or impoverishment pattern of each clone in the library. Both selection methods performed similarly, and three rounds of selection was sufficient for the enrichment of AQP4-binding clones. After three rounds of selection, the positive AQP-binders were enriched by over 2-fold, and the non-binder clones were depleted by over 10-fold. This research will help us better understand the disease and assist in the discovery of targeted therapeutics to treat patients with NMO.

RS08

Proinsulin conjugates with inhibitory receptor ligands to tolerize insulin-binding B cells

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B cells are a promising target for type 1 diabetes (T1D) immunotherapy as evidence points to their role in the loss of immune tolerance. However, clinical trials for B cell-depletion have relied on globally immunosuppressive therapies, including the anti-CD20 monoclonal antibody rituximab, that carry the risk of life-threatening infection. Therefore, our long-term goal is to evaluate molecular antigen-specific immunotherapies (ASITs) to tolerize autoreactive B cells for T1D while avoiding global immunosuppression. We aim to develop an ASIT that selectively targets and tolerizes insulin-specific B cells (IBCs) by conjugating an engineered proinsulin-variant to an immune response-inhibitory receptor ligand. This conjugate is proposed to target the IBCs via the B-cell receptor (BCR) and block B-cell activation by co-ligating the BCR with the immune inhibitory receptor CD22.

Proinsulin-variants have been engineered to reduce insulin-receptor B binding affinity, to reduce dimerization and formation of zinc-stabilized hexamers, and to incorporate sortase-mediated bioconjugation while maintaining key B cell epitopes. Each variant has been expressed in E. *coli* and preliminarily characterized. Proinsulin-multimers on bifunctional-PEG and 4-arm PEG scaffolds have been synthesized using a sequential sortase and copper-catalyzed azide-alkyne cycloaddition (CuAAC) conjugation strategy, and discrete species have been isolated by preparatory high-performance liquid chromatography. An azide-functionalized CD22 ligand (CD22L) has been synthesized by Dr. Mark Farrell's lab, and discrete 4-arm PEG-insulin/CD22L conjugates have been synthesized by CuAAC for preliminary *in vitro* assays. Following the synthesis of the proinsulin/CD22L constructs, each will be characterized and evaluated *in vitro* against IBCs isolated from 125Tg mice.

RS09
Antibody-antigen display libraries for antibody discovery

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Monoclonal antibodies are highly potent potential therapeutic agents for multiple pathogen infections like HIV, Ebola, malaria, and SARS-CoV-2. A major bottleneck of all current antibody discovery methods is that they can only screen antibody library against a limited number of antigens simultaneously. This limitation hampers the antibody screening process since the number of potential pathogen antigens and epitopes is enormous. Here, we propose to construct an ultra-high-throughput screening system to drastically increase the number of antigens we can screen against to a library scale (to at least 100). By leveraging a recently described yeast Synthetic Agglutination (SynAg) approach, we aim to achieve the screening of antibody libraries against antigen libraries (antibody-antigen library:library screening) in a single experiment.

The SynAg antibody-antigen library:library screening system was constructed based on natural yeast mating. We replaced the complementary natural yeast mating proteins with Fab region of the antibody and antigen. A Cre-Lox recombination system and a novel barcode system were incorporated to keep track of antibodies and antigens' identities in next-generation sequencing (NGS) analysis. To test the efficacy and reproducibility of the system, we prepared a small panel of known antibodies that target different pathogens and also a panel of different pathogen antigens. While most of the antibodies and small antigens showed good expression and binding, some large antigens didn't express well on the yeast surface. We are in the process of optimizing the mating condition and generating the bioinformatic algorithms to deconvolute the NGS data. In parallel, we also optimized our platform for generating the natively paired human antibody library which yielded around 90,000 unique antibody sequences from 2 million memory B cells after optimization. We anticipate the new system can drastically accelerate the antibody screening process and serve as a multifunctional toolkit for comprehensive antigen-specific immune response analysis, which will aid in the antibody discovery for human diseases that lack effective treatments.

RS10

Reconstitution of catalytic activities of two essential leishmanial cytochrome P450 enzymes involved in ergosterol biosynthesis

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CYP51 and CYP5122A1 are two cytochrome P450 (CYP) enzymes essential for the survival of *Leishmania donovani*, a major causative agent of human visceral leishmaniasis. CYP51 acts as a sterol 14α-demethylase in ergosterol biosynthesis in *Leishmania* parasites. It has been considered as an attractive target for development of new antileishmanial drugs. Studies showed that CYP5122A1 is also involved in the ergosterol biosynthesis pathway. However, its exact biological role remains unknown. To elucidate the biochemical function of this enzyme, it is important to reconstitute its activity with endogenous substrates and redox partner. In this study, recombinant *L. donovani* CYP51 and CYP5122A1 and *Trypanosoma brucei* NADPH-CYP reductase (TbCPR) were expressed in *Escherichia coli* and purified using chromatographic approaches. Purified CYP51 and CYP5122A1 exhibited characteristic UV-Vis spectrophotometric properties of CYP enzymes and were reduced by TbCPR in the presence of NADPH. Using lanosterol as the substrate, CYP51 and CYP5122A1 were reconstituted in vitro with TbCPR in the presence of NADPH. Their catalytic activities showed a pH-dependence, with a pH optimum of 6.2 – 6.6. These results indicate that TbCPR can be used as a surrogate redox partner of the two leishmanial CYP enzymes to study their functions. Experiments are ongoing to identify *L. donovani* CPR as the redox partner. Overall, this study provides an important foundation for elucidating the biochemical roles of CYP51 and CYP5122A1 in ergosterol biosynthesis in *Leishmania* and developing therapeutics targeting these essential enzymes.

Pharmacy Practice

RS11

Evaluation of procalcitonin laboratory reference range modification and antimicrobial utilization

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Procalcitonin is an established biomarker that helps distinguish bacterial infections from other disease states that present with similar features. It provides useful data that can aid in the decision surrounding continuation or cessation of antibiotics. In respiratory tract infections and in critically ill patients with sepsis, procalcitonin guidance has shown to be beneficial by reducing antibiotic length of therapy and mortality. Randomized trials have established certain thresholds that can help determine if antibiotic therapy is necessary. When procalcitonin is >0.26 ng/mL in a lower respiratory tract infection, it is likely bacterial in nature. In intensive care unit (ICU) patients with suspected sepsis, a procalcitonin >2.0 ng/mL is highly predictive of sepsis while a procalcitonin of >0.5-2.0 ng/mL indicates that sepsis is possible. In ICU patients, antibiotics can be safely discontinued when procalcitonin is <0.5 ng/mL. Levels ≤0.25 ng/mL may warrant discontinuation in community acquired pneumonia. Procalcitonin guidance helps to decrease cost associated with unnecessary antibiotic use, decrease antibiotic associated adverse effects, and to combat antimicrobial resistance.

Given the relationship between bacterial infection and procalcitonin, it is important for electronic medical records (EMRs) to display procalcitonin accurately. Our institution's EMR was found to flag any procalcitonin value >0.1 ng/mL as abnormal. This study aims to update the manner in which procalcitonin is displayed in the EMR in order to ensure that it is used in a way that is consistent with the evidence supporting procalcitonin guidance. The modification to the EMR involves removal of the inappropriate abnormal marking and the insertion of a comment that outlines the previously described procalcitonin thresholds.

This quasi-experimental chart review will primarily examine antibiotic utilization in patients before and after an adjustment to the way that procalcitonin is presented in the EMR. The secondary outcome will be effects on patient safety. Specifically, patients with procalcitonin levels between 0.11-0.25 ng/mL will be examined. The goal sample size will be 200-300 patients in the both the pre- and post-implementation groups.

Procalcitonin holds an important role in the decision making surrounding the use of antibiotics, therefore, how the laboratory value appears in the EMR may affect this decision making. This study aims to improve the utilization of antibiotics and patient care by updating the EMR's presentation of procalcitonin.

RS12

Implementation and Evaluation of Fixed Dosing Prothrombin Complex Concentrate for Warfarin Reversal

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Kcentra™ is a 4-factor prothrombin complex concentrate containing exogenous clotting factors II, VII, IX, and X, sourced from human plasma. Kcentra™ may be used for the reversal of warfarin in the setting of acute bleeding or emergent surgery. Currently, the Kcentra™ package insert recommends weight-based dosing (units/kg) for the reversal of warfarin; however, recent data suggests a fixed dosing strategy may be used.

In a 2020 American College of Cardiology Expert Consensus Decision Pathway, 1000 units for a major non-cranial bleed and 1500 units for all other major bleeds are recommended. In August 2020, The University of Kansas Health System implemented a fixed dosing protocol for Kcentra™ in the setting of warfarin reversal. Our protocol now recommends 1500 units for intracranial hemorrhage, and 1000 units for all other bleeds, in conjunction with vitamin K. Providers have the option to give an additional 500 units based on patient specific factors, such as pre-treatment INR, weight, presence of continued bleeding, and if the INR remains above goal post-treatment.

A single-center, retrospective chart-review will be used to evaluate the efficacy and adherence to fixed dose Kcentra™. Patients 18 years and older will be included if they received Kcentra™ for reversal of warfarin after policy implementation. The primary outcome assessed will be achievement of an INR less than 2 post fixed dose Kcentra™. Secondary outcomes to be assessed include time from Kcentra administration to achievement of goal INR, total dose of Kcentra™ administered (including additional supplemental doses), compliance with the approved policy regarding indications for Kcentra™ and corresponding dosing, and predicted cost-savings. Data collection following this implementation is still underway; however, we expect the fixed dosing protocol will lead to achievement of goal INR. We predict a significant cost savings as an additional result of the implementation of this protocol. We will use data regarding use of additional bolus doses and time to INR reversal to assess appropriateness of the protocol implemented.

Fixed doses of Kcentra™ used for warfarin reversal has shown to have similar efficacy as weight based dosing. In implementing this new protocol, we expect to see attainment of goal INR.

RS13

Impact of Utilization Guidelines on Injectable Hydralazine Use in Hypertensive Crises

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Patient safety concerns exist regarding the use of injectable hydralazine. According to The Journal of Clinical Hypertension, "Aggressive dosing with oral agents, or even intravenous agents to rapidly lower blood pressure is not without risk to the individual patient." Per the product labeling, injectable hydralazine has been associated with prolonged hypotension and reflex tachycardia, leading to concerns if used and monitored inappropriately.

Injectable hydralazine is FDA approved as an alternative treatment for hypertensive emergency and has no approved indication for use in hypertensive urgency. Per the 2017 ACC/AHA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults, hypertensive emergency and hypertensive urgency are defined as a blood pressure ≥ 180/120 mmHg, with and without signs of target organ damage, respectively. A single center drug utilization evaluation at the University of Kansas Health System revealed that only 36.1% of doses were administered to patients experiencing a blood pressure ≥ 180/120 mmHg. Additionally, post-dose blood pressure monitoring was performed according to institution guidelines for only 61% of administered doses. This information revealed opportunities for improved patient safety through increased appropriate use and monitoring of injectable hydralazine. To address these patient safety concerns, utilization guidelines were developed and supporting medication order questions were added to the electronic medical record with the goal of increasing the appropriate utilization and monitoring of injectable hydralazine at our institution.

Data collection is in process. We are conducting a quasi-experimental, single center chart review of adult patients to evaluate the impact of hydralazine utilization criteria built into the electronic medical record on the appropriate use and monitoring of injectable hydralazine at the University of Kansas Health System. Appropriate use is defined as use in patients with blood pressure ≥ 180/120 mmHg. Appropriate monitoring is defined as adherence to institution-specific blood pressure monitoring requirements.

Injectable hydralazine brings the risk of prolonged hypotension and reflex tachycardia, especially if used and monitored inappropriately. This study will inform whether the implementation of utilization criteria built into the electronic medical record can improve the appropriate use and monitoring of injectable hydralazine. This information will help to inform and guide the implementation of utilization criteria for other high-risk medications in our health system. Additionally, the barriers to the implementation of and adherence to medication utilization guidelines in a health system and possible solutions to those barriers will be discussed.

RS14

Oral Vancomycin for Clostridium difficile Prophylaxis in Allogenic Hematopoietic Cell Transplant

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Neutropenia and antibiotic use put patients at risk for *Clostridium difficile* infection (CDI) following allogenic hematopoietic cell transplant (alloHCT). CDI following alloHCT has been associated with acute graft versus host disease (GVHD), a significant cause of morbidity and mortality in this population. We sought to evaluate if prophylactic oral vancomycin reduces the incidence of CDI in alloHCT recipients.

One study of 145 alloHCT patients at the University of Pennsylvania looked at the benefit of prophylaxis on CDI. They found favorable results with oral vancomycin, but due to study size at a single institution, further research is required to show reproducibility. We are conducting a single center retrospective chart review to compare the effectiveness of oral vancomycin prophylaxis versus no prophylaxis in alloHCT recipients at the University of Kansas Health System (TUKHS). Vancomycin for CDI prophylaxis was implemented in early 2018 at TUKHS. Review of 100 consecutive alloHCT patients before and after this implementation will be used to compare outcomes. Patients received oral vancomycin 125 mg twice daily starting on the day of inpatient admission for alloHCT and continued until discharge.

The primary outcome is the incidence of CDI in patients with oral vancomycin prophylaxis compared to those who did not receive prophylaxis during hospital admission for alloHCT. The secondary endpoints include the incidence of acute grade 2-4 GVHD, relapse, non-relapse mortality, and overall survival (OS) for each arm.

Data collection is still underway; however, prophylaxis with oral vancomycin is expected to be effective in decreasing rates of CDI in alloHCT recipients. We do not expect to see a difference in the risk of GVHD, disease relapse, non-relapse mortality, or OS between arms

The retrospective study will inform whether the use of vancomycin prophylaxis during hospital admission in the alloHCT setting is effective at reducing rates of CDI without negatively affecting post-transplant outcomes.

RS15
Intravenous Iron Order Set Optimization at an Academic Medical Center

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Iron products are utilized for iron deficiency anemia (IDA), which can occur from decreased iron absorption or increased iron loss (such as hemorrhage, inflammatory bowel disease (IBD), chronic kidney disease).[1] Oral iron products are more cost effective; however, they can cause gastrointestinal (GI) upset and have slower absorption rates.[2] Intravenous (IV) iron products are ideal for patients who cannot tolerate oral iron; however, there is risk for infusion reactions and iron overload.[3] Established clinical guidelines support the use of IV iron for chronic kidney disease (CKD) patients and patients with heart failure (HF).[4,5,6] Some guidelines state either oral or IV iron supplementation is acceptable, such as the NCCN guidelines for cancer and chemotherapy-induced anemia.[7,8] For GI disorders, oral or IV supplementation is acceptable post-GI bleed; however, oral iron may exacerbate underlying conditions such as IBD.[9]

A drug utilization evaluation of adult patients receiving IV iron products at The University of Kansas Health System discovered differences in prescribing patterns among services and indications. Seventy-one percent of orders were not ordered though the IV iron order set, which removed monitoring parameters, nursing vital checks, and infusion reaction medications. Twenty-seven percent of patients receiving orders for IV iron products did not have iron studies (serum iron, ferritin, transferrin saturation, and total iron binding capacity) within the 14 days prior. Twenty-three percent of patients received orders that provided greater 1000 mg in a 14-day period, especially when considering iron supplied from blood transfusions.[10]

Interventions will be implemented to restrict IV iron to the order set, populate iron studies within the past 14 days, add oral iron plus ascorbic acid order options, and include indication-specific guideline recommendations for appropriate utilization of IV iron products. Education has been provided to internal medicine pharmacists and a multi-disciplinary committee of acute care providers. Post-implementation data will be collected after the intervention go-live date. The goal of this research is to compare pre- and post-intervention appropriateness of IV iron utilization, safety outcomes, cost to healthcare system, and promotion of utilizing oral iron products or referring to outpatient management when appropriate.

The IV iron order set interventions aim to optimize appropriate utilization of IV iron products in the inpatient setting, improve safety outcomes, reduce cost to the healthcare system, and promote alternative methods of IDA management in comparison to previous DUE data.

Pharmacology & Toxicology

RS16
Mapping Brain-Wide Synaptic and Cellular Activity Correlates of Visual Experience

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Brain-wide maps of neuronal connectivity provide an entry point to mechanistic investigations of information processing associated with perception and memory. Current methods allow for mapping structural connections between neurons at the scale of individual synapses. In contrast, brain-wide maps of functional connections, or temporally correlated neuronal activity elicited by a context or stimulus, are generated only at the cellular or circuit scale.

This project addresses this methodological gap by developing a novel transgenic mouse model that allows for tagging either the cell bodies or the presynaptic terminals of active neurons within a specific time window, providing the first ever brain-wide synaptic scale functional connectivity maps. When used in parallel with brain-wide cellular scale functional connectivity mapping, this approach may reveal regional memory- or perception-evoked changes in the relationship between active synaptic input distribution and active cellular output distribution across the brain. Initial experiments show that this system is effective for mapping synaptic-scale functional connectivity through mouse development up to at least one month of age. This system will first be applied to map synaptic correlates of visual experience and memory. Additionally, this tagging system may be combined with mouse models of disease, showing promise as an important tool for investigating regional changes to the distribution of input/output activity in pathological states.

RS17

Extended amygdala corticotropin-releasing hormone activity underlies stress-induced pair bond impairments in male prairie voles (*Microtus ochrogaster*)

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Meaningful social connections and relationships provide a multitude of emotional and mental health benefits. However, social conflict can lead to a state of social aversion that may negatively impact an individual's ability or motivation to form and maintain long-lasting social connections. This study uses the *social defeat* model of social conflict in the prairie vole (*Microtus ochrogaster*), a socially monogamous rodent that forms selective and long-lasting social connections between mating pairs known as a *pair bond*, in order to investigate the neurobiology underlying the connection between social conflict and impairments in relationship formation. Following social defeat experience, pair bond formation is diminished in male voles and accelerated in females, as measured by a selective preference for affiliation with the partner. Cohabitation with an opposite-sex mate for 24 hours, a time period sufficient to observe pair bond-related behaviors, led to an increase of corticotrophin-releasing hormone (CRH) in the bed nucleus of the stria terminalis (BNST) in stress-naïve animals. CRHergic neurons in the BNST are known to be associated with both the behavioral response to stress and pair bond formation in prairie voles; thus, it was hypothesized that CRHergic activity in the BNST underlies changes in pair bond formation induced by stress experience. Chemogenetic manipulation of BNST CRHergic neurons revealed that activity of these neurons promotes pair bond formation in stress-naive conditions but inhibits bonding in chronic stress conditions in male prairie voles. The results of this study demonstrate a novel "switch" function of BNST CRH neurons that either promote or prevent pair bond formation depending on prior social conflict experience.

RS18

Clusterin interacts with microglia and downregulates inflammation in in vitro models

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Clusterin (CLU), also known as apolipoprotein J, is one of the top 3 genetic risk factors for the development of late-onset Alzheimer's disease (LOAD); however, how its genetic variants influence the risk of LOAD is unknown. Apart from the well-characterized amyloid and tau pathologies, the presence of extensive neuroinflammation mediated by microglia, the brain-resident immune cells, has been emerging as a major contributor in the pathogenesis of LOAD. Therefore, modulation of microglial activation is crucial for retaining microglial homeostasis and maintaining overall brain health. In this study, using mouse primary astrocytic and microglial cultures as well as a microglial cell line, we analyzed the expression profile of CLU and the role of CLU on microglial response to inflammatory challenge induced by lipopolysaccharide (LPS). We found that CLU is abundantly expressed in and secreted from astrocytes, however, it is neither expressed in nor secreted from microglia. Furthermore, both astrocyte-secreted and recombinant CLU glycoprotein interacted with microglia and reduced the level of LPS-induced microglial inflammation. These findings indicate that CLU may play an important immunomodulatory role in the brain.

RS19
ApoE2 reverses glycolytic deficit in ApoE4-expressing neuronal cells

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Continued clinical failures in the search for a successful treatment of Alzheimer's disease (AD) raise questions about the validity of currently focused therapeutic targets, underscoring the importance of an alternative concept that emphasizes on the neuroprotective mechanism that promotes brain resilience against the onset of AD. We have recently demonstrated that human ApoE genetic isoforms, ApoE2 ApoE3 ApoE4, differentially modulate neuronal glycolytic metabolism with the ApoE2-expressing cells exhibiting the most robust while the ApoE4-expressing cells display the most deficient profile. Our follow-up study revealed that these ApoE isoforms-mediated glycolytic differences directly correlated to cellular overall health status, as indicated by both metabolic activity and morphological phenotype of the cells. In the present study, we investigated metabolic changes in cells stably expressing hApoE4 upon a transient transfection of pCMV-hApoE2 or administration of recombinant human ApoE2 (rhApoE2) protein. Our results show that a 48hr exposure to hApoE2 induced a significant increase in both the expression and enzymatic activity of hexokinase when compared to the vehicle-treated control group, indicating that introduction of hApoE2 can potentially reverse the glycolytic deficit in neuronal cells that express ApoE4. These in vitro data provide a proof of concept for the potential of translating ApoE2-mediated neuroprotective mechanisms into a novel therapeutic approach in the prevention and early intervention of AD.