2nd ASPET/ADDC
Academic Drug Discovery Colloquium

Fueling Innovation: Public Programs Driving Drug Discovery

October 12-13, 2017
Bethesda, MD

ABSTRACTS

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A colloquium hosted by:

#1 *Molecular Design and Synthesis Group at Rutgers University Translational Sciences.*

D.J. Augeri, A. Bencivenga, B. Cao, J. Gilleran, R. Fondekar, J. Roberge, Q. Xin, V. Smeraglio, D. Kimball. Molecular Design and Synthesis Group, Rutgers University Translational Sciences.

Rutgers University has built Rutgers Translational Sciences (RTS), an interdisciplinary team of scientists together with business development. Consisting of medicinal chemistry (Molecular Design and Synthesis), imaging, and histopathology/toxicology that collaborate with Principal Investigators, the goal is increased funding by generating preliminary results to strengthen research proposals to NIH and other funding agencies. With a group of 7 experienced medicinal chemists, the Molecular Design and Synthesis group currently has 7 programs where we synthesize probe molecules to investigate novel biology. We build SAR and attempt to provide a molecule that is successful in providing proof of principle and these preliminary results have resulted in several funded proposals, including p53 mutant reactivators (oncology), plasmodium kinase G (PKG, malaria) and others. Several of these successful collaborations will be presented.

#2 *Lamotrigine-Resistant Corneal Kindled Mice: A Model Of Pharmacoresistant Partial Seizures For Moderate-Throughput Antiseizure Drug Discovery.*

M. Barker-Haliski, Z. Koneval, K. Knox, and H. S. White. University of Washington.

Rationale: Despite numerous therapies for the treatment of epilepsy, over 30% of patients remain resistant to available antiseizure drugs (ASDs) and are considered pharmacoresistant. ASD discovery has primarily relied on demonstration of efficacy in acute in vivo seizure models (e.g. 6 Hz) before advancing a compound into etiologically-relevant chronic models of epilepsy. Traditional pharmacoresistant rodent models, such as the focal kainic acid mouse or lamotrigine (LTG)-resistant kindled rat, are labor- and resource-intensive, whereas the 6 Hz seizure model does not exhibit the pathophysiological or behavioral changes associated with chronic seizures. Given that over 30% of patients with epilepsy are currently resistant to available therapies, there is a high unmet need for novel ASDs that are effective in patients with pharmacoresistant epilepsy. The early identification of novel ASDs should thus be conducted in etiologically-relevant models of pharmacoresistant chronic epilepsy.

The traditional 60 Hz corneal kindled mouse (CKM) represents a well-characterized preclinical model of chronic seizures that has contributed to the identification and development of numerous ASDs; however, this model is typically not considered pharmacoresistant. To provide a moderate-throughput, etiologically-relevant model that exhibits the pathophysiological and behavioral alterations associated with epilepsy, we developed a pharmacoresistant CKM that is potentially suitable for frontline ASD discovery.

Methods: Male CF-1 mice (n = 30/ group) were administered either vehicle (VEH; 0.5% methylcellulose) or LTG (8.5 mg/kg, i.p. b.i.d.) 30 min prior to twice-daily corneal kindling (3 mA, 60 Hz, 3 sec stimulation) until mice achieved kindling criterion (5 consecutive Racine stage 5 seizures). An additional cohort of mice were not administered either VEH or LTG. Handling controls (no electrical stimulus delivered at corneal electrode placement; n = 5/treatment group) were also included for all treatment groups. Upon achieving kindling criterion, mice were allowed 5-7 days of washout before determining the response to prototype ASDs: carbamazepine (CBZ); phenytoin (PHT); lamotrigine (LTG); levetiracetam (LEV); valproic acid (VPA); diazepam (DZP); phenobarbital (PB); and retigabine (RTG). Fluoxetine, celecoxib, and minocycline were also evaluated as compounds with mechanisms of action of interest to ASD discovery.

Results: Administration of LTG did not delay acquisition of the corneal kindled seizure, consistent with the LTG-resistant amygdala-kindled rat. Following the wash-out period, administration of 17 mg/kg (i.p.) LTG did not block the expression of the secondarily generalized kindled seizure in the LTG-kindled mice (mean seizure score (MSS) of 4.90.05 SEM), whereas VEH-treated mice were highly sensitive to this dose (MSS: 2.30.30; t=10.8, p<0.0001); thus, confirming LTG-resistance. Furthermore, the seizures of LTG-resistant CKM were resistant to treatment with single i.p. doses of CBZ (20 mg/kg), RTG (10 mg/kg), and VPA (300 mg/kg) that elicited significant reductions in seizure score in VEH-treated kindled mice. The MSS for VEH- vs. LTG-kindled mice at these ASD doses was as follows: CBZ – 3.400.29 vs. 4.80.17; RTG – 3.20.70 vs. 4.70.20; VPA – 1.10.58 vs 2.60.53. The full pharmacological profile of this model will be discussed.

Conclusions: The pharmacoresistant LTG-resistant corneal kindled mouse provides an early platform to identify compounds before advancing to more resource-intensive models of pharmacoresistant epilepsy.

#3 *Opioid Analgesia: Concurrent Evaluation of Antinociception and Behavioral Impairment.*
J. Bergman, B. Kangas, S. Withey, C. Paronis. McLean Hospital/Harvard Med School, Belmont.

Opioids remain the most widely prescribed drug class in the management of moderate to severe pain, yet their clinical application is often hindered by a broad profile of undesirable effects. The response to thermal stimuli remains the most common method for assaying opioid antinociception across species. However, the behaviorally disruptive effects of opioids that may accompany antinociception --an important consideration in their clinical application --typically are not measured in this type of assay. To address this consideration, we have developed two antinociception assays in squirrel monkeys. In the first, to evaluate nociceptive threshold, we employ an operant task requiring the subject to pull down a heated cylindrical thermode for a specified duration to obtain reinforcement; nociceptive threshold varies with the required duration and is defined as the temperature at which the subject fails to complete the response.   In the second procedure, we have modified the conventional warm water tail withdrawal assay (Dykstra and Woods, 1986) to concurrently determine the effects of opioids on both tail withdrawal latency (antinociception) and measures of food-maintained behavior (reinforcement density, response rate). Opioid agonists produce dose-dependent antinociceptive effects in both procedures. In the first procedure, conventional morphine-like opioids and, as well, NOP-selective agonists produce dose-related increases in nociceptive threshold at doses below those that markedly impair performance. In the second procedure, dose-dependent increases in tail withdrawal latency and behavioral impairment occur concurrently. In particular, data indicate that the ratio of ED50 values for the two measures is similar for morphine-like high-efficacy agonists (0.82-1.14) and highest for the opioid partial agonist nalbuphine (4.88), reflecting significant antinociception with only minimal behavioral disruption. These studies illustrate the utility of operant procedures for increasing the efficiency and predictive value of analgesiometric assays. (supported by USPHS DA035857)

#4 *Structure-Guided Design for Agonist Selectivity of the α7-nAChRs.*
K. Kaczanowska, M. Harel, L. Kohs, L Bendiks, L. Doan, L. Huber, C. Stokes, R. L. Papke, P. Taylor. Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical.

Structure-Guided Design for Agonist Selectivity of the α7-nAChRs. Gisela Andrea Camacho-Hernandez1,2, Katarzyna Kaczanowska1, Michal Harel1, Larissa Kohs1, Larissa Bendiks1, Lisa Doan1,

Laura Huber1, Clare Stokes3, Roger L. Papke3, Palmer Taylor1. 1Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA. 2Facultad de Ciencias Químicas e Ingeniería, Universidad Autóma de Baja California, Tijuana, BC, México. 3Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, USA.
Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels present in the peripheral and central nervous systems. Pre- and post-synaptic nAChRs modulate CNS functions, selective ligands for nAChR subtype may form useful therapies for chronic neurodegenerative diseases like Alzheimer's and Parkinson's, and be useful therapeutics to ameliorate ymptoms in schizophrenia. In our previous study we identified a series of 4,6-disubstituted 2-aminopyrimidines interacting with Acetylcholine Binding Proteins (Lymnaea stagnalis AChBP) soluble surrogate protein of the extracellular domain of α7-nAChR, in a cooperative fashion [Kaczanowska et al., Proc.Natl. Acad. Sci. USA 111, 10749 (2014)]. Taking advantage of these findings we developed a new landscape for 4,6 di-substituted 2-aminopyrimidines that not only interact with the acetylcholine binding protein (AChBP) but also activate α7-AChRs. These agents show Kd and Ka values for both AChBP binding and α7−nAChR activation between 10 and 70nM. To examine the structural basis of agonist selectivity, we employ easurements of ligand binding to AChBP, and analysis of ligand binding poses from X-ray crystal structures, along with activation of HEK cells transfected with cDNA's encoding one of three requisite receptor subtypes: α7-nAChR, α4β2-nAChR and 5HT3A [Kaczanowska, Camacho-Hernandez. et al., J Am Chem Soc. 139, 3676 (2017)]. We were able to assess fast activation of lead candidates in an oocyte system, revealing an agonist behavior distinguishing them form silent agonists and ago PAM's. Crystal structures suggest a distinct binding pose is required for α7-nAChR activation enabling us to define the molecular determinants giving rise to activation and selectivity for the α7 subtype. By combining the di-picolyl substitution at the 4-position of the pyrimidine and departing from the symmetry of the molecule, we are able to discriminate between α7 activation and generalized binding at the subunit interface. The 4, 6-disubstituted 2-aminopyrimidines have high selectivity for α7-nAChR. Crystallographic studies using the soluble AChBP, reveal three distinctive binding interactions and poses of nicotinic agonists: 1) cation-π interactions for quaternary amines and an aromatic nest of side chains at the subunit interface, 2) stabilization of secondary amines and imines by a hydrogen bond from its protonated basic nitrogen to the backbone carbonyl of a conserved tryptophan on the principal subunit face, 3) interaction of less basic multi-ring pyrimidine-pyridine heterocycles at the binding interface. This new family of 2-aminopyrimidines enables us to define receptor subtype selectivity and achieve a distinctive structural landscape for α7 selective activation amongst a plethora of CNS nAChR subtypes. We are continuing to expand the library of ligands to obtain a more detailed insight of the structural determinants that dictate the selective activity.

#5 *Developing Small Molecule Therapeutics Targeting Exchange Protein Directly Activated By Cyclic AMP.*
X. Cheng, F. C. Mei. The University of Texas Health Science Center at Houston.

The pleotropic second messenger cAMP is a major stress response signal that regulates a multitude of physiological functions under normal and diseased conditions. The major cellular effects of cAMP are mainly transduced by two ubiquitously expressed intracellular cAMP receptors, protein kinase A (PKA) and exchange protein directly activated by cAMP (EPAC). Using genetically engineered EPAC knockout mouse and various disease models, we have demonstrated that EPAC proteins, while not essential for normal development and survival, play important roles in the development of major human diseases therefore, are ideal therapeutic targets. Furthermore, we have developed first-in-class EPAC specific inhibitors with favorable in vivo pharmacological and toxicological profiles. Applications of EPAC

inhibitors in various animal disease models recapitulate the genetic phenotypes of EPAC knockout mice, validating the therapeutic potentials of small molecule EPAC specific inhibitors. Our current efforts of preclinical drug development of EPAC inhibitors and their applications in the treatment of chronic pain, bacterial infection and cardiovascular diseases will be discussed.

#6 *Recombinant Human Tissue Inhibitor of Metalloprotease -2; a Novel Anti-cancer Therapeutic.*
A. Chowdhury, B. Wei, Y. Yamada, N. Castro, R. Brinson, J. Schneider, D. Salomon, W. G. Stetler-Stevenson. National Cancer Institute.

Tissue inhibitor of Metalloprotease-2 (TIMP-2), a soluble endogenous metalloprotease inhibitor, exhibits anti-cancer properties by metalloprotease inhibition in tumor microenvironment, direct receptor-mediated growth inhibition, increased chemo –sensitivity and decreased cell motility. Herein we report a complete production-characterization process, biophysical analysis, anti-MMP activity and anti-proliferative activity of rhTIMP-2 in cell lines and mouse models of breast and lung cancer. Further we report the development and characterization of rhTIMP-2 extended release peptide hydrogels and desferoxamine(des) labelled rhTIMP-2 for dosage form and PET scan probe development respectively. Codon optimization and stable clonal selection resulted in about 7-fold increase in rhTIMP-2 His6 expression compared to identical culture with WT rTIMP-2 cDNA transfected HEK-293F cells. Purification by IMAC Ni2+ chromatography and RP-HPLC yielded TIMP-2 His6 with greater than 90% (96.21% recovery) and 99% (81.75% recovery) percentage purities respectively. Purified rTIMP-2 exhibited single band and peak in SDS-PAGE gel and by ESI-TOF mass spec.; and was immuno-reactive against anti-TIMP-2 and poly-histidine antibodies. Circular Dichorism spectra indicated high degree of β-pleated sheet in rhTIMP-2 2° structure. 2D NMR of rhTIMP-2 His6 in natural abundance indicated a well folded monodisperse rhTIMP-2 with ~10-15% population of alternate rhTIMP-2 species. All production batches of the rTIMP-2 were negative in MAP testing with Endotoxin levels at 3.702 EU/ml (<10 EU/ml acceptable in cGMP). TIMP-2 effectively displayed dose dependent inhibition of MMP-2 (type IV collagenase) activity with an IC50 of < 50nM; with no MMP-2 inhibitory observed in the Ala+TIMP-2 (N' terminal Ala appended TIMP-2 with no MMP inhibitory activity). In- vitro pre-treatment with 50 nM rTIMP-2 in gelatin coated plates suppressed rhEGF induced growth of A549 (lung cancer) and JygM(A) (triple negative breast cancer cell lines) to statistically significant levels. Athymic nude mice were injected with JygM(A) breast cancer cell lines under the mammary fat pads. Mice with palpable tumors were randomized and treated with 3 ug rhTIMP-2 /day/ mouse intra-peritonealy up to a period of 35 days that lead to ~30% reduction of tumor volume compared to the HBSS (vehicle control). We have also evaluated the effects of encapsulation and delivery of rhTIMP-2 from cationic peptide hydrogel – which shows extended release to up to 28 days with little or no loss of its biologic activity. Similarly, little or no loss of biological activity was observed upon desferoxamine(des) conjugation to rhTIMP-2. Zn89 attachment to the des subunit has been completed and the Zn89-rhTIMP-2 is being used for uptake, distribution and metabolism studies. Conclusively we have developed a robust, scalable bioprocess method for rhTIMP-2 (and analogs) production, characterization to support the availability and analysis through pre-clinical development. In-vitro and in-vivo results shows reduction in cell proliferation and tumor volume in breast, lung cancer cell lines and tumors. Extended release dosage forms and Zn89 labelled TIMP-2 are now being used to characterize the PK-PD and distribution of the biologic. With all supportive data, reagents and in-depth biophysical analysis we present a comprehensive report of rhTIMP-2 as a novel biologic therapeutic for cancer treatment and we plan to move forward towards continued pre-clinical/clinical development.

#7 *Intracellular Targeted Delivery of DON Using Novel Prodrug Strategies for Cancer Therapy.*
R. Dash, A. J. Gadiano, J. Alt, L. Tenora, S. Zimmermann, P. Majer, B. Slusher, R. Rais. Johns Hopkins University, Baltimore and Institute of Organic Chemistry and Biochemistry, Prague.

Purpose

The glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) broadly blocks glutamine utilizing reactions in cancer cells, and has shown robust efficacy in both preclinical cancer models and exploratory clinical studies. The development of DON was halted due to its dose-limiting gastrointestinal toxicities. Given DON's promising efficacy in treating "glutamine addicted" tumors, we identified a strategy to deliver DON selectively to the tumor/lymphoid cells while minimizing peripheral toxicity. Novel prodrugs of DON were developed to target tumor/lymphoid cells which were designed to circulate intact as inert prodrugs in plasma and preferentially biotransformed to DON in tumor cells, permitting significant dose reduction and improved therapeutic index.

Methods

Several prodrugs with different promoeities were synthesized. The prodrugs were evaluated for in vitro metabolic stability in plasma, and liver and gut swine homogenates for their potential to release the active DON. Briefly, prodrugs were spiked in plasma and tissue homogenates and incubated at 37°C. Aliquots of the mixture in triplicate were removed at predetermined times and prodrug disappearance was monitored using LC-MS/MS. The prodrugs were also evaluated for cell partitioning using P493B cancer cells and PBMCs. To evaluate the partitioning of DON prodrugs into PBMC or P493 lymphoma tumor cells, prodrugs were spiked into human or pig whole blood (PBMC partitioning) or in human plasma containing 10 million P493 cells and incubated at 37°C for 30-60 min. For the PBMC experiments, whole blood was processed for separation into plasma and PBMCs. For the lymphoma experiments, the cell plasma mix was centrifuged prodrug and DON in cells and plasma was quantified using our validated LC/MS/MS method. Since prodrugs are known to have species-specific metabolism where larger (non-rodent) species more closely mimic human metabolism, we found miniature swine to closely resemble humans in DON prodrug metabolism. For in vivo evaluation, DON prodrugs were evaluated in miniature swine for PBMC partitioning by administering via i.v. infusion and blood samples were taken at several time points post administration. Blood samples were subsequently processed for plasma and PBMCs as described above and analyzed for DON by LC-MS/MS.

Results

We have successfully synthesized intracellular targeted DON prodrugs, the best of which delivers ~30-fold more DON to PBMC and P493 cells versus human plasma. Moreover, our lead prodrugs also show minimal release of DON in the gut tissue (toxic site). A tissue distribution/tolerability study conducted in swine confirmed PBMC targeting. In a head-to-head comparison of prodrug versus equimolar DON, the prodrug showed enhanced DON delivery to PBMCs and reduced delivery to GI tissues, resulting in less GI pathology and fewer clinical symptoms.

Conclusion

We have shown a successful prodrug strategy for the development of a novel, robust and safe inhibitor of glutamine metabolism that will provide a new approach for the treatment of lymphoma and other "glutamine addicted" tumors.

#8 *Leukemia Inhibitory Factor-loaded Nanoparticles with Enhanced Cytokine Metabolic Stability and Anti-Inflammatory Activity for Ischemic Stroke Treatment.*

S.M. Davis, D. Reichel, Y. Bae, K.R. Pennypacker. University of Kentucky.

Purpose: To prolong the stability and activity of leukemia inhibitory factor (LIF), an anti-inflammatory cytokine that increases neural cell survival and improves motor skill function after permanent ischemic stroke.

Methods: LIF was packaged in nanoparticles made of poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) polymer to form LIF-loaded nanoparticles (NanoLIF). The surface of NanoLIF was also modified with the CD11b antibody (CD11b-NanoLIF) targeting activated peripheral macrophages to increase cytokine delivery into activated macrophages. ELISA was used to quantify bioactive cytokine inside and releasing from NanoLIF. Bioactivity of NanoLIF was measured using the M1 murine myeloid leukemia cell proliferation assay.

Results: NanoLIF and CD11b-NanoLIF had an approximately 30 nm diameter, neutral surface charge, and physicochemical stability retaining biological activity of the cytokine during incubation at 25°C for 12 h. NanoLIF particles released LIF relatively fast from 0-6 h after incubation at 37°C followed by slow release from 24-72 h according to a two-phase exponential decay model. NanoLIF and CD11b-NanoLIF significantly decreased M1 cell proliferation over 72 h compared to free LIF.

Conclusions: NanoLIF and CD11b-NanoLIF preserved the metabolic stability and biological activity of LIF in vitro, and these results are promising to improve the therapeutic potential of LIF in treating neurodegenerative and inflammatory diseases.

#9 *Identification and Characterization of a Novel Series of Exceptionally Selective and Potent D2 Dopamine Receptor Antagonists.*

M. M. Day, A. E. Dulcey, D. Bayron, C. R.T. Stang, A. E. Moritz, T. Doyle, J. A. Javitch, N. T. Southall, M. Ferrer, J. J. Marugan, D. R. Sibley, R. B. Free. National Institutes of Health.

FDA-approved antipsychotics are primarily D2 dopamine receptor (D2R) antagonists. Despite the clear clinical importance of the D2R, there are currently few D2R-selective antagonists, as most of these compounds generally interact with other GPCRs and dopamine receptor subtypes. Structural similarities in the orthosteric binding sites of D2-like (D2R, D3R, D4R) receptors and other biogenic amine receptors are partially responsible for this lack of selective D2R compounds. To find better therapeutic candidates, as well as probe compounds to investigate D2R pathophysiology, we sought to identify highly selective D2R antagonists by implementing a high-throughput screening (HTS) campaign using more than 400,000 unique compounds in the Molecular Libraries Screening Center Network library. Most of the hit compounds that were identified as antagonists of the D2R were also antagonists of the closely related D3R. However, several compounds were found to exhibit high affinity for the D2R with low affinity for the D3R. One such compound (MLS6916) served as our lead scaffold for optimization. MLS6916 is ~700 fold selective for the D2R vs. D3R as determined in functional β-arrestin recruitment assays and radioligand binding competition assays. Importantly, MLS6916 was also examined for functional activity at 168 different GPCRs and showed no agonist activity on any GPCR tested, and only exhibited antagonist activity at the D2-like receptors. Preliminary investigations, however, revealed that this initial hit compound was metabolically unstable. To optimize this hit scaffold, we conducted an iterative

medicinal chemistry campaign with the goals of increasing selectivity, potency, and engendering metabolic stability. Over 80 analogs of MLS6916 were synthesized to dissect the influence of various constituents of the molecule and to construct structure-activity-relationships. Analogs were analyzed for affinity and potency on the D2-like receptors using functional and radioligand binding competition assays, as well as tested for solubility, permeability, and metabolic stability. Several lead compounds with high affinity for the D2R and >1,000-fold selectivity vs. the D3R were identified. In β-arrestin recruitment functional assays, these selectivity trends were mirrored. Analogs with structural deviations from the MLS6916 scaffold had maintained or improved selectivity, adequate solubility and permeability, but most remain metabolically unstable. We are further optimizing this analog series to improve stability while maintaining D2R selectivity. Further analysis of lead compounds will provide new insight into highly selective D2R antagonists for the treatment and understanding of dopamine-related disorders.

#10 *Discovery of Inhibitors of the Histone Methyltransferase NSD1.*

K.M. Drake, A. Kisielewski, D.M. Cartledge, S. Langhans, E.A. Kolb, J.T. Koh, A.D. Napper. University of Delaware and Nemours AI duPont Hospital for Children, Wilmington, DE.

The mammalian nuclear SET domain containing (NSD) proteins are histone methyltransferases known to methylate lysine 36 of histone 3 (H3K36). Fusion of the C-terminal half of NSD1 to NUP98 is present in 5-15% of pediatric acute myeloid leukemia (AML) patients and strongly associated with a poor prognosis. The NUP98-NSD1 fusion protein induces leukemogenesis in mice, and in vitro the fusion has been shown to sustain self-renewal of myeloid stem cells in an undifferentiated state through H3K36 methylation and activation of transcription of the HoxA and MeisI genes. Therefore, small molecules that inhibit the enzymatic activity of NSD1 should be effective in reversing activation of these genes, allowing normal myeloid differentiation to proceed.

We have developed a high-throughput assay to discover inhibitors of NSD1 using an enzyme-coupled luminescent read-out. S-adenosylhomocysteine (SAH) produced during the methylation reaction is hydrolyzed by S-adenosylhomocysteine hydrolase (SAHH) to adenosine and homocysteine. Adenosine is phosphorylated by adenosine kinase (AK), and the concomitant consumption of ATP is measured using reagents from the Kinase Glo assay kit (Promega). This assay is highly sensitive, allows for the use of full length histones, octamers, or recombinant nucleosomes as substrates, and should be universally amenable for use with all S-adenosylmethionine (SAM) dependent methyltransferases. Results of our high-throughput screening of 200,000 compounds and characterization of hits will be presented.

#11 *Identification and Characterization of ML321: A Novel and Selective D2 Dopamine Receptor Antagonist with Predicted Antipsychotic Efficacy.*

R. B. Free, J Xiao, M. Ferrer, N. Southall, G. Stanwood, R. Mach, Jeremiah Bertz, James H. Woods, Juan J Marugan, William Wetsel, and David R. Sibley. Molecular Neuropharmacology Section, NINDS, Bethesda, MD, National Center for Advancing Translational Sciences, NIH, Rockville, MD, Florida State University College of Medicine, Tallahassee, FL, University of Pennsylvania School of Medicine, Philadelphia, PA, University of Michigan School of Medicine, Ann Arbor, MI, Duke University School of Medicine, Durham, NC.

Dopamine receptors (DARs) belong to the G protein-coupled receptor (GPCR) superfamily, whose members play a critical role in cell signaling, especially modulating the transfer of information within the nervous system. Amongst DARs, the D2R receptor is arguably one of the most validated drug targets in neurology and psychiatry, and there is a strong correlation between the clinical doses of antipsychotics and their affinities for blocking D2Rs. However, current FDA-approved antipsychotic drugs typically cross-react with other GPCRs, leading to many off-target side effects. A potent and highly selective D2R antagonist would thus be particularly valuable for the treatment of psychosis and related CNS disorders. Using high throughput screening techniques, we have now identified, optimized, and characterized a lead D2R antagonist with high selectivity – ML321. In a functional profiling screen of 168 different GPCRs, ML321 showed relatively little cross reactivity beyond inhibition of the D2R, and to a lesser extent the D3R, demonstrating its exceptional selectivity. Interestingly, we found that the binding of ML321 to the D2R is entirely dependent on Na+. PET imaging studies in non-human primates demonstrated that ML321 can penetrate the CNS and occupy the D2 DAR in a dose-dependent manner. Preliminary in vitro ADME studies showed that ML321 is metabolically stable and did not block hERG channels, whereas pharmacokinetic (PK) studies in mice demonstrated elevated plasma and brain levels of ML321 for up to 8 hrs. Behavioral paradigms in rats demonstrated that that ML321 can selectivity antagonize a D2R-mediated response (hypothermia) while not affecting a D3R-mediated response (yawning) using the same dose of drug, thus demonstrating good in vivo selectivity. We also investigated the effects of ML321 in animal models that are predictive of antipsychotic efficacy in humans. We found that ML321 can attenuate both PCP and amphetamine-induced locomotor activity and pre-pulse inhibition (PPI) in a dose dependent manner. Importantly, using doses that were maximally effective in the locomotor and PPI studies, ML321 promoted little-no catalepsy compared with the non-selective antipsychotic haloperidol. This observation suggests that ML321 may produce fewer extrapyramidal side effects (EPS), a common problem with FDA-approved D2R antagonists. Overall, these results suggest that the ML321 scaffold could serve as a lead compound for the development of an improved therapeutic with fewer side effects for treating schizophrenia and other psychotic syndromes.

#12 *Enhanced CNS Uptake of Prodrugs of the GCPII Inhibitor 2-PMPA Following Intranasal Administration.*
A. J. Gadiano, S. C. Zimmermann, R. P. Dash, C. Garret, J. Alt, C. Rojas, A. G. Thomas, Y. Wu, P. Majer, R. Rais, B. S. Slusher. Johns Hopkins Drug Discovery & National Institute on Drug Abuse.

2-(Phosphonomethyl)pentanedioic acid (2-PMPA) is a potent and selective inhibitor of glutamate carboxypeptidase-II (GCPII), a membrane-bound zinc metalloprotease that plays a key role in regulating extracellular glutamate availability in the brain. As disrupted glutamate homeostasis is implicated in several neurological and psychiatric conditions, GCPII is a promising drug target. Several independent laboratories have shown robust neuroprotective efficacy with 2-PMPA in dozens of preclinical neurological and psychiatric disease models (e.g. neuropathic pain, peripheral neuropathy, stroke, schizophrenia, addiction, multiple sclerosis, traumatic brain injury), when given at very high systemic doses (>100 mg/kg) or when directly injected into the brain. However, the clinical development of 2-PMPA has been hampered by its low brain penetration, presumably due to its multiple acidic functionalities. We recently reported an improvement in the brain-to-plasma ratio of 2-PMPA after intranasal (IN) dosing in both rodents and primates. We also recently employed a 2-PMPA prodrug strategy to decrease its polarity and showed that we could improve its oral availability by >20-fold in both rodents and dogs We therefore hypothesized that combining a IN delivery with a prodrug approach could further enhance permeability and increase drug exposures in the brain. Herein, we have undertaken the synthesis of several 2-PMPA prodrugs with masked -carboxylates and performed

pharmacokinetic evaluations of these compounds in Wistar rats and rhesus macaques. Our results show that the prodrugs investigated have enhanced lipophilicity and further improve brain as well as systemic delivery of 2-PMPA after IN administration. When compared to IN 2-PMPA in rats at 1 hour post-administration, the prodrug -(4-acetoxybenzyl)-2-PMPA (JHU 144), resulted in substantially higher 2-PMPA delivery to both plasma (4.1-fold) and brain (11-fold). Subsequent time-course evaluation of JHU 144 also showed high brain and plasma 2-PMPA exposures with the brain/plasma ratios of 1.45, 0.23, and 0.20 for olfactory bulb, cortex, and cerebellum, respectively. Further, in primates IN administration of JHU 144 more than doubled 2-PMPA concentrations in the CSF relative to previously reported levels and provided a CSF to plasma ratio of 1. In conclusion, the results of these experiments provide a promising strategy of enhancing delivery of drugs that demonstrate therapeutic potential for neurological and psychiatric disorders.

#13 *Investigating the Effects of Gator01 on Triple-Negative Breast Cancer.*
A. Graves, A. Truong, L. Herring, Y. Liu, A. Tikunov, J. Macdonald, I. McDonald, M. East, L. Krulikas, E. Iwanowicz, L. Graves. University of North Carolina at Chapel Hill and Madera BioSciences.

Breast cancer is the most common type of cancer among women. One subtype of breast cancer, triple-negative breast cancer (TNBC), is particularly aggressive and has poor prognoses. This is due in part to the fact that this subtype lacks the targets that are exploited in other breast cancer cell lines. Currently, there are no effective targeted therapies for triple-negative breast cancer.
ONC201/TIC10 (Oncoceutics) is a drug that is currently in phase 2 clinical trials (ClinicalTrials.gov Identifier: NCT02863991). ONC201 has shown anti-tumor effects in several cancer cell lines and has been shown to perturb several key signaling pathways, including Akt and ERK. By inhibiting the Akt and ERK pathways, it has been demonstrated that ONC201 mediates upregulation of TRAIL, a death ligand that induces cellular apoptosis. ONC201 has also been demonstrated to be a dopamine receptor antagonist. Despite this evidence, questions remain about the specific targets and mechanism of ONC201 action.

A structural derivative of ONC201, Gator01, was developed by Madera BioSciences as a potential alternative to ONC201 with increased potency. In this study, we examined the effectiveness of Gator01 on multiple breast cancer cell lines. Dose-dependent inhibition of cell growth was measured by MTS cell viability assays in a TNBC cell line (SUM159) using standard 2D monolayer cultures. The efficacy of Gator01 was also tested using 3D co-cultures of mCherry-expressing MDA-MB-231 cancer cells and GFP-expressing cancer associated fibroblasts (CAFs).
The results of the 2D cell viability assays demonstrated that Gator01 was approximately 60-100-fold more potent than ONC201. However, under similar conditions, Gator01 did not inhibit the Akt/ERK pathways or induce TRAIL similarly to ONC201. Interestingly, a distinct acidification and yellowing of the media (48-72 hours) was observed in response to Gator01 treatment, but not in response to ONC201 treatment or vehicle control. To further investigate the mechanism of Gator01 action, kinome and metabolic profiling was performed. The results of these studies will be discussed in the poster. Support provided by: P30 CA016086.

#14 *Inhibiting Tau Phosphorylation Using Synthetic Peptides: Developing Peptide-based Inhibitors of Microtubule Affinity Regulating Kinase 2 (MARK2).*
R.A. Colvin, N. Alqaeisoom, J. M. Holub. Ohio University.

The microtubule-affinity regulating kinase (MARK) proteins are a family of Ser/Thr kinases that play critical roles in regulating signal transduction and cellular polarity. A primary function of MARK2 is to

phosphorylate microtubule-associated proteins such as MAP2, MAP4 and Tau within their microtubule-binding repeat (R) domains. In neurons, Tau proteins act to preserve the structural integrity of the cytoskeleton by binding and stabilizing microtubules. The phosphorylated state of Tau is important in regulating its interaction with tubulin; Tau that is post-translationally phosphorylated by MARK2 dissociates from tubulin, leaving polymeric microtubules in highly dynamic states. Under pathological conditions, Tau proteins can become abnormally phosphorylated, leading to irreversible changes in microtubule dynamics and neuronal cell death. Furthermore, hyper-phosphorylated Tau proteins can form prion-like oligomers known as neurofibrillary tangles, which have been implicated in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) and frontotemporal dementia. Indeed, elevated activity of Tau kinases, including MARK2, has been documented in patients with AD. In this study, our objective was to develop a cell-permeable, peptide-based inhibitor of MARK2 function. This peptide (tR1) was designed as a direct sequence mimic of the Tau R1 domain to selectively inhibit the MARK2-mediated phosphorylation of endogenous Tau. In vitro experiments showed that tR1 inhibits the MARK2-mediated phosphorylation of Ser262 within the Tau R1 microtubule-binding domain. It was further demonstrated that tR1 is internalized by rat primary cortical neurons via endocytosis and can be delivered to the cytoplasm when co-treated with small-molecule endosome disruptors such as bafilomycin A1 or chloroquine. More critically, the tR1 peptide was found to inhibit the phosphorylation of endogenous Tau at Ser262 in rat primary cortical neurons displaying hyper-active MARK2. Finally, the effects of tR1 were found to be selective for inhibiting Tau phosphorylation at MARK2-dependent sites, as tR1 did not interfere with the activity of other kinases, such as GSK-3B, that phosphorylate Tau at Thr231. Importantly, these results have established tR1 as a novel peptide-based kinase inhibitor that has significant therapeutic potential and the promising capacity to be developed as a tool to dissect the complex nature of MARK/Tau biology.

#15 *Abuse Liability and Anti-Addiction Potential of the Atypical Mu Opioid Receptor Agonist IBNtxA.*
A. Islam, V. Verchio, C. Wu, G. Moura-Letts, B. Fischer, T. M. Keck. Rowan University, Glassboro, NJ.

OBJECTIVE: IBNtxA is a μ opioid receptor (MOR) agonist structurally related to the classical MOR antagonist naltrexone. Recent studies suggest IBNtxA preferentially signals through truncated MOR splice variants, producing a unique pharmacological profile resulting in potent analgesia with reduced side effects, including no conditioned place preference (CPP) when tested at a single dose. The purpose of this study is to 1) evaluate a range of IBNtxA doses to more fully assess its abuse liability and 2) determine the effects of IBNtxA on morphine CPP expression and reinstatement of morphine CPP.

METHODS: IBNtxA was synthesized and compared to morphine in standard CPP expression assays. Following morphine CPP training, IBNtxA was tested for its effects in inducing CPP reinstatement on its own or attenuating morphine-primed reinstatement. Drug discrimination studies are also underway.

RESULTS and CONCLUSIONS: IBNtxA represents an intriguing lead compound for preclinical drug development specifically targeting MOR splice variants, potentially creating effective analgesics with reduced side effects. Furthermore, IBNtxA could have use as an adjunct therapy in agonist replacement strategies (e.g., methadone). Current collaborative efforts are aimed at developing novel analogues of IBNtxA for further analysis and understanding ligand-receptor interactions across MOR splice variants using molecular modeling.

#16 *Myxopyronin-Inspired Phloroglucinol Derivatives, Novel Inhibitors of Bacterial RNA Polymerase Switch Region, Are Active Against Staphylococcal Biofilms.*
H. Kim, D. Degen, B. Li, J. Shen, S. Ho, R.H. Ebright. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ. Summer Undergraduate Research Fellowship, Rutgers University, Piscataway, NJ.

The CDC and NIH estimate that around 65-80% of all bacterial infections involve biofilms. Bacterial biofilms are sticky matrix-enclosed bacterial populations that form when bacteria colonize foreign material, such as human tissue and medical devices. Exhibiting decreased antibiotic susceptibility, biofilm infections often require surgical removal of the infected tissue or replacement of the device - procedures that add dramatically to the morbidity and mortality associated with these infections. Of particular concern is the ease with which mutations confer resistance to the rifamycin antibiotics, inhibitors of bacterial RNA polymerase that are first-line treatments for biofilm infections. Given that bacterial RNA polymerase may be a favorable drug target for clearing biofilms, this study seeks to investigate the in vitro activity of synthetic phloroglucinol derivatives, novel inhibitors of bacterial RNA polymerase that target the switch region with no overlap from the rifamycin target (and thus exhibit no cross-resistance), against clinically relevant biofilms of Staphylococcus epidermidis and Staphylococcus aureus. Susceptibility testing in a peg-lid biofilm reactor revealed that these compounds had anti-biofilm activity, whereas most of the comparator compounds, such as methicillin, linezolid, and gentamicin, were unable to eradicate the biofilm. Co-administration of rifampin and a phloroglucinol derivative did not result in significant enhancements of anti-biofilm activity. However, serial passaging with planktonic cells demonstrated that 1:1 combinations of rifampin and a phloroglucinol prevented resistance emergence for at least 40 days. This suggests that co-administration of rifampin and a phloroglucinol can also suppress biofilm resistance emergence. These compounds - either by themselves or in combination with rifampin - are promising candidates for the development of antimicrobial therapies for biofilm infections. Supported by ASPET, NIH ES020721, and NIH AI109713.

#17 *Identification of Anti-TB Therapy Induced ADRs Genetic Markers Using In-Silico Approaches.*
K. Kishor, R. K. Sahu. Open Source Drug Discovery Unit New Delhi, India.

Adverse drug reactions (ADRs) are associated with clinical morbidity and, in severe cases, even mortality. Globally billions of dollars are spent on managing these ADRs for common and uncommon diseases. The developing world suffers from a high burden of tuberculosis, which requires 6-8 months of multi-drug treatment. In spite of most cases being treatable the problem persists mainly due to a high attrition rate associated with ADR mediated complications. Due to these reasons drug resistant strains have emerged and are now a serious challenge to TB eradication. To effectively deliver the available treatment regimen and ensure patient compliance it is important to manage ADRs more efficiently. Recent studies have demonstrated that drug outcomes are patient-specific and can, therefore be predicted. A few of these drugs, including a few administered for TB, have shown excellent correlation with response rates and development of ADRs. In this review, we profile information available in public domain for existing anti-TB drugs to understand the genesis of ADRs and patient response. Additionally, human genome variation databases have been used to correlate the frequency of these markers and their genomic variants in different populations.

#18 *Shear-thinning Beta-hairpin Peptide Hydrogels As 3D Cell Culture Scaffolds For Automated High-throughput Screening.*

S.A. Langhans, P. Worthington, K.M. Drake, Z. Li, A.D. Napper, D.J. Pochan. Nemours/AI duPont Hospital for Children and University of Delaware.

Automated cell-based high-throughput screening (HTS) is a powerful tool in drug discovery, and it is increasingly being recognized that three-dimensional (3D) cell culture models, which more closely mimic in vivo-like conditions, are desirable screening platforms. One limitation hampering the development of 3D HTS is the lack of suitable 3D culture scaffolds that can readily be incorporated into existing HTS infrastructure. We now show that b-hairpin peptide hydrogels can serve as a 3D cell culture platform that is compatible with HTS. MAX8 b-hairpin peptides can physically assemble into a hydrogel with defined porosity, permeability and mechanical stability with encapsulated cells. Most importantly, the hydrogels can then be injected under shear-flow and immediately reheal into a hydrogel with the same properties exhibited prior to injection. The post-injection hydrogels are cell culture compatible at physiological conditions. Using standard HTS equipment and medulloblastoma pediatric brain tumor cells as a model system, we show that automatic distribution of cell-peptide mixtures into 384-well assay plates results in evenly dispensed, viable MAX8-cell constructs suitable for commercially available cell viability assays. Since MAX8 peptides can be functionalized to mimic the microenvironment of cells from a variety of origins, MAX8 peptide gels should have broad applicability for 3D HTS drug discovery. This work was supported by ACS RSG-09-021-01-CNE, the NIH IDeA program, with grants from the National Institute of General Medical Sciences NIGMS (P20-GM103464, P30-GM114736, and U54-GM104941), the DO Believe Foundation and the Nemours Foundation. This work utilized facilities supported in part by the National Science Foundation under Agreement No. DMR-0944772.

#19 *New Drug Leads for Ewing's Sarcoma from High-Throughput Library Screening.*

M. Leid, E. Esfandiari, M. Nazari, W.K. Vogel, M. Davare. Oregon State University and Oregon Health & Science University.

Ewing's Sarcoma (EwS) is the second most common type of primary bone cancer in children and young adults between ages 10 and 20. With early diagnosis, approximately 70% of children with EwS can be cured with high-dose chemotherapy. However, subgroups of patients have poor prognoses even with early diagnosis and aggressive follow-up; survival rates for children diagnosed with metastatic disease and adolescent patients (15-19 years) are around 30% and 50%, respectively. Further, a durable cure is more difficult to achieve in children with EwS tumors located in the pelvis, ribs or spine.

The causative event for 85% of EwS cases is a reciprocal t(11;22)(q24;q12) chromosomal translocation between genes encoding EWS (a RNA-binding protein) and FLI1 (an ETS family member), resulting in expression of a EWS/FLI1 chimeric transcription factor. Chromosomal translocations involving the EWS gene and other ETS genes contribute to EwS; these include EGR (10% of EwS cases) and ETV1, ETV5, and FEV, the latter three cumulatively account for the remaining 5% of cases. The oncogenic EWS/FLI1 fusion protein regulates expression of a large network of genes that underpins EwS pathogenesis. EwS cells exhibit "onco-addiction" to EWS/ETS fusion proteins, however, fusion proteins involving transcription factors are challenging pharmacologic targets. Despite knowledge of the molecular etiology of EwS for more than 30 years, a precision medicine-based treatment strategy for EwS has been elusive, although it is highly amenable for such an approach because the fusion protein is not expressed in normal cells. Current treatment protocols for EwS patients include a panel of nonspecific, cytotoxic chemotherapy agents. Despite success of these regiments in eliminating EwS and providing beneficial long-term

prognoses for some patients, survivors often have long-term treatment-related adverse sequelae. Development of targeted therapies for treatment of EwS is a top priority in the field.

We identified new drug leads for treatment of EwS by conducting a high-throughput screen of a highly diverse chemical library composed of over 246,000 compounds. We have characterized one of these leads from this screen, ML111, in detail and this drug appears to be a very promising agent for treatment of EwS. We have also synthesized many more compounds that exhibit even greater potency and more drug-like physicochemical properties than ML111. The ML111 family of compounds inhibit STAT protein phosphorylation, induce a G2/M cell cycle arrest, destabilize the EWS/FLI1 fusion protein, and launch a rapid program of apoptotic EwS cell death with nanomolar potency, while exerting minimal toxicity in non-transformed cell types.

The ML111 family of compounds has the potential to transform treatment protocols for EwS patients, particularly when combined with other targeted therapies, and may eliminate or reduce the need for use of non-specific chemotherapy agents.

#20 *Structurally Diverse Positive Allosteric Modulators Of The D1 Dopamine Receptor Potentiate G-Protein And β-Arrestin-Mediated Signaling.*

K. D. Luderman, J. L. Conroy, R.B. Free, N. T. Southall, M. Ferrer, J. Aubé, K. J. Frankowski, and D. R. Sibley. NIH and NINDS.

The D1 dopamine receptor (D1R) is linked to a variety of neuropsychiatric disorders and represents an attractive drug target for the enhancement of cognition. Agents that enhance D1R signaling in the prefrontal cortex may be useful in the treatment of cognitive decline in schizophrenia, Alzheimer's disease and other disorders. Unfortunately, orthosteric D1R agonists have proved problematic as they frequently induce hypotension and exhibit a narrow therapeutic window. An alternative approach is to potentiate D1R activity using allosteric modulation. Hypothetically, a positive allosteric modulator of the D1R may exhibit highly selective actions and exhibit a larger therapeutic window. To identify allosteric ligands of the D1R, we implemented a high throughput screen of the NIH Molecular Libraries Program 400,000+ small molecule library and identified two structurally diverse lead compounds, MLS1082 and MLS6585. These compounds were selected and characterized using endogenous G-protein (cAMP stimulation) and β-arrestin (recruitment) signaling pathways. MLS1082 and MLS6585 potentiate dopamine-stimulated G-protein and β-arrestin-mediated D1R signaling, increasing the potency of dopamine by 3-5 fold for stimulating cAMP accumulation and 6-8 fold for β-arrestin recruitment. Both compounds also increased the maximum dopamine-stimulated responses by 20-30%. Neither compound displayed any intrinsic agonist activity in either assay. Further, the two compounds potentiated the binding affinity of dopamine for the D1R by 3-6 fold and displayed minimal to no ability to inhibit radioligand binding to the D1R orthosteric-binding site on their own. Both MLS1082 and MLS6585 potentiated the agonist activity of other full and partial D1R agonists in a dose-dependent manner with EC50 values in the micromolar range. Importantly, they showed no ability to potentiate forskolin-stimulated cAMP accumulation, or β-arrestin recruitment by the D2-like dopamine receptor subtypes. Experiments using maximally effective concentrations of MLS1082 and MLS6585 in combination were used to determine if the compounds were acting at separate or similar binding locations. The combination of MLS1082 + MLS6585 caused an additional potentiation of dopamine's potency for stimulating β-arrestin recruitment (11-20 fold) and cAMP accumulation (4-6 fold), compared to MLS1082 and MLS6585 alone, suggesting the two compounds are acting at separate sites on the receptor. Repeating these combination experiments with Compound B, a known D1R PAM, showed

additive activity with only MLS6585 and not MLS1082, further suggesting that there are two separate PAM binding sites on the D1R. Thus, MLS1082 and MLS6585 may serve as important scaffolds for the future development of optimized D1R PAMs for in vivo use and the discovery of therapeutic lead compounds.

#21 *Chk1 Inhibitor LY2606368 and Histone Deacetylase Inhibitor Vorinostat as a Combination Therapy for Solid Tumor Cancer.*

N. McArthur, S.A. Flanagan, D.S. Shewach. Department of Chemical and Biomolecular Engineering, North Carolina State University and Department of Pharmacology, University of Michigan Medical School.

Cancer develops as the result of the accumulation of mutations in genes that regulate cell growth and differentiation allowing for uncontrolled cell replication. Pancreatic cancer is one of the most difficult cancers to treat, and despite aggressive therapies, the five-year survival rate is less than 9%. Standard treatment includes DNA damaging drugs and mitotic inhibitors, but there is a great need for more effective therapies. New approaches include histone deacetylase inhibitors (HDACis), such as vorinostat, which can alter the expression of many genes resulting in diverse effects including cell cycle dysregulation, upregulation of apoptosis, and increased replicative stress in the form of DNA damage, stalled replication forks, and the activation of dormant replication origins. We hypothesized that vorinostat could interact well with other compounds that cause replication stress through different mechanisms. We tested this hypothesis by combining vorinostat with a drug that directly interferes with DNA production and is used in pancreatic cancer treatment, gemcitabine, or with a checkpoint kinase 1 (Chk1) inhibitor, LY2606368, that can also cause replication stress. Chk1 is a serine/threonine-specific protein kinase that is activated primarily by ATR, a protein that recognizes single-stranded DNA and initiates the DNA damage response pathway, in response to replication stress. Activation of Chk1 results in the initiation of the S and G2/M cell cycle checkpoints and cell cycle arrest, DNA damage response, and cell death. The inhibition of Chk1 causes the deregulation of DNA replication and the loss of a cell's ability to pause the cell cycle and repair damaged DNA. LY2606368 is advantageous because it is an ATP-competitive kinase inhibitor that prevents Chk1 autophosphorylation at serine 296, a site at which phosphorylation is required for specific downstream Chk1 functions. Other Chk1 inhibitors lack specificity and act on the Chk1 ATP-binding site leading to off-target effects. The effects of these drugs alone or in combination on cell survival was measured using a colony formation assay. Drug interactions were evaluated by isobologram analysis. Vorinostat was combined with gemcitabine or LY2606368 over a broad concentration range in the human pancreatic cancer cell lines Panc1 and BxPC3. BxPC3 cells were more sensitive to vorinostat, gemcitabine, and LY2606368 (IC50 = 8 ± 2.8 µM, IC50 = 61 ± 12 nM, and IC50 = 3 nM, respectively) than Panc1 cells (IC50 = 18 ± 5.3 µM, IC50 = 244 ± 69.1 nM, and IC50 not yet determined, respectively). Results demonstrated that vorinostat and gemcitabine interacted synergistically in these cell lines but that synergy was highly dependent on schedule and the ability of the cells to accumulate in S-phase. Ongoing studies are aimed at combining the Chk1 inhibitor with vorinostat at concentrations and in an order that will promote S-phase accumulation during drug exposure. Research reported in this publication was supported, in part, by the National Institutes of Health's National Center for Advancing Translational Sciences, Grant Number UL1TR000433. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

#22 *Identification and Optimization of a Potent and Highly Selective D3 Dopamine Receptor Agonist.*

A. E. Moritz, R. B. Free, W.Weiner, E. Akano, M. Bachani, T. Doyle, N. Southall, M. Ferrer, J. A. Javitch, J. Steiner, J. Aubé, K. Frankowski, D. R. Sibley. Molecular Neuropharmacology Section, NINDS, Neurotherapeutic Development Unit, NINDS, NIH, Bethesda, MD, University of North Carolina, Chapel Hill, NC, NCATS, NIH, Rockville, MD, Columbia University, New York, NY.

Dopamine receptors (DARs) regulate diverse physiological functions including cognition, movement, and reward-related behaviors, and are involved in the development and/or treatment of neuropsychiatric disorders including schizophrenia and Parkinson's disease (PD). They are composed of two classes based on structure and pharmacology: D1-like (D1R and D5R) and D2-like (D2R, D3R, and D4R). The D2R and D3R display high sequence homology within their orthosteric binding sites where the endogenous agonist dopamine binds, making discovery of truly subtype selective compounds difficult. In addition, known D2R-like ligands can also exhibit high degrees of cross reactivity with closely related GPCRs, leading to the potential for side effects and uncertainty as to the roles each DAR subtype plays in normal and pathological processes. Thus, a highly selective D3R agonist that lacks efficacy at other DARs may have great utility for both therapeutic and basic science pursuits. As such, our lab sought to discover selective D3R agonists by employing high throughput screening paradigms using the NIH Molecular Libraries Program 400,000+ small molecule library and a D3R enzyme complementation-based β-arrestin recruitment assay. Hits were counter screened against the D2R to allow elucidation of compounds that activate the D3R without effects on the D2R. Orthogonal confirmation and counter-screens were also performed to obtain an initial assessment of selectivity and mechanisms of action. We identified 62 potentially selective hit compounds. The most promising compound was chosen for a full optimization study and investigation of its structure-activity relationships. 375 analogs were synthesized and screened in the β-arrestin assay in an effort to increase both affinity and selectivity. The lead compound identified through this process, ML417, acts as a full agonist at the D3R with a potency of 36 nM, while having minimal effects on D2R-mediated β-arrestin translocation. ML417 also acts as a potent and selective D3R agonist in a separate BRET-based assay of β-arrestin recruitment. Importantly, the compound also exhibits potent and selective agonist activity in D3R-G protein-mediated signaling responses as demonstrated using Go-BRET-based assays and pERK assays. ML417 was further assessed for receptor cross-reactivity using panels of other GPCRs, and was found to have limited liability for either functional interactions with, or displacement of a radioligand from numerous GPCRs. As D3R-preferring orthosteric agonists show promise as neuroprotective and neurorestorative agents, we conducted preliminary studies using ML417 in a neuroprotective assay, and found that it displays neuroprotective properties. This highly selective and potent D3R agonist will prove useful as a research tool to identify biological processes governed by D3R activation, and may show utility as a therapeutic drug lead.

#23 *Discovery of Novel Chemotheraputic Agents for Hospital Acquired Infections.*

O.A. Olaleye. H. Xie. Texas Southern University, Houston, Texas.

Enterococcus faecalis (E. faecalis) is a major cause of hospital acquired infections (HAI). The emergence of drug resistant HAIs have reduced the efficacy of available chemotherapeutic options. Therefore, novel drug targets are imperative to identify new chemical entities for treatment of HAIs, caused by E. faecalis. Our study focuses on Methionine Aminopeptidase (MetAP) a new drug target for treatment of E.

faecalis related infections. MetAP is a metalloprotease that removes the initiating methionine after protein translation. The essential role of MetAP in post-translational biology, makes it a promising antibiotic target. Our hypothesis is that inhibition of MetAP in E. faecalis will treat and/limit the progression of HAIs caused by E. faecalis. Previously, using a target based approach we screened over 175,000 small molecule inhibitors against E. faecalis MetAP (unpublished data). We discovered several structurally diverse inhibitors with potent activity against MetAP. To evaluate the therapeutic potential of MetAP inhibitors, we determined the minimum inhibitory activity of some of the inhibitors against E. faecalis culture. To further evaluate the activity of EfMetAP inhibitors in vivo, we developed a novel formulations of the novel lead compound suitable for pharmacokinetic and efficacy studies. We found that the lead compound and its derivatives have potent activity against E. faecalis MetAP with MICs in the low micro molar range. In addition we have developed formulations for the lead compound suitable for intravenous, subcutaneous and oral administrations. The clinical significance of this study is that the therapeutic targeting of EfMetAP in HAIs - E. faecalis related infections could accelerate the development of new agents to treat HAIs.

#24 *Developing Shorter Acting Cannabinoids as Potential Therapeutics.*
C.A. Paronis, S.P. Nikas, R. Sharma, S. Kulkarni, A. Makriyannis, J. Bergman. McLean Hospital / Harvard Medical School and Northeastern University, Boston, MA.

Cannabinoids such as nabilone and dronabinol are FDA-approved to treat nausea and weight-loss however their unpredictable onset and long duration of action sometimes limits compliance. We have previously demonstrated that the introduction of a labile ester in the side-chain of experimental cannabinoid compounds results in more rapid deactivation of the compounds and, hence, a shorter duration of action of behavioral activity. Here we compare effects of nabilone and derivatives designed to have either shorter (AM7444) or longer (AM2389) durations of action in different in vivo assays of cannabinoid activity in rodents and nonhuman primates. Changes in colonic temperature were measured in groups (n=6) of either rats or mice for up to 6 hours following single injections of novel compounds. Antinociceptive effects were determined in mice (n=4-8) using a warm-water tail withdrawal procedure with water held at 52oC. Lastly, the abilities of the drugs to produce CB1-like subjective effects in animals were determined in a group of nonhuman primates (n=4) trained to discriminate the CB1 agonist AM4054 from saline. Results show that all drugs produced hypothermia, with the highest doses decreasing body temperature by ≥ 4oC. All drugs also had antinociceptive effects, ranging from 70-100% of the maximum possible effect (8 sec). Likewise, all drugs dose-dependently substituted for the 0.01 mg/kg AM4054 training stimulus in the drug discrimination assay. However, the drugs did differ in terms of potency and duration of action. Incorporation of a cyclobutyl group in the side chain of nabilone (AM2389) yielded a compound that was both more potent and longer lasting than nabilone. In contrast, carboxylation of the side chain (AM7444) yielded a compound that was equipotent with nabilone, but with a slightly more rapid onset and offset of behavioral effects. In summary, these data show that the in vivo duration of action of cannabinoids can be predictably modified by alterations to the parent molecule designed to increase their metabolism, and decrease the accumulation of active metabolites. These molecules are presumed to retain their therapeutic value while limiting some of their abuse-related effects. (Supported by DA035411)

#25 *Bioengineering of Single RNA Molecule to Target miR-21a and Introduce miR-34a in NSCLC Cells.*
H. Petrek, P.Y. Ho, Z. Duan, N. Batra, J. L. Jilek, A.Yu. Department of Biochemistry & Molecular Medicine, Comprehensive Cancer Center, UC Davis School of Medicine, Sacramento, CA.

Lung cancer is the second most common cancer among both men and women in the United States. The majority of lung cancer cases are classified as non-small cell lung cancer (NSCLC). Current NSCLC treatments include resection surgery, radiation, and pharmacotherapy which all provide some benefits and have limitations. As a result, lung cancer caused deaths account for about 27% of all cancer deaths in the US. Therefore, new therapeutic agents are highly demanded for NSCLC. MicroRNAs (miRNA or miR) are a class of genome-derived, small noncoding RNAs in cells that govern target gene expression through mRNA degradation or translational inhibition. MiR-34a is commonly downregulated in NSCLC and targets various oncogenes such as c-MYC, c-MET, BCL-2 and SIRT. On the other hand, miR-21a is upregulated in NSCLC and reduces the expression of many tumor suppressors such as PTEN and PDCD4. Aiming at targeting oncogene miR-21a and introducing tumor suppressor miR-34a simultaneously in NSCLC cells, we employed our recently developed RNA bioengineering technology to produce a single RNA molecule containing both miR-34a and a siRNA against miR-21a (anti-miR21a). Our data showed this anti-miR21a/miR-34a agent was highly expressed in E. coli cells. After purification by FPLC method, anti-miR21a/miR-34a agent was transfected into human NSCLC cells. We further defined the levels of cellular anti-miR21a, miR-21a and miR-34a levels, the effects on miR-21a and miR-34a target gene expression, and the impact on NSCLC cell proliferation. Using a single RNA agent for dual targeting represents a new strategy for the development of cancer therapeutics.

#26 *Identification Of Residues In The Fifth Transmembrane-Spanning Domain Of The D2 Dopamine Receptor That Regulate Signaling Bias.*
M. Sanchez Soto, E. C. Gonye, A. M. Moore1, L.S. Chun, A. E. Moritz, H. Yano, L. Shi, R. B. Free, and D. R. Sibley. NINDS/NIH.

The D2 dopamine receptor (D2R) signals through a variety of second messenger pathways making it challenging to discern which are linked to specific effects of D2R-targeted drugs; however, this complexity provides a unique opportunity to develop pathway-selective therapeutics. Our laboratory previously described MLS1547 as a functionally selective D2R ligand that robustly activates G-protein signaling with minimal recruitment of ß-arrestin. Structure-activity analyses of a series of analogs with varying bias, coupled with molecular dynamics, led to a molecular model for biased signaling including a hydrophobic binding pocket formed by residues I184, F189, and V190, all located within the fifth transmembrane region (TM5) of the D2R. In the current study, we used mutagenesis techniques to test this model and investigate the role of TM5 in regulating D2R signaling bias. We constructed single point mutations (I184A, V190A, F189A, F189L, and F189Y) in the D2R and studied their G protein-mediated signaling and ß-arrestin recruitment using BRET and enzyme complementation-based technologies. While there was a minimal change in potency, mutation of either I184 or V190 showed no effect on dopamine's efficacy for ß-arrestin recruitment or activation of G-protein-mediated signaling. Interestingly, when we tested the F189A mutant, the ability of dopamine and other D2R agonists to recruit ß-arrestin was undetectable, while the G protein-signaling efficacy was maintained. Dopamine-stimulated ß-arrestin recruitment was similarly undetectable in the F189L mutant, but maintained in the more conserved F189Y mutant receptor. G-protein activation efficacy was unaffected by the F189L or F189Y mutations. These data demonstrate that the F189A and the F189L D2R mutants are biased towards G protein-mediated signaling and suggest that the D2R F189 residue is important for stabilizing an activated state for recruiting ß-arrestin. We extended our study to other D2-like receptors and found that mutation of an analogous TM5 residue in the D3R (F188) or the D4R (Y192), resulted in similar findings (i.e., loss of agonist-stimulated ß-arrestin recruitment, but no effect on G protein-mediated

signaling efficacy). Taken together, these results suggest that conformational changes in TM5 can act as a molecular switch for receptor signaling via ß-arrestin recruitment, which may have implications for the design of novel biased compounds for the treatment of D2R-related disorders.

#27 *Identification of Novel Lead Compounds for the Inhibition of the RNA Binding Protein HuR.*
M. Tranter, S.R. Anthony, G.Yoshida, W.L. Seibel. University of Cincinnati College of Medicine and Cincinnati Children's Hospital Medical Center.

The RNA binding protein HuR has been implicated in numerous human diseases including cancer, neurological disorders, and cardiovascular disease. Our laboratory has recently identified HuR as a suitable therapeutic target for the reduction of hypertension-induced heart failure. In vitro mechanistic work has demonstrated that one of the ways in which HuR reduces pathological hypertrophy of primary cardiac myocytes is by preventing activation of the transcription factor NFAT.

The goal of this work was to identify novel lead compounds for the inhibition of HuR. To do this, we first applied a predictive molecular docking approach to screen the University of Cincinnati compound library (acquired from P&G Pharmaceuticals and containing over 250,000 unique compounds) for likely binding fits into the HuR RNA binding pocket based on the structure of a known experimental inhibitor. The top 50 hits from this approach were screened (at a concentration of 20 uM) for their ability to inhibit HuR-mediated NFAT transcriptional activation in primary rat cardiac myocytes. Our results show that 7 of the 50 selected compounds demonstrated at least a 50% inhibition of NFAT activation, and 2 of the 50 compounds yielded a more efficacious inhibition than the known experimental inhibitor.

Future work will confirm specific binding within the HuR RNA binding pocket, potency and efficacy, and a physiological reduction of myocyte hypertrophy for each of these positive hits. In summary, our results have identified novel lead compounds that may have therapeutic value for the inhibition of HuR.

#28 *Immune Modulation By Mixed Aryl Phosphonate Butyrophilin Prodrugs.*
A. J. Wiemer, B. J. Foust, M. M. Poe, N. A. Lentini, C. H. Hsiao, D. F. Wiemer. University of Iowa and University of Connecticut.

The B7 family protein butyrophilin 3A1 (BTN3A1) is an immune co-receptor that binds intracellular pathogen-derived molecules such as (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP). Receptor binding to these ligands promotes a multifaceted immune response including activation of Vγ9Vδ2 T cells. To understand further the underlying structure activity relationships, we have characterized a number of novel butyrophilin ligands and their prodrugs. Studies of aryl phosphonate derivatives of a butyrophilin 3A1 ligand have resulted in identification of a potent stimulant of Vγ9Vδ2 T cells. This compound, a mixed ester bearing one pivaloyloxymethyl substituent and one 1-naphthyl ester displayed sub nanomolar activity as a stimulant of T cell proliferation and nanomolar activity in an assay designed to measure interferon gamma production. This is the most potent butyrophilin ligand prodrug yet reported, and thus it should be a valuable tool for manipulation of T cell function. Furthermore, mixed aryl/acyloxyalkyl esters may represent a new class of phosphonate prodrugs with high efficacy.

#29 *Behavioral Pharmacology of Muscarinic and Nicotinic Receptors.*
J.H. Woods, G.D. Winger, A. Coop, M. Ansari, C. Johnson, E. Jutkiewicz, B. Kangas, J. Bergman. Univ of Texas, San Antonio; Univ of Maryland, Baltimore; McLean Hospital/Harvard Med Schl, Belmont.

The projects encompass research that is a combined effort of three laboratories. All of the projects evolve from muscarinic and/or nicotinic cholinergic concepts. One project attempts to discover potential antidepressants based on compounds described earlier by Merck (Freedman etal. Europ. J. Pharmacol. 215:135-136, 1992). Other projects are related to nicotinic compounds described earlier by Targacept-based research (Gatto etal., CNS Drug Rev. 10: 147-166, 2004) which might repurposed for smoking cessation.

#30 *Discovery of Novel Phosphodiesterase 2 Inhibitors For Treatment of Depression And Anxiety.*
Y. Xu, Y. Xiaokaiti, L. Liu, X. Zhu, H.T. Zhang, J.M. O'Donnell. The State University of New York at Buffalo.

Major depression affects nearly 18-20% of people during their lifetimes in the world. It will become the second leading cause of social disability around the world by the year of 2020. Although increasing new antidepressant drugs have been applying in clinic since 1980's, the efficacy of currently available antidepressants is limited. Only a third of the patients achieve remission at the first mediation, remaining at least one third of other patients never responds to antidepressants. The low remission rates of symptom amelioration for current antidepressants highlight the necessity of developing novel antidepressants with improved efficacy and better therapeutic profiles. Regulation of cyclic AMP and cGMP signaling is catching our attention for treatment of major depression and anxiety disorders. Phosphodiesterase 2 (PDE2) is a dual substrate PDE, which hydrolyzes both cAMP and cGMP. PDE2 inhibitors including Bay 60-7550 enhance cyclic nucleotide signaling and upregulation of downstream molecules that protect neurons against various stimuli induced cell lesion. However, the poor brain penetration of Bay 60-7550 limits its therapeutic utility. The aim of this study is to develop lead compounds targeting PDE2 as therapeutics for treatment of major depression and evaluate antidepressant- and anxiolytic-like effects of novel PDE2 inhibitors in vitro and in vivo. We performed structure-based design of a new type of PDE2 inhibitors, such as Hcyb1 and XOGA, on the basis of known structures of other PDE families, and optimized the design by computational docking. The in vitro results suggested that the cyclic AMP and GMP levels were increased after treatment with both Hcyb1 and XOGA in HT-22 cells. Further studies suggested that the elevated phosphorylation of VASP and CREB was significant after treatment with these two PDE2 inhibitors in HT-22 cells. The in vivo study suggested that Hcyb1 at doses of 0.5, 1 and 3 mg/kg (p.o.), and XOGA at doses of 1, 2 and 5 mg/kg (p.o.) significantly decreased the immobility time in tail suspension and forced swimming tests, in dose-dependent manner. Both of them at same doses exhibited anxiolytic-like effects, as evidenced by increased head dips, percentage of open arm entries and percentage of time spent in open arms in the hole board and elevated plus maze tests. These results suggested that these two lead PDE2 inhibitors produced antidepressant- and anxiolytic-like effects, which may be related to enhancing cAMP/ cGMP-CREB signaling in the brain.

#31 *Rutgers University Molecular Imaging Center (RUMIC).*

E. J. Yurkow, S. Wang, D. Adler. Rutgers University, Piscataway New Jersey.

The Rutgers University Molecular Imaging Center (RUMIC) is a multiuser preclinical, small animal, imaging resource utilized by researchers at Rutgers University and the greater academic community as well as by the local biotech and pharmaceutical industries. Located on the Livingston Campus in Piscataway NJ, the RUMIC provides a non-invasive approach to study various biological and disease models in living systems, ex vivo tissues and physical objects. Our comprehensive imaging modalities for the basic sciences include: MRI, PET/CT, MicroCT, Optical/X-ray Imaging and High-Resolution Ultrasound technologies. The facility allows researchers to generate multiple, spatially-resolved anatomical, functional, and molecular-level readouts from a single study. Image reconstruction, quantitative image analysis, 3D display and 3D printing are also available. The Center provides animal holding facilities for serial imaging, anesthesia, surgery and veterinary care. In addition to consultation and experimental services, the Center offers periodic training and conducts research to improve existing imaging technologies. Structural and functional images of in vivo & ex vivo subjects as well as physical objects generated at the Center are highlighted here.

#32 *Marine Natural Products for Comorbid Pain and Depression.*

K.J. Tidgewell, B.J. Kolber. Graduate School of Pharmaceutical Sciences and Bayer School of Natural and Environmental Sciences, Duquesne University, Pittsburgh, PA.

Chronic pain and psychiatric illnesses (e.g. depression and anxiety) are each associated with a greatly reduced quality of life. The negative impact from these conditions is amplified when they are comorbidly expressed. A growing body of evidence demonstrates that there is a similar means of development and maintenance of these conditions with higher incidences of depression occurring in patients suffering from chronic pain than in the general population (18-56% vs 5-10%). A major gap exists in our understanding and application of the shared molecular mechanisms these diseases. Evidence suggests that targeting serotonin (5-HT) receptors may modulate pain and depression, but most studies have used established 5-HT targeting agents with antidepressive OR analgesic effects, but not with both antidepressant and analgesic activities.

The overall goal of our research is to understand the mechanisms of chronic pain and psychiatric comorbidity with the aim of discovering novel non-opioid therapeutics. Our project utilizes natural products to drive the pathophysiological investigation of the 5-HT system and its effects in pain and depression and our understanding of the comorbidity will influence our targets for natural products discovery. The identification of psychoactive compounds from terrestrial plants has been extensively studied but the microbial world may also be a valuable source of CNS-active compounds. Marine cyanobacteria produce a variety of secondary metabolites and we have identified several cyanobacterial fractions and compounds from Caribbean locations that show marked affinity for 5-HT receptors and alter pain-related affective behavior. By accessing the marine environments of Panama, Curaçao and other Caribbean locations, we will utilize the rich and underexplored biodiversity in these areas for the discovery of novel analgesic and anti-depressive 5-HT compounds from collected and cultured microbes. By focusing on analgesic and/or anti-depressant 5-HT receptor targets (e.g. 5-HT2C and 5-HT7) that have not been extensively studied in the context of comorbidity, we will develop more effective and targeted approaches for treatment. We test marine natural products and extracts, which have shown affinity for 5-HT receptors, in mouse models of anxiety, depression, and pain. In addition to screening new fractions, we also explore the underlying biology with known agents to test the hypothesis that pain and depression share common molecular mechanisms regulated by the 5-HT system. Our research will help

better understand the linkage between the serotonergic system and chronic pain as well as identify novel leads for non-opioid management of symptoms.

#33 *Utilizing DPPH Assay of Crude Berry Extracts in Order to Determine Strength of Antioxidants.*
L.A. Rufino. University of Massachusetts Dartmouth, Dartmouth.

Neurodegenerative diseases such as Alzheimer's, and Huntington's are relative to the progressive loss of neurons over a host's life span. Proteins within the host have a propensity to fold incorrectly over time, specifically, tau proteins. When Tau proteins are exposed to genetic mutation, posttranslational modification, or intracellular environmental changes, Tau proteins are vulnerable forming into Neurofibrillary tangles (NFT's) or paired helical filaments (PHF). Hyper phosphorylation occurs when the regulatory signaling mechanism in mitosis fails and causes multiple phosphorylated sites saturate. Thus, faltering in their ability to transport nutrients or vital materials to neighboring neurons. Without pathways to transport materials and nutrients to support these neurons, then perish neighboring neurons. Therefore, under consideration that compounds behave as strong antioxidants may be effective in preventing mild cognitive impairment. Trolox, an antioxidant standard, and demonstrated an EC50 value of 9.24 µg/mL. Extracts of polar desugared blueberry/cranberry samples exhibited EC50 values of 3 µg/mL for both the desugared blueberry/cranberry samples. Funded by the office of undergraduate research grant.

#34 *The Neuroscience Catalyst as an Innovative Model of Public-Private Partnership to Advance Drug Discovery in Mood Disorders and Alzheimer's Disease.*
N. Kabli, R. Yu, G. Seabrook, R.A. Ross. Centre for Collaborative Drug Research, University of Toronto, King's College Circle Room.

Mood disorders and Alzheimer's disease are chronic diseases that impact hundreds of millions of Canadians and people worldwide. By 2040, these debilitating brain diseases could surpass cancer as the second leading cause of death. Advancements in this area of unmet therapeutic need require deeper and innovative collaborative partnerships between academia and the pharmaceutical sector, two industries with complementary value propositions.

To this end, the Neuroscience Catalyst program was designed to accelerate the pace of developing and bringing new drugs to market and providing treatments for major neuropsychiatric disorders by leveraging the collective skills and resources of academia and industry. The Neuroscience Catalyst, an open innovation partnership between the University of Toronto through the Centre for Collaborative Drug Research, and Janssen Inc. ("Janssen") as facilitated by Johnson & Johnson Innovation, LLC ("JJI"), is to our knowledge, the first open innovation drug discovery collaboration between a University and a multinational pharmaceutical company in Ontario. The Neuroscience Catalyst funds pre-competitive, early-stage investigations that can progress to clinical treatments for mood disorders and Alzheimer's disease. Research projects are subject to due diligence expert review by a joint committee of scientists, clinicians, and research innovation and industry professionals from the University, Academic Health Sciences Network, commercialization professionals, Janssen, and JJI.   To ensure project progression, research excellence and industry rigor, funded researchers are supported by an industrial and academic mentor. Project and alliance managers ensure timely resolution of program-related issues, report to funders and drive program objectives.   The Neuroscience Catalyst has generated interest from academic researchers keen to advance their basic science discoveries into the clinic. In the first call, a

total of 94 basic scientists and clinicians joined forces to collaborate on research proposals from 24 cross-disciplinary, cross-institutional and international research teams from the University and its affiliated research hospitals. To date, the program has funded 7 milestone-driven and commercialization-oriented collaborative projects across the University and academic health sciences network. One project has spun off an investigator-led company that was accepted into JLABS @ Toronto. Early interim research progress reports show that the funding supported researchers in creating 8 new jobs, retaining 2 jobs and enabling 2 process improvements in their laboratories. In conclusion, the Neuroscience Catalyst represents a nimble, milestone-driven approach to the translation and commercialization of early stage innovation and is a scalable and efficient public-private model not only for advancing academic drug discovery but also for building capacity and stimulating the local entrepreneurial ecosystem.

#35 *A Sensitive Microflow LC/MS/MS Method for the Analysis of Fluticasone Propionate in Human Plasma.*
R. Burgoyne, A. Doneanu, M. Donnegan, J. Murphy. Waters Corporation, Milford, MA.

Fluticasone propionate is a glucocorticoid indicated for the prophylactic treatment of asthma. It is administered by inhalation from an aerosol-type device or powder inhaler. Due to its low administered dose and the corresponding low circulatory levels, a difficult bioanalytical challenge is presented to correctly define the pharmacokinetics in plasma (<10 pg/mL).

To obtain the required sensitivity for this assay, microflow LC/MS was utilized. Using this technique we were able to demonstrate a lower limit of quantification (LLOQ) in human plasma of 0.25 pg/mL for fluticasone propionate. This enhanced level of sensitivity allows for the accurate determination of the pharmacokinetics of the fluticasone in plasma.

#36 *Trifunctional Building Blocks for Streamlined Chemical Probe Synthesis.*
J. Fetter, K. Wilke, B. Glasspoole. MilliporeSigma.

To enable development of probes for target identification and other biological research applications, we have developed a series of trifunctional probe building blocks. The scaffolds possess three important components: a connectivity group to link to a pharmacophore or ligand; a reactive group for covalent labeling; and an alkyne handle for bioorthogonal tagging. Not only do the building blocks reduce the number of synthetic steps to incorporate multiple functional groups, the use of a series with the same connectively group streamlines the generation of probe libraries. For the chemists developing probes for target ID/validation, protein profiling, imaging, or other uses, this growing collection of building blocks facilitates the synthesis of many analogs to enable discovery of the optimal probe for your biological needs.

#37 *NIMH IRP Translational Neuropsychopharmacology Initiative.*
J.A. Clark , S.G. Amara. National Institute of Mental Health Intramural Research Program, Bethesda, MD.

Background: The need for novel therapies for central nervous system (CNS) disorders with improved efficacy, safety, and tolerability is unquestionably high, as it is widely recognized that, despite currently recognized treatments, CNS disorders are major contributors to the global burden of illness and incur

high economic costs. Unfortunately, over the past two decades, CNS drug discovery has been, with a few exceptions, relatively unsuccessful in delivering new chemical entities especially for the treatment of psychiatric disorders. The National Institute of Mental Health (NIMH) has become increasingly aware of the reduced investment by pharmaceutical companies in the development of therapeutics for treating psychiatric disorders despite the unmet medical need. In response to the reduced efforts in the pharmaceutical industry towards psychiatric drug discovery the NIMH Intramural Research Program (IRP) is proposing to re-invigorate psychiatric drug discovery by facilitating and de-risking the discovery and development of novel treatments. Support for the discovery and development of new treatments for psychiatric disorders including target validation, biomarker development, IND enabling studies, and Phase I safety / tolerability and Phase II proof of concept studies are all in scope for this important NIMH IRP initiative.

Methods: After completing a confidentiality agreement, interested parties including academic groups, small and large pharma or biotech may apply by submitting a proposal including detailed information on: the psychiatric indication, therapeutic rationale, target engagement, current stage of development, a development plan, project milestones, intellectual property and patent landscape, future objectives and anticipated outcomes. In addition, a compound `report card' with details on the chemical properties of the compound of interest is completed and submitted with each proposal.
Results: To steer this initiative the NIMH IRP has established the NIMH Translational Neuropsychopharmacology Task Force (TNTF). The TNTF is comprised of a panel of neuroscience drug discovery/development experts from the pharmaceutical industry and the NIH who prioritize, critically review, and recommend new proposals for support to the NIMH IRP Leadership. Supported proposals will either be sourced by NIMH IRP Principle Investigators and Staff or the desired work may be supported by outsourcing under the direction of the IRP.   The NIMH IRP has experience in the clinical testing of novel therapies for the treatment of psychiatric diseases including generalized anxiety disorder (GAD), treatment resistant depression (TRD), bipolar disorder (BPD), perimenopausal depression (PMD). In addition, the NIMH IRP has extensive CNS imaging capabilities including PET and fMRI that are readily integrated into clinical studies to answer questions regarding target engagement or better understand disease pathology.

Discussion: The NIMH is committed to engaging with the scientific community and reinvigorating the development of new therapies for the treatment of psychiatric disorders. The current reduction in psychiatric drug discovery and development in the pharmaceutical industry provides an opportunity for the NIMH IRP to contribute in an important way to de-risking novel therapeutics and engage industry in an effort to get more effective treatments to patients.

#38 *Technology Transfer and Industry Partnerships - Innovation. Collaboration. Commercialization.*
J. Conrad, T. Dick, J. Hewes, M. Newton, L. Prestia, R. Sons, M. Salgaller. National Cancer Institute Technology Transfer Center, Rockville, MD.

The National Institutes of Health (NIH) is the largest basic science research institute in the world, the epicenter for discoveries around the fundamental nature of living things. Our research protects and improves health. We develop drugs. We fund research around the world. With the largest number of hospital beds in the country, patients treated at the Clinical Center receive cutting-edge care at no cost.

All this is true. But, if this is all you know about NIH, then you don't know NIH.

NIH's more than 100 technology transfer professionals help our thousands of researchers - from basic to translational (and yes) to clinical - file patents on their discoveries so that the Bayh-Dole Act of 1980 can help move them towards the patient and - concurrently - towards the marketplace. It is well known that Bayh-Dole allows a business to obtain patent rights from federal government-funded research. It is not as well known that NIH is part of this revolution.

The NCI Technology Transfer Center (TTC) within NIH is committed to facilitating collaborative partnerships and supporting industry and academic research from bench to bedside to market. NCI TTC represents 10 of the 27 NIH Institutes/Centers. Long thought of as the go-to place for academics to work with a thought-leader and publish in a top-tier journal, NCI TTC has established itself as open for business - as one of the go-to places for the healthcare industry to work with a thought-leader, overcome a technology or knowledge gap, and get their products to market. Yes, we develop drugs. But we also develop devices, diagnostics, research tools, wearable and digital health solutions, and software.

We are the largest provider of in-kind support in the world. Research tools - mice, cell lines, antibodies - are provided at little or no cost. Our largest clinical center in the world? We work with companies to run dozens of clinical validation studies where each side pays its way. When it comes to being competitive, there are thousands of technologies available for licensing that require little or no up-front equity. Our royalty rates are highly competitive. An array of cooperative agreements is successfully executed at a rate of more than ten a day.

Look again at our mission statement. It may be surprising that it includes accelerating and promoting economic development. That means working with companies to positively impact their chances as an equity investment, an M&A target, an active employer. We might not be the first place a company thinks of to bolster their pipeline or solve that development problem - but perhaps we should be.

#39 *Animal Studies with Desformylflustrabromine, a Naturally Occurring Nicotinic Acetylcholine Receptor Positive Allosteric Modulator.*
A. Jackson, F. Deba, D. Bagdas, S.L. Peterson, D.E. Potter, M.I. Damaj, A.K. Hamouda. Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA,Department of Pharmaceutical Sciences, College of Pharmacy, Texas A&M HSC, Kingsville, TX.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels. The α4β2 subtype of nAChRs plays an important role in the mediation of several nicotine-evoked effects such as dependence and antinociception. Agonists and partial agonists of α4β2 nAChRs show efficacy in animal models of dependence and pain. There is a growing body of evidence pointing to allosteric modulation of nAChRs as an alternative strategy to direct agonism. Desformylflustrabromine (dFBr) is a positive allosteric modulator (PAM) at α4β2 nAChRs that enhances agonist responses without activating receptors. In animal studies, dFBr was well tolerated and reduced intravenous nicotine self-administration in rats. dFBr isolated originally from the marine bryozoan Flustra foliacea then its chemical synthesis and interaction with nAChRs in vitro were illustrated. In two-electrode voltage clamp recording from Xenopus oocyte, dFBr potentiated ACh-induced currents of low-sensitivity (α4)3(β2)2 and high-sensitivity (α4)2(β2)3 nAChRs maximally by ~300 and ~400% and with IC50s of ~0.4 and ~2 μM, respectively. We investigated the antinociceptive and anti-allodynic effects of dFBr in rat model of acute pain and in the mouse chronic constriction injury (CCI)-induced neuropathic pain model, respectively. dFBr dose-dependently increased time for hind paw-licking behavior in hot-plate test (~300% at 20 mg/kg). s.c. administration of dFBr failed to reduce pain behavior on its own. dFBr in combination with

nicotine, but not alone or in combination with morphine, significantly reversed neuropathic pain behavior dose- and time-dependently without motor impairment. This effect was blocked by the α4β2 antagonist dihydro-β-erythroidine. We also assessed the effect of dFBr on nicotine withdrawal symptoms in mice. dFBr produced dose-dependent reversal of nicotine withdrawal signs in mouse model of spontaneous nicotine withdrawal. The present results suggest that allosteric modulation of α4β2* nAChR may provide new strategies in chronic neuropathic pain and support the potential clinical use of this novel class of nAChR-based therapeutics as smoking cessation aid.

#40 *Dietary Restriction Of Fat Increases Efficacy Of Tumor-Specific Irreversible Inhibitors Of Stearoyl CoA Desaturase.*
N.S. Williams, S. Voruganti, S.S. Gonzales, E. Capota, P. Theodoropoulos, L.K. Morlock, H. Chen, P.L. Mallipeddi, J.D. Minna, B.A. Posner, J.M. Ready, D. Nijhawan. UT Southwestern Medical School.

The increased energetic and biosynthetic demands of cancer cells relative to their normal counterparts results in altered metabolism in these cells. While this metabolic reprogramming supports cancer cell survival and proliferation, it also leaves them highly susceptible to perturbations of their metabolic pathways. In particular, cancer cells are highly dependent on fatty acid biosynthesis, and several enzymes involved in these pathways are predicted to be targets for effective cancer therapeutics. We recently published data identifying two chemical series, benzothiazoles and oxalamides, which were selectively toxic to the same four of 12 non-small cell lung cancer (NSCLC) lines in a high throughput screen (Theodoropoulos, et al, 2016, Nat. Chem. Biol., 12:218). Using click chemistry and shotgun mass spectrometry, the compound series were shown to bind to Stearoyl CoA Desaturase (SCD). Tumor cell sensitivity to these inhibitors was predicted by expression of CYP-4F11, whose activity resulted in generation of O-demethylated metabolites, which irreversibly and selectively inhibited SCD. SCD has shown promise as a biological target in cancer and metabolic disease; however, known SCD inhibitors cause toxicity to skin sebocytes. Mouse sebocytes were unable to activate the benzothiazoles and oxalamides to their active form, suggesting a therapeutic window for inhibiting SCD may exist in vivo. In xenograft models with mice bearing CYP-4F11hi sensitive H2122 tumors or CYP-4F11lo resistant H1155 tumors, only the sensitive H2122 tumors showed modest growth inhibition in response to therapy with a bioavailable benzothiazole, and sebocyte function, although slightly reduced, was still intact. While these initial efficacy trials were encouraging, we hypothesized that tumors in vivo may scavenge fatty acids from dietary lipids provided by the bloodstream. We, therefore, placed NOD SCID mice on a no-fat diet prior to implantation of tumor and therapy with our prodrug inhibitor of SCD. Mice were given a control or non-fat diet and administered vehicle, or the benzothiazole prodrug SCD inhibitor either once or twice daily. While dietary restriction of fat had no effect on tumor growth itself, the combination of twice daily administration of the benzothiazole compound with a no-fat diet resulted in nearly complete inhibition of tumor growth. Tumor efficacy correlated with lowered plasma levels of triglycerides and non-esterified fatty acids. As expected sebocyte function was impaired but not eliminated after treatment with the prodrug SCD inhibitor. However, there was no effect of diet on sebocyte levels, and the combination of diet with an SCD inhibitor did not uniformly exacerbate toxicity. Finally, there was no overt liver toxicity evidenced. The results presented herein present a principle to pharmacologically target SCD in cancer, taking advantage of high CYP expression in a subset of NSCLC in combination with a dietary restriction of fat, and illustrate how knowledge of the target can inform rationale strategies to improve therapeutic effect.

#41 ***Accelerating Academic Drug Discovery with Computer-Aided Design.***
M. R. Landon, J. Vendome, J. Chambers, P. Kontrafouris, V. Feher, C. D'Cruz.

Here we present case studies of partnerships between academic drug discovery centers and Schrödinger, a leader in computer-aided design. These partnerships have accelerated drug discovery in a cost effective way and led to new company formation by providing modeling support, educational opportunities, and access to cutting edge software. Key to the success of these relationships is LiveDesignTM, Schrödinger's proprietary real-time collaboration platform. We will show how our partners have leveraged LiveDesign to expedite the design/synthesize/test cycle.

#42 ***Novel, Highly Biased Mu Opioid Receptor Compounds Minus Side Effects for the Treatment of Pain***
B.A. Tounge, S. Bayoumy, R. Besthof, S. Dax, L.C. Kuo. Mebias DiscoveryJ.E. Barrett. Drexel University College of Medicine.

Mu-opioids are the most effective analgesics, but their clinical effectiveness is severely hampered by side effects. Addressing these problems with two technologies – native GPCR purification and solution dynamic protein nuclear magnetic resonance – Mebias Discovery has identified a series of 'biased agonists' of the human mu-opioid receptor as pre-development candidates for the treatment of pain. MEB‑1166 and MEB-1170 are efficacious with drastically diminished on-target adverse effects of respiratory depression, constipation, and sedation.
MEB-1166 and MEB-1170 were profiled for efficacy in the rat tail flick assay. Both Trevena's Oliceridine (TRV130) and morphine were included. Compounds were administered sub‑cutaneous for all studies at their respective concentrations that equal to ED80 and 4x ED80 of morphine as measured in the tail flick study. At these dosages, we found that of MEB-1166 (4.5 and 18 mg/kg) and MEB-1170 (5 and 20 mg/kg) had longer duration of action than morphine (1 and 5 mg/kg) and TRV-130 (0.3 and 1.2 mg/kg). In accelerating rotarod assays, both Mebias compounds prompted no sedation at either low or high concentration, whereas morphine and Oliceridine induced significant sedation at their respective high concentration.

To determine in vivo translation of the Mebias compounds, we tested gastrointestinal motility and respiratory depression in SD rats. In measuring charcoal transit in the proximal small bowel, MEB-1166 was indistinguishable from vehicle, whereas MEB-1170 and Oliceridine showed negligible changes at the high concentration and morphine significantly impacted charcoal transit at 4X ED80. However, the Mebias compounds were clearly differentiating in the respiratory depression studies, validating the bias agonism determined with our platform. MEB‑1166 and MEB-1170 displayed no respiratory depression at their respective high and low concentrations as reflected by pO_2, pCO_2, pH, minute volume, tidal volume, and respiratory rate. At their high concentrations, both morphine and Oliceridine drove the pO_2 levels below 75 mmHg.

These compounds provide a unique opportunity to address existing challenges with opioid analgesics.