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Svetlana	Panina	RU	*Mitophagy-Inducing Compounds as Potential Agents for Leukemia Eradication*
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Aditya	Singh	UTMB	*Development of Allosteric Modulators of the Voltage-Gated Sodium Channel 1.1 to Rescue Perturbations of the Excitatory/Inhibitory Tone of the Nucleus Accumbens*
Aditya	Singh	UTMB	*Peptide Mimicking Crucial Residues of the β12 Sheet of Fibroblast Growth Factor 14 Modulates Accumbal Excitability via Interactions with the Voltage-Gated Na^+ Channel 1.6*
Joe	Tolar	RU	*Characterizing Novel Mitochondria-Targeting Small Molecule Drugs in Caenorhabditis elegans*

First Name	Last Name	Institution	Abstract Title
Paul	Wadsworth	UTMB	*Developing Pharmacological Probes for Interrogation of the Circuitry of the Nucleus Accumbens*
Yang	Wang	TSU	*A UHPLC-MS/MS Method for The Quantification of JIB-04 in Rat Plasma: Development, Validation and Application to Pharmacokinetics Study*

Population Pharmacokinetics of Vincristine and its Metabolite in Kenyan Pediatric Cancer Patients

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Background: Vincristine (VCR) dosing strategies are largely empirical regardless of its extensive use in pediatric oncology. Little information is known about its disposition and optimal therapeutic dosing. Renbarger and associates reported that CYP3A5 enzyme metabolizes VCR to M1 more efficiently than CYP3A4 enzyme. This may be clinically significant as CYP3A5 expression varies among Kenyans (90%), African Americans (AA, 70%) and Caucasians (10-20%).

Hypothesis/Goals: It is essential to characterize the disposition of M1 in humans to provide an insight of the inter-ethnic variability in VCR metabolism and clearance, which will be helpful for future rational dosing regimen optimization. Pharmacokinetic (PK) dispositions of VCR and its M1 metabolite were characterized through PK co-modeling for Kenyan pediatric cancer patients with acute lymphoblastic leukemia or nephroblastoma.

Methods: *Pharmacokinetic Study:* Dried blood spot (DBS) samples were collected, via finger stick at various time points depending on the feasibility, from 25 Kenyan pediatric cancer patients (13 males/12 females, 1-14 years, BSA of 0.36 - 1.3 m^2) after an IV dose of VCR (1.6 – 3.1 mg/m^2). Concentrations of VCR and M1 from DBS were simultaneously quantified using a validated LC-MS-MS assay. *Pharmacokinetic Modeling:* A population PK (pop PK) co-model was developed using Phoenix® NLME™ to simultaneously capture and predict the PK of VCR and M1 concentrations. A correlation between the observed and predicted concentrations of VCR and M1 PK was established by pop PK fitting. Model discrimination was performed by visual inspection, goodness of fit plots and AIC value.

Results: The best fit pop PK co-model for VCR and its M1 metabolite was established. PK parameter estimates were derived. Volumes of distribution for VCR and M1 were 0.12 L (33.55 %CV) and 249.07 L (33.42 %CV), respectively. The elimination rate constants for VCR and M1 were 5.6 hr^{-1} (22.81 %CV) and 0.00015 hr^{-1} (66.85 %CV), respectively. The conversion rate constant of VCR to its M1 metabolite was 9.66 hr^{-1} (32.49 %CV). Clearance values were 0.67 L/hr for VCR and 0.04 L/hr for M1.

Conclusions: VCR and its M1 metabolite exhibit distinct PK characteristics, in that M1 distribution is substantially larger than that of VCR (249 vs 0.12 L), and clearance is slower than that of VCR (0.04 vs 0.67 L/hr). The conversion kinetics of VCR to M1 is characterized for the first time, which may potentially offer a rationale for ethnic disparity in VCR therapy. In addition, a large interpatient variability is documented even within the same ethnic Kenyan population. After model refinement and validation, our model could potentially serve as a tool for rational VCR dosing regimen modification for Kenyan/AA pediatric cancer patients.

Acknowledgements: Funding from Institute for Drug Education and Research (IDER), University of Houston, College of Pharmacy; 2020 AACR Scholar-in-Training Award

PBPK Modelling of Matrine in Healthy Adults and Buccal Delivery Formulation for Prevention of Oral Cancer

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Background

Oral cavity carcinomas are the sixth most common cancer in the world, approximately 600,000 new cases are diagnosed every year with a mortality rate of 40-50%. Antitumor B (ATB, Zeng Sheng Ping, ACAPHA), is a natural herbal supplement composed of six plants. Recent clinical studies showed that ATB is active against oral cancer. Matrine (Matr) was listed as the quality control marker of Antitumor B (ATB). However, little information has been known about the pharmacokinetics of ATB in human.

Hypothesis/Goals

The aims of this study are (1) to develope a PBPK model and characterize the pharmacokinetics profile of Matr in healthy adults and (2) an alternative formulation is needed to enhance the delivery of ATB to the targeted oral tissues efficiently.

Method

An open-label PK study of ATB 2400 mg single dose was conducted on 8 healthy adults to investigate the safety and PK of ATB compounds in healthy adults. The study was approved by the IRB of University of Houston, Texas, USA. Each participant took 8 ATB tablets of 300 mg each with 250 mL water after overnight fasting. Plasma and saliva samples were collected pre-dosing, 0.5, 1, 2, 3, 4, 6, 8 and 24 hrs post dosing. The GastroPlus® 9.7 and PK-Sim 8.0 were used to build the PBPK model for Matr. The buccal delivery patch of ATB was prepared using solvent casting method.

Results

ATB was found to be safe after oral administration of a single dose 2400 mg on 8 healthy volunteers. Matrine was detected at quantifiable concentrations in human plasma and saliva. Significant higher concentration of Matr in the saliva sample suggested an active secrection of this compound to saliva. The PBPK model adequately described Cp-time profiles of Matr. The developed PBPK model adequately captured the observed Matr Cp time profile following oral administration under fasted state in healthy subjects. The formulation could release drug within 30 mins.

Conclusion

This study contributes to the pharmacokinetics of Antitumor B herbal supplement in healthy adults. The plasma concentration of matrine were adequately described using physiological and drug-specific parameters. The model successfully captured the dose and time-dependent PK of Matr in human plasma. Pharmacokinetics of Matr could be tracked with saliva sampling. More information of the transporter in the salivary gland compartment is needed for the model to be extended for quantitative prediction of the drug concentration in saliva. The buccal patch formulation is promising to locally deliver ATB to the target site.

Rationally Designing Peptidomimetics Derived from the β12 Sheet and β8-β9 Loop of Fibroblast Growth Factor 14 (FGF14) to Develop Allosteric Modulators of the Voltage-Gated Na^+ Channel 1.6

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Background: Disruption of protein:protein interactions (PPI) between voltage-gated Na^+ (Na_v) channels and their auxiliary proteins causes channelopathies. Despite restoration of these perturbed PPIs serving as a novel therapeutic approach, efforts to develop such PPI modulators are vexingly difficult for two main reasons. Firstly, PPI interfaces are structurally divergent from the surfaces that most conventional small molecules are designed to target on account of their expansive size and relatively featureless topography. Secondly, insights into where along a PPI interface to target with a small molecule are typically hindered by a lack of structural information regarding motifs of binding partners that confer functionally relevant modulation.
Hypothesis/Goals: Focusing on the PPI between $Na_v1.6$ and its auxiliary protein fibroblast growth factor 14 (FGF14), we previously identified that the PLEV and EYYV motifs of the β12 sheet and β8-β9 loop of FGF14, respectively, are predicted to disproportionately contribute to the Gibbs energy of FGF14:$Na_v1.6$ complex assembly. Based upon these structural insights, peptidomimetics derived from these motifs were designed, synthesized, and pharmacologically evaluated in an effort to develop allosteric modulators of $Na_v1.6$.
Methods: Combinatorial chemistry, split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: Peptidomimetics derived from the PLEV motif were first pursued. In total, 30 analogs of the tetrapeptide were designed and synthesized. Of these analogs, nine were shown using the LCA to inhibit FGF14:$Na_v1.6$ complex assembly at low micromolar concentrations. Of these nine analogs, PW0564 stood out for its ability to bind to both FGF14 and the C-terminal domain (CTD) of $Na_v1.6$ using SPR. Subsequent functional evaluation of PW0564 as a modulator of $Na_v1.6$ channels using whole-cell patch-clamp electrophysiology in heterologous cells revealed that, in the absence of FGF14, PW0564 exerted phenotypes similar to those observed by co-expression of FGF14, indicating the ability of PW0564 to serve as a pharmacological mimic of the β12 sheet of FGF14. Having developed an allosteric modulator of $Na_v1.6$ that exerted its effects via pharmacological mimicry of the β12 sheet of FGF14, analogs of the EYYV motif were subsequently pursued. In total, 12 analogs of the motif were designed and synthesized. Of the 12 analogs, PW201 stood out for its ability to inhibit FGF14:$Na_v1.6$ complex at low micromolar concentrations and for its ability to bind to both the CTD of $Na_v1.6$ and FGF14. Similar to PW0564, subsequent functional evaluation of PW201 as a modulator of $Na_v1.6$ channels using whole-cell patch-clamp electrophysiology revealed that it served as a negative allosteric modulator (NAM) of $Na_v1.6$ by suppressing $Na_v1.6$-mediated peak current density.
Conclusions: Using combinatorial chemistry, in tandem with techniques for *in vitro* pharmacological evaluation, including LCA, SPR, and whole-cell patch-clamp electrophysiology, we have identified NAMs of $Na_v1.6$ derived from β12 sheet and β8-β9 loop of FGF14. With further chemical optimization to improve the drug-like properties of these analogs, it is envisioned that these pharmacological tools used for interrogating the biophysical properties of $Na_v1.6$ could be developed into PPI-based neurotherapeutics.
Acknowledgments: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

The Effects of $ATRX^{Loss}$ and $IDH1^{R132H}$ when Combining Radiotherapy with the $IDH1^{R132H}$ Inhibitor Ivosidenib (AG-120) in Glioma Stem Cells

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Background: Glioblastoma Multiforme (GBM) is the most prevalent and aggressive form of malignant glioma in the USA with only a 15 month median survival time.[1,2] The recent discovery of glioma stem cells (GSCs), a subpopulation of undifferentiated, self-renewable, tumor initiating cells in GBM tumors known to promote chemoradioresistance, metastasis, and tumorigenesis, presents a new target for therapeutic intervention.[3,4] Importantly, 70-80% of low-grade glioma (LGG) and secondary GBM patients harbor $IDH1^{R132H}$, a mutation that occurs early in tumorigenesis and results in the elevated production of 2-Hydroxyglutarate (2-HG), a metabolite that contributes to intracellular oxidative stress and impaired post-radiation DNA damage repair (DDR) via Homologous Recombination (HR).[5-9] Although $IDH1^{R132H}$ is associated with improved patient survival and clinical response to chemotherapy,[10] astrocytoma (LGG) patients specifically also harbor $ATRX^{Loss}$, a genetic alteration that inactivates the ATRX chromatin remodeling protein and ultimately impairs DDR via non-homologous end joining (NHEJ).[6,11-13] Interestingly, the recent development of Ivosidenib, an $IDH1^{R132H}$ inhibitor shown to impair tumor cell growth and promote GSC differentiation by inhibiting 2-HG production, represents a potential treatment modality to incorporate alongside conventional X-ray radiotherapy in glioma patients.[14,15] However, the mechanisms by $IDH1^{R132H}$ and $ATRX^{Loss}$ impact the efficacy of X-ray radiotherapy (XRT) when combined with Ivosidenib in GSCs are not well understood. **We hypothesize that $ATRX^{Loss}$ is required to successfully sensitize GSCs to the cytotoxic effects of XRT in the presence of Ivosidenib.**

Methods: Isogenic MGG18-$IDH1^{WT}$, MGG18-$IDH1^{R132H}$, TS543-$ATRX^{WT}$, TS543-$ATRX^{Loss}$, and GS818-$IDH1^{R132H}$/$ATRX^{Loss}$ GSC neurospheres were treated with 3-6 Gy XRT using the XRAD 320 irradiation platform and/or 5 nM Ivosidenib, at 24 hours prior to irradiation. GSC self-renewal, based on neurosphere formation frequency, was quantified using extreme limiting dilution analysis (ELDA).[16]

Results: $ATRX^{Loss}$ or $IDH1^{R132H}$ alone were not sufficient to inhibit GSC self-renewal after XRT irradiation compared to $ATRX^{WT}$ or $IDH1^{WT}$ respectively (Pairwise Testing $Pr(>\chi^2)<0.0001$). The combination of XRT and Ivosidenib in MGG18-$IDH1^{R132H}$ GSCs promoted XRT resistance by increasing self-renewal compared to XRT or Ivosidenib alone (Pairwise Testing $Pr(>\chi^2)<0.01$). Finally, we demonstrated that combining XRT and Ivosidenib only impairs self-renewal compared with Ivosidenib alone (Pairwise Testing $Pr(>\chi^2)<0.0001$) in GS818-$ATRX^{Loss}$/$IDH1^{R132H}$ GSCs.

Conclusions: We conclude that $ATRX^{Loss}$ is required for the GSC sensitivity to XRT and Ivosidenib, thereby supporting the benefits of this regiment for astrocytoma patients. Our future experiments will further elucidate the mechanisms by which XRT and Ivosidenib impact GSC apoptosis and DNA damage.

Acknowledgements: A. Garcés acknowledges Drs. Grosshans, Bhat, Guan, Sawakuchi, Lin, and Bronk for their continued mentorship and support throughout this project. GSCs were generously donated to A. Garcés by Dr. Krishna Bhat and Dr. Jason Huse at MD Anderson Cancer Center as well as Dr. Daniel Cahill at Massachusetts General Hospital. This research was funded by the NIH R01 grant entitled "Synaptic basis of deficits in attention and executive function following cranial radiation."

References

1. Mesfin FB, Al-Dhahir MA: Cancer, Brain Gliomas, StatPearls. Treasure Island (FL), StatPearls Publishing

2. Tamimi AF, Juweid M: Epidemiology and Outcome of Glioblastoma, in De Vleeschouwer S (ed): Glioblastoma. Brisbane (AU), Codon Publications

3. Liebelt BD, Shingu T, Zhou X, et al: Glioma Stem Cells: Signaling, Microenvironment, and Therapy. Stem Cells Int 2016:7849890, 2016

4. Arnold CR: The role of cancer stem cells in radiation resistance. 2020

5. Philip B, Yu DX, Silvis MR, et al: Mutant IDH1 Promotes Glioma Formation In Vivo. Cell Rep 23:1553-1564, 2018

6. Sulkowski PL, Corso CD, Robinson ND, et al: 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. Sci Transl Med 9, 2017

7. M Gagné L, Boulay K, Topisirovic I, et al: Oncogenic Activities of IDH1/2 Mutations: From Epigenetics to Cellular Signaling. Trends in cell biology 27:738-752, 2017

8. Alan Mitteer R, Wang Y, Shah J, et al: Proton beam radiation induces DNA damage and cell apoptosis in glioma stem cells through reactive oxygen species. Scientific reports 5:13961-13961, 2015

9. Malta TM, de Souza CF, Sabedot TS, et al: Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications. Neuro-oncology 20:608-620, 2018

10. Khurana R, Rath S, Singh HB, et al: Correlation of Molecular Markers in High Grade Gliomas with Response to Chemo-Radiation. Asian Pacific Journal of Cancer Prevention 21:755-760, 2020

11. Haase S, Garcia-Fabiani MB, Carney S, et al: Mutant ATRX: uncovering a new therapeutic target for glioma. Expert opinion on therapeutic targets 22:599-613, 2018

12. Nunez FJ, Mendez FM, Kadiyala P, et al: IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. Sci Transl Med 11, 2019

13. Koschmann C, Lowenstein PR, Castro MG: ATRX mutations and glioblastoma: Impaired DNA damage repair, alternative lengthening of telomeres, and genetic instability. 3:e1167158, 2016

14. Rohle D, Popovici-Muller J, Palaskas N, et al: An Inhibitor of Mutant IDH1 Delays Growth and Promotes Differentiation of Glioma Cells. Science 340:626-630, 2013

15. Popovici-Muller J, Lemieux RM, Artin E, et al: Discovery of AG-120 (Ivosidenib): A First-in-Class Mutant IDH1 Inhibitor for the Treatment of IDH1 Mutant Cancers. ACS Medicinal Chemistry Letters 9:300-305, 2018

16. Hu Y, Smyth GK: ELDA: Extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. Journal of Immunological Methods 347:70-78, 2009

Splicing Factor 1 (SF1) Levels Influence Colon Polyp Development in Mice

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Background:
Colorectal cancer (CRC) is the third most common cancer in the US, with the second highest in terms of mortality rates, and with higher incidence and mortality rates in African Americans. Although great progress has been made in the screening and detection of this cancer, there are still many aspects to be elucidated regarding the genetic susceptibility factors of this cancer.
Splicing Factor 1 (SF1) is an alternative splicing (AS) factor that is ubiquitously expressed and conserved across species. It is involved in early spliceosome assembly and may function as a constitutive splicing factor in lower eukaryotes but as an alternative splicing (AS) factor in mammalian cells. It interacts with U2 snRNP auxiliary factor ($U2AF^{65}$) to co-operatively bind to the branch point sequence and polypyrimidine tract within the intron of pre-mRNAs during splicing.

Hypothesis/Goals:
Mutational inactivation of APC (adenomatous polyposis coli) is frequent in colon cancers. Our goal is to utilize mouse models to assess genetic factors that influence colon tumorigenesis. We developed a mouse strain *Sf1+/-* that expresses reduced levels of splicing factor 1 (SF1) protein. We tested whether lower levels of SF1 could affect APC mediated colon cancer.

Methods:
$Apc^{Min/+}$mice develop numerous intestinal polyps by 4 months. We collected 2 cohorts of mice: $Apc^{Min/+}$and $Sf1+/-;Apc^{Min/+}$ mice in which the number and sizes of intestinal polyps were counted.

Results:
Results showed that $Sf1+/-;Apc^{Min/+}$ mice had lower numbers of intestinal polyps compared to $Apc^{Min/+}$. Fewer, smaller sized polyps were detected in intestines of $Sf1+/-;Apc^{Min/+}$ but the numbers of larger sized polyps were similar in both cohorts. Thus, lowered SF1 levels reduced incidence of colon polyps, likely by decreasing the number of polyps that are initiated in the intestine.

Conclusions:
Our results show that congenitally reduced SF1 levels resulted in reduction in intestinal polyp development in $Apc^{Min/+}$ mice and also suggest that SF1 levels influence cellular transformation and polyp development.

Acknowledgements:
GRANT SUPPORT
NCI R21CA186033 and NIMHD 5G12MD007605

Removal of Trace Levels Emerging Contaminants (ECs) From Water By Green Synthesized Acalypha Indica Silver Nanoparticles

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Emerging contaminants (ECs) include but are not limited to water disinfectant byproducts, gasoline additives, manufactured nanoparticles, pesticides, herbicides, UV-filters, human and veterinary pharmaceutical products. They have been found in trace amounts (ppt to ppb) in treated wastewater and have been detected in water samples – they are therefore difficult to remove. We have synthesized silver nanoparticles (Ag NPs) using Acalypha indica leaf extracts; and use them to treat pesticides and hormones in water samples to determine efficiency of removal. Ag-herbal extract nanoparticles was synthesized by incubation of Acalypha Indica extract with $AgNO_3$ at 80^0 C for 1 hour until the change of color from pale yellow to dark brown indicated the formation of colloidal silver nanoparticles. The synthesized Ag NPs were characterized by UV-Vis spectrophotometer, Zetasizer (ZS 90, Malvern, UK) for particle size and zeta potential of the Ag NPs. To investigate the effects of the Ag NP's on the removal of ECs from water, a 6 ml portion of the silver nanoparticle solution was centrifuged at 10,000 rpm for 7 minutes. The supernatant was discarded and to the residue, 5 ml of 5 ppm and 10 ppm pesticide/hormone standard solution was added and then allowed for continuous shaking for 1 hour. Then centrifuge at 1500 rpm for 5 min. 1ml of supernatant solution was collected, samples were filtered using 0.45µm syringe filter into HPLC vials for analysis. This experiment was repeated and allowed for continuous shaking for 24 hours to measure ECs removal/degradation efficiency of Ag NP's using HPLC analytical methods. Our results indicated that the Ag NP synthesized showed Absorption spectrum (absorbance peak) at about 400 nm, which confirmed the formation of Ag NP. The average size and zeta potential analyzed by dynamic light scattering techniques (DLS) showed the sized to be 132.6 nm and the Zeta potential as -ve 13.6 mV suggesting higher stability of Ag NPs. After treatment there is a significant reduction in the peak areas observed in samples treated with A. indica Ag NP's. Among the selected hormonal compounds and pesticides, there is a 100% removal of 5ppm cortisone, aldrin, endrin and 10 ppm cortisone after 24 hours of treatment. After 1 hour of treatment 92% of 5 ppm cortisone, 75.5% of 10 ppm cortisone, 58.8% of 5 ppm endrin, 78% of 10 ppm endrin were removed. After 24 hours 47.7% of 5 ppm testosterone, 35% of 5 ppm atrazine, 58.2% of 10 ppm estrone, 37.7% of testosterone, 38.4% of 10 ppm atrazine, 66% of 10 ppm aldrin and 78% 10 ppm endrin were removed. This indicates the removal of ECs by Ag NP's depends upon the nature and concentration of ECs and the removal mechanism is time dependent. Overall, Ag NP's achieved 100% removal of some targeted ECs by simple treatment method. This is a novel effort that need further investigation for broader application in water treatment processes to remove contaminants and in environmental bioremediation.

Key words: Emerging Contaminants, Acalypha Indica, Silver Nanoparticles

Acknowledgement/funding: This research was partly supported by supplies funded by Tittle III Award Number P031B090216; Title III, Part B, Historically Black Graduate Institutions (HBGI) (CFDA No. 84.031B), United States Department of Education

In Vitro Dissolution Considerations Associated with Nanoparticle-based Drug Delivery Systems (NDDS)

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Nanoparticle-based Drug Delivery Systems(NDDS) have emerged as a promising formulation approach for drug delivery, by increasing drug solubility and dissolution rates with large surface area-to-volume ratios, by improving passive diffusion across biological membranes with reduced particle size, and by utilizing liposomal- and lipid-based nanocarriers to enhance drug permeability across phospholipid cellular membrane. A widely accepted modeling tool in pharmacokinetic studies, *in vitro in vivo* correlation (IVIVC), is often used to predict *in vivo* pharmacokinetic profiles of a study drug by building mathematical models based on *in vitro* drug release in a dissolution apparatus.

However, significant challenges exist in IVIVC of NDDS due to incomplete understanding of nanoparticles' biochemical characteristics and how they impact experimental protocols. Unlike that of conventional dosage forms, the *in vivo* disposition of nanocarriers is highly intricate, differing greatly from their *in vitro* behavior due to the interplay between the materials and the local biological environment. An example would be the outset of spontaneous surface-adsorbed layer of biomolecules around the nanoparticles, termed as protein-corona (PC), that causes large deviation from traditional, well-studied pharmacokinetic profiles. Owing to numerous physicochemical characteristics of these nanoparticles (e.g. size, shape, surface charge, topology, hydrophobicity), standardization of *in vivo* nanoparticle interactions becomes difficult, especially when concerning phagocytic pathways. Therefore, to obtain reliable and realistic IVIVC modeling data, existing protocols must be executed under physiologically relevant *in vitro* conditions, which are not well described in available literature to date.

Characterizing drug safety profiles for nanoparticles is another aspect of this review. While NDDS release can be sustained for months to decrease the dosing frequency and reduce adverse events associated with concentration peaks, the long-term fate of study compounds, including elimination, accumulation, and biodegradation, should be scrutinized to develop safer nanomedicines.

Nanotechnology offers promising solutions to the development of potential drug candidates and to the maintenance of exclusivity after expiry of patents associated with referenced products. Hence, it becomes indispensable that we clearly understand the impact of bio-nano interactions, and design *in vitro* dissolution strategies capable of making accurate prediction of *in vivo* pharmacokinetic profiles. Reliable IVIVC will properly correlate nanoparticle physicochemical properties with its performance in a physiologic environment, with favorable economic implications. This review aims to describe various challenges associated with characterizing nanoparticle properties during *in vitro* dissolution studies, to elucidate the impacts of bio-nano interactions, and to propose strategies to develop reliable pharmacokinetic modeling of nanoparticles.

Acknowledgements-This study was funded in part by Cancer Prevention & Reseach Institute of Texas (CPRIT) Core Facilities Support Awards under Award Number RP180748 and the National Institute on Minority Health and Health Disparities of the National Institute of Health (NIH) under Award Number 2U54MD007605. The content is solely the responsibility of the authors and does not necessarily represent the official views of the CPRIT and NIH.

Identification and Validation of a Novel Antivirulent that Binds to Pyoverdine and Inhibits its Function

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Background: *Pseudomonas aeruginosa* is a multidrug resistant, nosocomial pathogen that frequently causes ventilator-associated pneumonia in intensive care units and chronic lung infections in cystic fibrosis patients. The rising prevalence of drug-resistant bacteria demands new therapeutic avenues to treat *P. aeruginosa* infections. The major siderophore pyoverdine is a key virulence factor in the pathogen that can be targeted for therapeutic intervention. Pyoverdine not only provides the bacterium with ferric iron, a micronutrient necessary for growth, but also regulates the production of secreted toxins. Furthermore, we have recently demonstrated that pyoverdine disrupts *Caenorhabditis elegans* iron and mitochondrial homeostasis even in the absence of live pathogen. Due to a combination of these factors, pyoverdine production is necessary for full *P. aeruginosa* virulence against various mammalian and invertebrate hosts.

Hypothesis/Goals: We hypothesized that inhibitors of pyoverdine function would attenuate the production of pyoverdine-regulated virulence factors and mitigate *P. aeruginosa* pathogenesis against *C. elegans*.

Methods: Pyoverdine functional inhibitors were identified using high-throughput biochemical screen that was based on quenching in intrinsic fluorescence of pyoverdine. Specifically, we measured fluorescence after partially purified pyoverdine was treated with approximately 45,000 compounds from small molecule diversity libraries. *C. elegans* host death upon *P. aeruginosa* exposure was visualized via fluorescence microscopy after staining dead organisms with cell impermeant dye. ^{1}H-^{15}N and ^{1}H-^{13}C 2D NMR were used to identify the inhibitor binding sites on pyoverdine. Molecular docking and molecular dynamic simulations were used to further analyze small molecule – pyoverdine interactions.

Results: Twelve hits were identified from the screen, and top three were chosen for initial follow-up using structure-activity relationship analysis. Having tested various chemical analogues of these hits, we identified PQ3c, an analogue that exhibited greater affinity towards pyoverdine than its parental compound. PQ3c was able to attenuate the production of pyoverdine-regulated virulence factors (the translational inhibitor exotoxin A and protease PrpL) and rescue *C. elegans* hosts during *P. aeruginosa* exposure. PQ3c did not exhibit overt toxicity against *C. elegans* or human bronchial epithelial cells. Using NMR spectroscopy, we were able to demonstrate that PQ3c occupies a shallow groove on pyoverdine formed by the chromophore and N-terminal residues of the peptide chain. Using molecular docking and molecular dynamics simulations, we modeled the putative pyoverdine-inhibitor complex and predicted its interactions with the pyoverdine outermembrane receptor FpvA.

Conclusions: Antivirulence therapeutics, such as pyoverdine functional inhibitors, can be identified from high-throughput biochemical screens. PQ3c may serve as a scaffold for the development of pyoverdine inhibitors with higher potency and specificity.

Acknowledgements: This study was supported by National Institute of Health grants K22AI110552 and R35GM129294, by the Welch Foundation Grant C-1930, and John S. Dunn Foundation Collaborative Research Award Program administered by the Gulf Coast Consortia.

Co-modeling of Calcipotriol and Paclitaxel in Blood and Tumor of Kras^{G12D} Mouse Model of Pancreatic Cancer Post I.V. Dose of Micellar Co-formulation

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Background: Stroma-modulating agents in combination with standard chemotherapy have emerged as a potential treatment for pancreatic cancer, and we are developing a combination therapy of Calcipotriol (Cal) and paclitaxel (PTX). Cal, a stroma-modulating agent de-activates the cancer associated fibroblasts, shifting the tumor microenvironment into a dynamic milieu that aids PTX delivery. However, Cal can cause hypercalcemia at high doses.

Hypothesis/Goals: To mitigate toxicity and further improve tumor accumulation, we developed a micellar co-formulation (M-Cal/PTX).

Methods: Orthotopic Kras mouse model was developed. Healthy and tumor bearing mice were administered an IV bolus dose of M-Cal/PTX through the tail vein (5 mg/kg PTX and 0.5 mg/kg Cal). Whole blood samples were taken, as well as tumor, liver and spleen samples were harvested from tumor bearing group at predetermined time points post dose for simultaneous PTX and Cal quantifications by using a developed and validated LC-MS/MS assay. Compartmental analysis was performed to compare PK in healthy and diseased animals. To better understand the distribution kinetics of M-Cal/PTX, we co-modeled blood and tissue concentrations using Phoenix NLME (version 8.0).

Results: Cal from M-Cal/PTX (M-Cal) was measurable in the tumors and livers but not blood in orthotopic murine Kras^{G12D} mouse model 24 h post the IV bolus dose (0.5 mg/kg Cal, 5 mg/kg PTX). In contrast, Cal was not measurable from non-formulated free Cal. PTX was measurable in blood at 24 h in both formulated and non-formulated groups, but only measurable in the tumors of mice administered M-Cal/PTX. Our analysis showed PK differences of M-Cal in normal versus tumor-bearing mice. The elimination half-life ($t_{1/2}$) of M-Cal in diseased mice was >4X shorter than that in healthy ones (0.089 h vs 0.38 h), which corresponded with the increased uptake into the peripheral tissues. M-Cal persisted in tumor and liver because of the slow eliminations from these organs. Unsurprisingly, our model estimated that the blood AUC in tumor-bearing mice was 5X less than that in the healthy counterparts. A similar analysis of PTX from M-Cal/PTX showed that the elimination $t_{1/2}$ was about 2X shorter in diseased mice when compared to their healthy counterparts (0.58 h vs 1.2 h). This corresponded with a modest blood AUC decrease in diseased mice. Of the three organs evaluated, our model estimated PTX liver uptake to be the highest, followed by the spleen and then tumor. The rates of eliminations from the three tissues varied with the tumor being the highest. PTX could be measured in all three sites 24 h after M-Cal/PTX injection.

Conclusions: We conclude that 1) PK differences exist for Cal and PTX from M-Cal/PTX between tumor-bearing and healthy mice with faster elimination in the former. 2) Reduced Cal AUC in blood and its presence in tumors are expected to potentially reduce systemic toxicity and increase efficacy profiles, respectively, because Cal toxicity is directly related to the elevated systemic exposure that exacerbates off-target vitamin D receptor activation. Future studies will focus on demonstrating the proof-of-concept therapeutic merits of our combination regimen in the orthotopic Kras^{G12D} mouse model of pancreatic cancer.

Acknowledgement: Gilson Longenbauch Foundation Funding; 2020 AACR Scholar-in Training Award

*Development and Validation of a Sensitive, Specific and Reproducible UPLC-MS/MS Method for Quantification of a Potent Inhibitor of Methionine Aminopeptidase***.**

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Background: OJT007 is a potent and selective inhibitor of Methionine aminopeptidase 1 (MetAP1) with potent activity against *Leishmania Major (L. major)*, the causative infectious agent of Cutaneous Leishmaniasis (CL). Inhibition of MetAP1 interferes with *L.major* amastigotes and promastigotes proliferation. This makes OJT007 a promising lead molecule for potential development of novel agents for the treatment of CL.

Goals: To facilitate the quantification of lead molecules during the different stages of drug development, it is necessary to develop a bioanalytical assay. The objective of this study is to develop and validate an LC-MS/MS method for the quantification of a novel leishmanicidal agent in rat plasma.

Methods: The ultra-performance LC-MS/MS (UPLC-MS/MS) system consisted of a Shimadzu Nexera X2 UPLC system and a 4000 QTRAP® MS/MS system. Separation was achieved with an Acquity UPLC BEH C_{18} column (2.1 x 50 mm, 1.7 µm) using 0.1% formic acid in acetonitrile and 0.1% formic acid in water as mobile phase under gradient elution with a flow rate of 0.4 mL/min. Multiple Reaction Monitoring (MRM) data were collected under positive mode to detect transitions *m/z* 325→ *m/z* 205 for OJT007, and *m/z* 350→ *m/z* 101 for Voriconazole (Internal Standard). LC-MS/MS assay validated according to Center for Drug Evaluation and Research (CDER) "Guidance for Industry: Bioanalytical Method Validation" with respect to specificity, lower limit of quantification (LLOQ), linearity and range, accuracy and precision, extraction recovery, matrix effect, carryover effect, stability.

Results: The standard curves of OJT007 in plasma were linear in the concentration range of 5-1000 ng/mL. The mean extraction recovery for OJT007 were 101.71± 5.52 at the low QC (15 ng/ml), 98.99± 3.64 at medium QC (200 ng/ml) and 95.80± 1.72 at the high QC concentration (800 ng/ml) suggesting that sample preparation resulted in a high and stable extraction recovery. The mean matrix effect for OJT007 were 8.01± 4.87, 4.43± 2.19 and 7.97± 2.52 at the low, medium, and high QC respectively, suggesting sample preparation yielded no measurable matrix effect interfering with determination of drug in biological matrix. The intra- and inter-day accuracy (% relative error, %RE) ranged from 3.19-9.78% and 5.61-10.25%, respectively. The intra- and inter-day precision (coefficient of variation, CV%) ranged from 2.77-10.25% and 2.91-7.88%, respectively.

Conclusion: A sensitive, specific, and reproducible LC-MS/MS method was developed and validated for the quantification of a novel antileishmanial agent in rat plasma. This method was validated to be linear, accurate and precise over the concentration range 5-1000 ng/ml. The method displayed good recovery without interference from endogenous components in plasma. OJT007 remains stable under the expected sample handling, storage, preparation, and analysis conditions This method will subsequently be applied for in-vitro and in-vivo studies.

Acknowledgements: This study was supported by the National Institute on Minority Health and Health Disparities of the National Institute of Health under Award Number G12MD007605.

Mitophagy-Inducing Compounds as Potential Agents for Leukemia Eradication

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Background. Acute myeloid leukemia (AML) is a diverse group of hematological cancers, characterized by malignant clonal proliferation of immature myeloid progenitor cells in the bone marrow and peripheral blood. AML course is usually aggressive, and risk of relapse is >50% even for younger adults (Dohner et al. *Blood* 2010). New selective treatments are clearly essential to prolong relapse-free survival of both younger and elderly patients. Previously, we identified AML as a class of tumors that are particularly sensitive to mitochondria-targeted drugs (Panina et al. *Cell Death Dis* 2019). Furthermore, mitophagy as a selective autophagy of mitochondria has been shown to be altered in AML (Watson et al. *Cell Death Discov* 2015; Pei et al. *Cell Stem Cell* 2018) and activation of mitophagy might be a beneficial approach of leukemia eradication (Dany et al. *Blood* 2016).

Goal. To assess if novel mitophagy-inducing compounds identified in *C. elegans* model have a potential in eradicating leukemia cells.

Methods. In this study, we used mitophagy-inducing compounds (PINK-1 stabilizers, or PS) identified in *C. elegans* model (n = 8). Their cytotoxicity against AML cells (MOLM-13) and toxicity in normal blood cells (PBMCs) derived from the blood of healthy donors were determined. For each effective compound which induced at least 15% drop in viability at 10 μM under 72 h treatment, LD50 dose was computed. Using chemical structure of effective parental compounds as a basis, commercially-available analogs were purchased and analyzed similarly. After ranking based on their effectiveness, PS compounds with LD50 ≤ 5 μM in MOLM-13 cells and selectivity ratio ≥ 10 were tested in other leukemia cell lines as well as included in mechanistic studies, e.g. reactive oxygen species (ROS), ATP and MMP (mitochondrial membrane potential) measurements.

Results and conclusions. Out of 8 parental PS compounds from primary *C. elegans* screen, 6 (PS30, 34, 83, 103, 106, 127) passed the initial effectiveness criterion. Of these 6 compounds along with their 33 analogs (n = 39 in total), 12 molecules had LD50 ≤ 5 μM in MOLM-13 cells. Top 6 leads with highest effectiveness and selectivity included analogs of PS127 compound (PS127E, G, B, B1, F) and PS30B molecule. The latter did not affect viability of normal blood cells even at 100 μM at 72 h. Most effective leads were PS127B, E, G with LD50 of 200-500 nM in MOLM-13 cells. In addition, these drugs were also cytotoxic against other AML cell lines (MV-4;11, OCI-AML2, THP-1, MOLM-14), except PS30B in OCI-AML2. Mechanistically, PS127 analogs induced ROS generation in AML cells, but not PBMCs, under 24 h treatment with LD50 dose. At the same time, they did not significantly change MMP under these conditions. On other hand, PS30B did not affect ROS level, but decreased MMP in AML cells. At 16 h of treatment an estimated drop in ATP level was higher in MOLM-13 cells than PBMCs (being most profound in case of PS127B), reflecting selective effect of these compounds on leukemia mitochondrial metabolism. Finally, PS127B and E analogs exhibited strong synergetic effects specifically in AML cells when combined with Complex I inhibitor IACS-010759. This led to 2.3-4.6-fold higher level of death in AML cells. These data support the potential of these mitophagy-inducing compounds for chemical optimization and future therapeutic development.

Acknowledgments. The study was supported by the CPRIT grant RR150044, NIH NIGMS grant R35GM129294 to NVK, and NIH NIGMS grant T32GM120011 to JGT.

Utilizing Synergistic Potential of Mitochondria-Targeted Drugs for Leukemia Therapy

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Background: Acute myeloid leukemia (AML) is an aggressive group of cancers with high mortality rates and significant relapse risks. Current treatments for AML, called induction and consolidation, exhibit poor treatment outcomes on elder patients and are insufficient against relapse problem in AML. Novel and more effective treatments are clearly necessary.

Goals: Recent discoveries suggest that AML may be particularly sensitive to chemotherapeutics that target mitochondria. Development of new combinatorial therapeutic regimens utilizing this sensitivity may resolve some limitations of single drugs and improve treatment efficacy.

Methods: To investigate the mitochondria sensitivity in AML, six compounds that target mitochondria [IACS-010759, rotenone, cytarabine, etoposide, ABT-199 (venetoclax), and carbonyl cyanide m -chlorophenylhydrazone] were each paired with six compounds with other activities, including tyrosine kinase inhibitors (midostaurin and dasatinib), glycolytic inhibitors (2-deoxy-D-glucose, 3-bromopyruvate, and lonidamine), and the microtubule destabilizer vinorelbine. The resulting combinations were tested for synergistic cytotoxicity against AML, CML (chronic myelogenous leukemia), and ALL (acute lymphoblastic leukemia) cell lines in short-term (24 h) and long-term (72h) treatments. Mitochondria function, including respiration rate, membrane potential, and ATP level in AML cell lines and primary AML samples were measured after treatment and compared with healthy blood cell control, PBMCs.

Results: 22 of 36 combinations tested showed synergistic cytotoxicity in both MOLM-13 and OCI-AML2 cell lines, with four most selective combinations being IACS-010759/vinorelbine, rotenone/2-deoxy-D-glucose, carbonyl cyanide m -chlorophenylhydrazone/dasatinib, and venetoclax/lonidiamine. In 72 h treatment, the synergistic cytotoxicity of these combinations remained, even when decreasing drugs' concentrations to nanomolar scale. The survival of healthy PBMCs was dramatically higher than AML cell lines after treatment. Among these drug pairs, IACS-010759/vinorelbine impairs several mitochondrial function and decreased ATP level most profoundly, including in primary AML samples. Some of the selected treatments were also effective in K-562, KU812 (CML) and CCRF-CEM, MOLT-4 (ALL) cell lines, and in primary AML cells from patient samples.

Conclusions: Selected drug combinations pairs were effective against AML, CML, and ALL cell lines, suggesting that these combinations may have value in treating various forms of leukemia. Some of the selected drug combinations impaired several mitochondria functions, including oxygen consumption rate (OCR) and membrane potential. Some of the selected combinations also significantly inhibited basal mitochondrial respiration in primary AML sample. Lastly, two selected combinations retained high synergy and strong selectivity in primary AML cells from patient samples, suggesting that these treatments have potential.

Acknowledgements: The study was supported by the CPRIT grant RR150044, as well as Welch Foundation grant C-1930 and NIH NIGMS grant R35GM129294 to NVK.

Novel Immune Modulators Enhance Caenorhabditis elegans Resistance to Multiple Pathogens

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Background. Traditionally, treatments for bacterial infection have focused on killing the microbe or preventing its growth. As antimicrobial resistance becomes more ubiquitous, the feasibility of this approach is beginning to wane and attention has begun to shift toward disrupting the host-pathogen interaction by improving the host defense.

Hypothesis. We hypothesize that small molecules that can modulate key innate immune pathways will be able to enhance host survival in the presence of pathogens.

Methods. We performed a high-throughput, high-content, phenotypic, fragment-based screen to identify small molecules that alleviate *Pseudomonas aeruginosa*-mediated killing of *Caenorhabditis elegans*. Screen hits were placed into antimicrobial, anti-virulents, or immune modulator categories based on MIC/EC ratio (EC, effective concentration, minimum amount of compound required for statistically-significant rescue; MIC, minimum inhibitory concentration, amount of the compound required to interfere with bacterial growth), and expression of *C. elegans* host defense genes. Transcriptome profiling was performed for the five selected hits. Bioinformatic, cell biology, and genetic assays were used to identify target pathways for these molecules. Compunds' ability to rescue C. elegans from Gram-positive pathogens *S. aureus* and *E. faecalis* was also tested.

Results. We identified over 20 compounds that stimulated host defense gene expression. Five of these molecules were selected for further characterization. Four of five compounds showed little toxicity against mammalian cells or worms, consistent with their identification in a phenotypic, high-content screen. Each of the compounds activated several host defense pathways, but the pathways were generally dispensable for compound-mediated rescue in Liquid Killing, suggesting redundancy or that the activation of one or more unknown pathways may be driving compound effects. A genetic mechanism was identified for LK56, which required the Mediator subunit MDT-15/MED15 and NHR-49/HNF4 for its function. Interestingly, LK32, LK34, LK38, and LK56 also rescue *C. elegans* from *P. aeruginosa* in an agar-based assay, which uses different virulence factors and defense mechanisms. Rescue in an agar-based assay for LK38 entirely depended upon the PMK-1/p38 MAPK pathway. Three compounds, LK32, LK34, and LK56 also conferred resistance to *Enterococcus faecalis*, and the two lattermost, LK34 and LK56, also reduced pathogenesis from *Staphylococcus aureus*.

Conclusions. This study supports a growing role for MDT-15 and NHR-49 in immune response. It also paves the way for future characterization of the anti-infective activity of the molecules in higher organisms and highlight the compounds' potential utility for further investigation of immune modulation as a novel therapeutic approach.

Acknowledgements: The study was supported by the Welch Foundation grant C-1930, NIH NIGMS grant R35GM129294, and John S. Dunn Foundation Collaborative Research Award Program administered by the Gulf Coast Consortia to NVK.

Development of Allosteric Modulators of the Voltage-Gated Sodium Channel 1.1 to Rescue Perturbations of the Excitatory/Inhibitory Tone of the Nucleus Accumbens

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Background: The voltage-gated Na^+ (Na_v) channel isoforms 1.1, 1.2, and 1.6 serve as the fundamental molecular determinants of electrical signaling in the central nervous system (CNS). Crucially, these three isoforms are unevenly expressed in different neuronal subpopulations. Specifically, $Na_v1.1$ channels are abundantly expressed in inhibitory parvalbumin (PV) interneurons, whereas $Na_v1.6$ channels are abundantly expressed in excitatory pyramidal neurons of the striatum. Given the oftentimes opposing roles of $Na_v1.1$ and $Na_v1.6$ channels in regulating the excitatory/inhibitory (E/I) tone of the striatum, the development of isoform selective, allosteric modulators of $Na_v1.1$ channels could serve as a novel therapeutic strategy for the treatment of a diverse array of channelopathies.
Hypothesis/Goals: In an effort to develop small molecules capable of treating neurologic disorders conferred via perturbation of the E/I tone, a drug-discovery campaign that employed *in silico* to *ex vivo* methodologies was pursued to develop isoform selective, allosteric modulators of $Na_v1.1$ channels. In particular, the protein:protein interaction (PPI) interface between the C-terminal domain (CTD) of $Na_v1.1$ and fibroblast growth factor 14 (FGF14) was targeted, as PPI interfaces are flexible and dynamic surfaces that structurally diverge among Na_v and FGF isoform complexes.
Methods: *In silico* screening, surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: To begin this drug discovery campaign to develop allosteric modulators of $Na_v1.1$ by targeting its PPI interface with FGF14, a ligand-based high-throughput virtual screening of small molecules against the PPI interface was conducted using Autodock. In this virtual screening, a grid box encompassing a portion of the FENYYV sequence (residues 155-160) of FGF14 within a distance of 8Å from the Nav1.1 C-tail was targeted, a structural region of FGF14 that is predicted to be part of a druggable pocket within the FGF14:$Na_v1.1$ PPI interface. As a result of this *in silico* screening, which included 642,759 ligands, 1001 ZINC compounds were predicted to interact with this structural region of the PPI interface. Of these 1001 ZINC compounds, two lead compounds, ZINC1 and ZINC3, were identified on account of their binding scores and drug-like properties. Protein:ligand binding of these two compounds using surface plasmon resonance (SPR) confirmed that ZINC3, but not ZINC1, demonstrated appreciable binding to FGF14 at its predicted interaction site with the CTD of $Na_v1.1$. Functional evaluation of ZINC3 as a modulator of $Na_v1.1$ channel kinetics revealed that the small molecule altered $Na_v1.1$-mediated peak current density in the presence of FGF14-1b, whereas in the presence of FGF14-1a, the compound altered the $V_{1/2}$ of $Na_v1.1$ channel activation, biophysical perturbations that collectively make plausible that the compound would modulate intrinsic excitability of PV neurons of the nucleus accumbens.
Conclusions: ZINC3 regulates biophysical properties of $Na_v1.1$ channels in a fashion that is dependent upon the presence of FGF14 isoforms expressed, illuminating its selective modulatory effects. Collectively considered, these findings suggest that ZINC3 could serve as a chemical scaffold for the optimization of neurotherapeutics to treat channelopathies conferred via perturbation of the E/I tone of the NAc.
Acknowledgements: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

Peptide Mimicking Crucial Residues of the β12 Sheet of Fibroblast Growth Factor 14 Modulates Accumbal Excitability via Interactions with the Voltage-Gated Na^+ Channel 1.6

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Background: Voltage-gated Na^+ (Na_v) channels, with the support of a diverse ensemble of auxiliary proteins tightly regulating their function, serve as the fundamental molecular determinants of the initiation and propagation of action potentials in excitable cells. Foremost among auxiliary proteins that regulate the activity of $Na_v1.6$ channels is fibroblast growth factor 14 (FGF14), whose direct binding to the C-terminal domain (CTD) of $Na_v1.6$ has been shown to exert modulatory effects on the biophysical properties of these channels. As perturbation of the protein:protein interaction (PPI) between FGF14 and the CTD of $Na_v1.6$ has been shown to cause neural circuity aberrations implicated in the etiology of neurologic and neuropsychiatric disorders, this PPI interface stands out as a promising target for ion channel drug discovery campaigns.
Hypothesis/Goals: In an effort to pharmacologically modulate the PPI between FGF14 and the CTD of $Na_v1.6$, model-guided studies of the PPI interface were employed to identify peptides capable of regulating FGF14:$Na_v1.6$ complex assembly. Peptides predicted to be capable of modulating FGF14:$Na_v1.6$ complex assembly *in silico* were subsequently synthesized and tested *in vitro* to assess their effects on the biophysical properties of $Na_v1.6$ followed by *ex vivo* studies to elucidate their effects on the excitability of medium spiny neurons (MSN) in the nucleus accumbens (NAc).
Methods: *In silico* molecular modeling, split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: Based upon prior investigations indicating that the β12 sheet of FGF14 is a crucial structural determinant of the protein that disproportionately contributes to its Gibbs energy of protein binding to the CTD of $Na_v1.6$, the role of the FLPK motif of this structural region was investigated for its modulatory effects on the $Na_v1.6$ channel macromolecular complex. To do so, *in silico* molecular docking of the FLPK peptide was first employed, which revealed that it was predicted to interact with so-called "hot spot" residues of the FGF14:$Na_v1.6$ PPI interface. Given these *in silico* findings, the FLPK tetrapeptide was synthesized and tested *in vitro* for its modulatory effects using the LCA, which revealed that peptide inhibits FGF14:$Na_v1.6$ complex assembly at low micromolar concentrations. To investigate if FLPK conferred functionally relevant modulation of $Na_v1.6$ channels, the effects of the peptide were assessed using whole-cell patch-clamp electrophysiology in heterologous cells. Consistent with the findings of the LCA, FLPK reversed many of the modulatory effects on $Na_v1.6$-mediated currents conferred by FGF14. Most prominently, FLPK partially reversed FGF14-mediated suppression of $Na_v1.6$-mediated currents, indicating that the tetrapeptide disrupts FGF14:$Na_v1.6$ complex assembly in a functionally relevant fashion. To assess how the *in vitro* effects of FLPK on FGF14:$Na_v1.6$ complex assembly regulated excitability of MSNs of the NAc, the *ex vivo* effects of the tetrapeptide were assayed using whole-cell patch-clamp electrophysiology in acute brain slice preparations, investigations that revealed that the peptide derived from the β12 sheet of FGF14 increased accumbal excitability.
Conclusions: The FLPK tetrapeptide could serve as a chemical scaffold for the development of therapeutics targeted the FGF14:$Na_v1.6$ PPI interface.
Acknowledgements: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

Characterizing Novel Mitochondria-Targeting Small Molecule Drugs in Caenorhabditis elegans

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Background: Mitochondria are hubs for cellular energetics. Additionally, they play critical roles in other cellular homeostasis mechanisms, such as cellular signaling and proteostasis. Many common pathologies, such as neurodegeneration and metabolic disease, are driven by mitochondrial dysfunction. Enhancement of mitochondrial quality control can benefit cell heath by promoting the clearance of damaged organelles (Murphey and Hartley. *Nature Rev. Drug Discovery* 2018). Furthermore, recent results from our lab demonstrated that by inflicting mitochondrial damage (particularly in combination with glycolytic inhibition), we can selectively trigger cancer cell death (Panina et al. *Cell Death Dis* 2019). Identifying more compounds that can alter mitochondrial parameters could lead to novel therapeutic approaches.

Goal: We aim to identify and characterize novel mitochondria-targeting small molecules that could be used to improve therapies of cancers.

Methods: We screened ~50,000 small molecules to identify hits capable of activating mitochondrial autophagy (mitophagy). The screen was performed by using a *C. elegans* fluorescent reporter which visualizes the stabilization of PINK-1/PINK1 kinase. This key regulatory enzyme phosphorylates PDR-1/Parkin2, which is necessary and sufficient for targeting mitochondria for autophagic degradation. We validated hits by investigating their effects on mitochondrial morphology in *C. elegans* (mitoGFP) and distribution of autophagy components. To elucidate the mechanisms by which these novel compounds are inducing mitophagy, we measured changes in cellular and mitochondrial parameters including ATP level, ROS level, mitochondrial membrane potential, NADH/NAD^+ ratio. Additionally, we assayed the activation of a number of stress response pathways (UPR^{mt}, $MAPK^{mt}$, ESRE network, oxidative stress, and insulin signaling).

Results: We identified 8 compounds that triggered mitophagy (termed PINK1-Stabilizing (PS) compounds). These hits induced widespread fragmentation of mitochondria and autophagy machinery recruitment as assessed by BEC-1 localization (BEC-1::mRFP), indicative of autophagosome formation. We found that most compounds altered cellular ROS (elevated in PS30 PS34, PS83, PS103, PS127 and PS143; lowered in PS106; unchanged in PS135) and significantly decreased mitochondrial membrane potential. Interestingly, three PS compounds (PS34, PS127, and PS143) were potent activators of insulin signaling (inducing DAF-16 nuclear localization). Activation of insulin signaling was shown to be necessary to activate mitophagy as *daf-16(RNAi)* diminished mitophagy activation. Additionally, these compounds mildly induced oxidative stress response (as measured by *gst-4* activation). RNAi knockdown of this pathway decreased PINK-1::GFP stabilization during PS143 stress.

Conclusions: Our screening methodology effectively identified mitophagy-activating compounds. The resulting hits altered multiple cellular parameters that are closely associated with mitochondrial functions. Lastly, our data suggests that three of the compounds elicit their effect via activation of the insulin signaling pathway.

Acknowledgements: The study was supported by the CPRIT grant RR150044, NIH NIGMS grant R35GM129294 to NVK, and NIH NIGMS grant T32GM120011 to JGT.

Developing Pharmacological Probes for Interrogation of the Circuitry of the Nucleus Accumbens

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Background: Disruption of protein:protein interactions (PPI) within the voltage-gated Na^+ (Na_v) channel macromolecular complex gives rise to neural circuity aberrations that are implicated in the etiology of neuropsychiatric disorders. Bridging the gap between disruption of PPIs and depression- and anxiety-like behaviors, however, are hampered by a lack of targeted pharmacological probes.
Hypothesis/Goals: Focusing on the PPI interface between $Na_v1.6$ and its auxiliary protein fibroblast growth factor 14 (FGF14), we screened 44,480 small molecules against this surface to identify a lead compound that could be used to study how pharmacological modulation of the PPI altered neuronal excitability of medium spiny neurons (MSN) in the nucleus accumbens (NAc) and to study how these electrophysiological changes *ex vivo* caused changes in goal-directed behaviors *in vivo*.
Methods: Split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), electrophysiology, *in silico* testing of blood-brain barrier (BBB) permeability, *in vivo* pharmacokinetic (PK) and behavioral testing.
Results: Primary and counter-screening of the initial pool of 44,480 small molecules identified seven compounds capable of selectively modulating FGF14:$Na_v1.6$ complex assembly with low micromolar potency. Of these seven compounds, three small molecules were shown to reduce the binding affinity of FGF14 toward the C-terminal domain (CTD) of $Na_v1.6$ using SPR. Electrophysiological evaluation of the three compounds in heterologous cells revealed that only one, 7605, exhibited a mechanism of action (MOA) that was contingent upon the presence of FGF14. Subsequent electrophysiological evaluation of the effects of 7605 in acute brain slice preparations revealed that the small molecule suppressed intrinsic firing properties and persistent Na^+ currents in MSNs of the NAc in an FGF14-dependent manner. After an *in silico* investigation suggested that the structural features of 7605 were favorable for BBB permeability, the compound underwent *in vivo* testing to assess its PK properties and effects on behavior in rats. Although the results of the behavioral assays are pending, the PK assessment of 7605 indicates that the NAc is modestly exposed to the drug upon intravenous injection in rats, making plausible that the compound will exert modulatory effects on goal-directed behaviors.
Conclusions: Through this HTS campaign, a small molecule has been identified that selectively inhibits FGF14:$Na_v1.6$ complex assembly and resultantly leads to altered mesocorticolimbic activity. *In vivo* effects of 7605 on goal-directed behaviors are currently being assessed. These collective measures will continue to illuminate how PPIs within the Na_v channel macromolecular complex modulate neural circuity that, when perturbed, is implicated in the etiology of neurologic and neuropsychiatric disorders.
Acknowledgments: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

A UHPLC-MS/MS Method for The Quantification of JIB-04 in Rat Plasma: Development, Validation and Application to Pharmacokinetics Study

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Background: JIB-04 is a small molecule that pan-selectively inhibits lysine demethylases (KDMs), showing maximal inhibitory activity against KDM5A, and as secondary targets, KDM4D/4B/4A/6B/4C. Recently, it was found that JIB-04 also potently and selectively blocks HIV-1 Tat expression, transactivation, and virus replication in T cell lines via the inhibition of a new target, serine hydroxymethyltransferase 2.

Hypothesis/Goals: Pharmacokinetic characterization and an analytical method for the quantification of JIB-04 are necessary for the further development of this small molecule. The objective of this study is to develop and validate a UHPLC-MS/MS method for the quantification of JIB-04 in rat plasma, and to apply the method to the pharmacokinetic study of JIB-04 in rat plasma after intravenous (i.v.) and oral administration.

Methods: The assay was performed on a 6500+ Triple Quad LC-MS/MS System equipped with an ExionLC UHPLC unit (AB SCIEX LLC, CA, USA) and an ACE Excel 2 SuperC18 Column (50 x 2.1 mm, 2 μm, Advanced Chromatography Technologies Ltd., Aberdeen, Scotland, UK). The optimized method used binary gradient mobile phases with water (0.05% acetic acid) as mobile phase A and ACN (0.05% acetic acid) as mobile phase B. A flow rate of 0.5 mL/min was used with 5 μL of injection volume. Running time was 3 min. Multiple Reaction Monitoring (MRM) data were collected under positive mode to detect transitions m/z 309.1→ 230.1 for JIB-04, and m/z 310.1→ 231.0 for EPH8 (Internal Standard). The method was validated according to the FDA and the EMA guidelines for bioanalytical method validation.

Results: The linearity of the calibration curves was found in the range of 0.5 – 1000 ng/mL. The intra-day and inter-day accuracy (RE%) was -7.4 % to 3.7 %, and the precision (CV%) ranged from 1.9 % to 10.2 % at LLOQ (0.5 ng/mL) and QC (1, 50 and 800 ng/mL) levels. Matrix effects were ranged from 87.6 ± 1.8 % to 101.0 ± 10.5 %, and the recoveries were between 104.8 ± 1.5 % and 135.1 ± 2.7 %. Following i.v. administration, high JIB-04 concentrations (5270 ± 527.2 ng/mL at 2 min after dosing) in plasma was observed immediately after dosing followed by a rapid decrease. After 24 h, JIB-04 concentration was only 3.0 ± 1.0 ng/mL. Following oral administration, JIB-04 was quickly absorbed into blood. JIB-04 concentration reached 122.9 ± 51.0 ng/mL within 5 min, and reached the Cmax in 0.5 ~ 4 h, then rapidly decreased. Four out of six subjects showed a 2nd peak concentration, and two subjects only showed one peak concentration, which implies there is individual difference among subjects. The oral bioavailability of JIB-04 was calculated to be 44.4%.

Conclusions: A sensitive, specific, fast, and reliable UHPLC- MS/MS method for the quantification of JIB-04 in rat plasma samples was developed and fully validated according to FDA and EMA guidelines. The method has been successfully applied to pharmacokinetic studies for the quantification of JIB-04 after i.v. and oral administration in rats.

Acknowledgements: This study was funded in part by Cancer Prevention & Research Institute of Texas (CPRIT) Core Facilities Support Awards (RP180748), and the National Institute of Health's Research Centers in Minority Institutes Program (RCMI, U54MD007605) to H.X. and D.L.; as well as by the Welch Foundation (I-1878), and by the John P. Perkins, Ph.D Endowment in Biomedical Science to E.D.M. We acknowledge Dr. Jung-Mo Ahn's lab at University of Texas at Dallas for synthesizing and providing JIB-04.