ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

10TH ANNUAL RESEARCH SYMPOSIUM

COLLABORATIVE RESEARCH IN THE HEALTH SCIENCES

Podium Session, Friday, April 17, 2020

Morning Session

9:00-9:10 am		**Introduction and Opening Remarks** **Martha A. Hass, Ph.D.** Director of Research
9:15-9:30 am		**Matthew Yacobucci, Pharm.D., BCOP** Assistant Professor, Department of Pharmacy Practice **"Characterization and Impact of Pharmacy Student Participation on Hematology/Oncology APPE Rotations in Varied Practice Settings"**
9:35-9:50 am		**Catherine M. Phelps** Candidate for MS, Molecular Biosciences **"Thioredoxin A mediated modulation of Acinetobacter Baumannii Virulence and Antibiotic Resistance"**
9:55-10:10 am		**Christopher Cioffi, Ph.D.** Assistant Professor, Department of Basic and Clinical Sciences and Department of Pharmaceutical Sciences **"A Photoswitchable ORG25543 Congener Enables Optical Control of Glycine Transporter 2"**
10:15-10:30 am		**Margaret Doll, Ph.D., M.P.H.** Assistant Professor, Department of Population Health Sciences **"Impact of the New York State Repeal of Nonmedical Vaccination Exemptions on Schools: Perspectives from a Survey of School Administrators"**
10:35-10:50 am		**Vir Singh, Ph.D.** Assistant Professor, Department of Basic and Clinical Sciences **"HIV-Tat Induces Its Neurotoxic Effects by Downregulating Sonic Hedgehog Signaling in Brain"**
10:55-11:10 am		**Manish Shah, Ph.D.** Assistant Professor, Department of Pharmaceutical Sciences **"Functional and Structural Basis of Genetic Polymorphisms in Human Cytochrome P450 2C9: Insights into the Effect of *2 Variant"**
11:15-11:30 am		**Ebot Tabe, B.M.L.S., M.S., PH.D., MB(ASCP)** Instructor, Department of Basic and Clinical Sciences **"Caspase Activation and PARP Degradation: Evidence of Francisella Tularensis-Induced Apoptosis in Mice Macrophage"**
11:30 am-11:40 am		**Close of Morning Session** **Anuja Ghorapade, Ph.D.** Dean and VP of Academic Affairs
11:30 am-12:25 pm		**Break/Research Roundtable Discussion**

Afternoon Session

12:30 - 12:40 pm		**Opening of the Afternoon Session** **Martha A. Hass, Ph.D.** Director of Research
12:45 - 1:00 pm		**Matthew Higgs** Candidate for MS, Molecular Biosciences **"Elucidating the Role of Thioredoxin in the Oxidative Stress Response of Francisella Tularensis"**
1:05 - 1:20 pm		**Robert J. Haluska, Jr.** Candidate for MS, Molecular Biosciences **"Cellular Compartmentalization During the Hyperglycemic Shift from Apoptosis to Necroptosis"**
1:25 - 1:40 pm		**Dakota Paine** Candidate for MS, Molecular Biosciences **"The Amino Acid Transport xCT Protects Macrophages from HIV Infection Through Hyper-Activation of SAMHD1"**
1:45 - 2:00 pm		**Kideok Jin, Ph.D.** Assistant Professor, Department of Pharmaceutical Sciences **"Adipsin Promotes Tumor Progression in ESR1 Mutant Breast Cancer Cells"**
2:05 - 2:20 pm		**Sumit S. Kamat** Candidate for MS, Pharmaceutical Sciences **"Crystal Structure of the Human Cytochrome P450 2C9*8 Genetic Variant"**
2:25 - 2:40 pm		**Joseph Carreno, Pharm.D.** Associate Professor, Department of Pharmacy Practice **"Prospective Observational Cohort Evaluation of Turnaround Time and Outcomes Associated with a Rapid Diagnostic Test for Nucleic Acidemia in Critically Ill Patients with Sepsis"**
2:45 - 3:00 pm		**Closing Remarks** **T. Gregory Dewey, Ph.D.** President

A big thank you
to all the presenters for your hard work
and to all the attendees for taking the time
to attend our Research Symposium.
See you next year!

Poster Abstracts

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Characterization and Impact of Pharmacy Student Participation on Hematology/Oncology APPE Rotations in Varied Practice Settings

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Background/Rationale: During Advanced Pharmacy Practice Experiences (APPEs), pharmacy students meaningfully contribute to clinical, economic, and/or humanistic patient care outcomes. With an increasing number of oncology pharmacists, pharmacy students are frequently completing APPEs in oncology settings. The full scope/impact of student contributions in the oncology setting has been sparsely characterized. Further, working as a student in an oncology setting can be intellectually and emotionally challenging, and thus, what is its impact on the students' professional identity formation?

Objective(s): The aim of this study was to characterize and evaluate the impact of APPE student pharmacist contributions to varied oncology practice settings, and the impact of the experience on the pharmacy student's professionalization.

Methods: For three APPE cycles spanning 2016-2019, students from our affiliated pharmacy college who completed any hematology/oncology (h/o) APPE were identified. Data extracted from each student's APPE evaluation in CoreELMS database included: student self-reported list of rotation activities, and APPE self-reflection describing meaningful impact on the student. APPE grades were verified to provide evidence of student aptitude. Impact of rotation activities were categorized into direct and indirect patient care. To assess the impact of student contributions on the practice site, an electronic survey was created & disseminated to the 33 preceptors of the h/o APPE cohort. For each student, <3 reflection themes were recorded; thematic analysis was applied to quantify themes of impact.

Results: 171 students completed a h/o APPE in private or hospital-affiliated ambulatory care (133) and/or inpatient (38) settings; 11 APPEs were at NCI designated comprehensive cancer centers. All but seven students (0.04%) earned a grade of B+ or higher. 932 self-reported student activities were identified. The five most common activities were: 1) evaluating patient pharmacotherapy (209); 2) in-services to medical staff (132); 3) non-chemotherapy patient counseling (99); 4) answering drug information questions (96) and 5) chemotherapy patient counseling (82). A majority of activities (64.6%) involved direct patient care.

Survey results from 16 preceptors identified the top five most impactful student activities: 1) evaluating pharmacotherapy; 2) providing pharmacotherapy recommendations during inpatient rounds; 3) medication education/ adherence resources; 4) non-chemotherapy patient counseling and 5) in-service presentations. 400 reflection themes were extracted and categorized into seven thematic categories: Practice/Research Skills/Curricular Immersion (88); Self-awareness (75); Communication Skills/Teaching/Counselling (59); Patient Interaction/care (50); Interprofessional education/team-based collaboration (49); Professionalization (40); Career Development/Pharmacists' Roles (39).

Conclusions/Discussion: Pharmacy students make significant direct patient care contributions to h/o practice settings by evaluating pharmacotherapy and providing education to patients and HC personnel. Participation in h/o APPEs is highly influential to the professionalization of students, particularly in developing skills in oncology practice, patient interactions/communications, and empathy.

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Thioredoxin A Mediated Modulation of Acinetobacter Baumannii Virulence and Antibiotic Resistance

Catherine M. Phelps, Khadija Moussadek, Philip V. Truong, Erica J. Scholl, Morgan T. Hatch, Meenakshi Malik, and Nicole L. Shakerley
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Background: *Acinetobacter baumannii* has become a serious threat to global health due to its ability to rapidly develop antibiotic resistance and persist within the healthcare environment. Though antibiotics interfere with specific bacterial targets, a secondary effect of their action can be an alteration of the bacterial redox state. Pathogens resist host-derived ROS through the upregulation of their antioxidant enzymes. While traditional mechanisms of resistance have been examined in *Acinetobacter*, the antioxidant arsenal represents an unexplored contributor. In this study, we are investigating the mechanisms by which *A. baumannii* antioxidants modulate the bacterial redox environment in response to antibiotics thereby contributing to antimicrobial resistance.

Methods: We examined the contributions of antioxidant enzymes to antibiotic resistance of *A. baumannii* by utilizing transposon-insertion mutants of antioxidant proteins. Preliminary screening assays highlighted the thioredoxin system as a key component. Further studies were carried out utilizing a WT clinical isolate (Ci-79), gene deletion mutant (Δ*trxA*) and plasmid complemented strain (Δ*trxA* + *ptrxA*). These strains were characterized to examine how the loss of thioredoxin impacts *A. baumannii* antibiotic resistance. Checkerboard assays were also performed to examine the impact of thioredoxin inhibition in other clinical isolates with varying resistance patterns.

Results: Δ*trxA* has a growth disadvantage under normal growth conditions and exhibited significant increases in susceptibility to oxidative stressors. The Δ*trxA* mutant exhibited slowed growth and increased susceptibility to low doses of thiol oxidizing agent diamide. Additionally, Δ*trxA* mutant was more susceptible to several antibiotics across various categories including multiple carbapenems. Gene expression studies demonstrate Δ*trxA* upregulates compensatory antioxidant enzymes. Modulation of the thioredoxin system in other clinical isolates with diamide increases sensitivity of these strains to antibiotics. Pilot studies with trxB mutants exhibit a similar phenotype to Δ*trxA*.

Conclusions: Our results indicate that antioxidant enzymes contribute to the ability of *A. baumannii* to withstand various forms of oxidative stress, thereby impacting bacterial virulence and antimicrobial resistance. By characterizing the bacterial antioxidant enzymes that modulate antibiotic resistance, we can develop novel therapeutic strategies to combat the resistant nature of this pathogen.

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A Photoswitchable ORG25543 Congener Enables Optical Control of Glycine Transporter 2

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Glycine neurotransmission in the dorsal horn of the spinal cord plays a key role in regulating nociceptive signaling, but in chronic pain states reduced glycine neurotransmission is associated with the development of allodynia and hypersensitivity to painful stimuli. This suggests that restoration of glycine neurotransmission may be therapeutic for the treatment of chronic pain. Glycine Transporter 2 inhibitors have been demonstrated to enhance glycine neurotransmission and provide relief from allodynia in rodent models of chronic pain. In recent years, photoswitchable compounds have been developed to provide the possibility of controlling the activity of target proteins using light. In this study we have developed a photoswitchable non-competitive inhibitor of Glycine Transporter 2 that has different affinities for the transporter at 365 nm compared to 470 nm light.

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Impact of the New York State Repeal of Nonmedical Vaccination Exemptions on Schools: Perspectives from a Survey of School Administrators

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Background: In an effort to curb recent measles outbreaks, the New York State (NYS) legislature repealed the religious school-entry vaccination exemption for public and nonpublic school students. Potential indirect consequences of the law include changes in student enrollment and absenteeism related to compliance, increases in student medical vaccination exemptions, and an increased workload for school administration responsible for assuring compliance. This research aimed to assess the impact of the repeal on upstate NY schools from the perspective of school administrators, who represent the front-line workforce for its enforcement and assurance.

Methods: We sent an electronic survey to upstate NY (i.e. non-New York City) public and nonpublic school administrators in November 2019 using the REDcap secure survey and data collection tool. Survey questions were related to the school's experiences complying with the new legislation, and changes in student enrollment, absenteeism, and medical vaccination exemptions associated with the new law. The survey also included questions regarding the legislation's effect on school budgets for nonpublic schools charging tuition. Electronic reminders were sent to schools if the survey was not completed within 10 business days. Phone calls were made to identify correct administrator contact information for schools with undeliverable, invalid, or blank email addresses. We used basic descriptive statistics to analyze data from completed surveys, and compared survey responses between nonpublic and public schools using rate ratios and 95% confidence intervals (CIs), since we hypothesized that school experience may differ by school type.

Results and Conclusions: Electronic surveys were sent to 908 nonpublic and 2,901 public schools with administrator contact information, representing 3,809 (97.8%) upstate NY schools. As of March 15, 2020, 457 (12.0%) recruited schools completed the survey, and 31 (0.8%) invitations were undeliverable. Nonpublic schools were 2.5 (95% CI: 2.1, 3.0) times more likely to respond, with 22.3% and 8.8% response rates among nonpublic and public schools, respectively. On average, schools spent 15.0 (95% CI: 11.2, 18.9; median: 5, interquartile range [IQR]: 3, 15) hours on compliance meetings and/or written correspondence and 12.8 (95% CI: 10.1, 15.5; median 5, IQR: 2, 12) hours addressing questions from parents/guardians related to the legislation; results from nonpublic and public

schools were similar. A change in student enrollment was reported by 45.6% (95% CI: 40.9%, 50.3%) of schools, with nonpublic schools 1.6 (95% CI: 1.3, 1.9) times more likely to experience enrollment changes. The law affected student attendance at 30.2% (95% CI: 26.0%, 34.7%) of schools, with no difference by school type. A change in medical exemptions was reported at 16.2% (95% CI: 12.9%, 20.0%) of schools, with no differences between public or nonpublic schools. Among nonpublic schools charging tuition, 52.0% (95% CI: 44.6%, 60.0%) reported that enrollment changes financially impacted their school, affecting a mean of 10.7% (95% CI: 7.7%, 13.8%; median: 8%, IQR: 2%, 12%) of the school's operating budget. Collectively, these results indicate a significant proportion of schools experienced changes in student enrollment, absenteeism, and medical exemptions; however, these results may not be generalizable to survey nonparticipants.

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HIV-Tat Induces Its Neurotoxic Effects by Downregulating Sonic Hedgehog Signaling in Brain

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Background/Objectives: Efficacious combined antiretroviral therapy (cART) has prolonged the life span of Human Immunodeficiency Virus Type-1 (HIV-1) infected individuals. However, persistent inflammation and drug related toxicities, exacerbate age-related comorbidities such as cardiovascular and neurological disorders. More than 50% of HIV infected individuals display neurocognitive impairment specifically related to memory and learning. Despite undetectable viral load, HIV infected individuals exhibit significant levels of Tat protein in plasma as well as in cerebrospinal fluid. Previous studies from our lab as well as others have established that HIV-Tat has neurotoxic properties and mediates BBB dysregulation as well as activation of CNS resident cells including astrocytes and microglia. Further, our recent studies have indicated that HIV-1 can cause BBB damage by downregulating Sonic hedgehog (Shh) signaling. Thus, in this study, we investigated if Tat exerts its neurotoxic effects via dysregulating Shh signaling. We used a conditional, CNS-specific Tat transgenic murine model to physiologically recapitulate HIV disease in post- cART era.

Method: HIV-Tat+ and Tat- mice were exposed to HIV-Tat for 3 weeks by feeding doxycycline diet. Tat+ Transgenic mice conditionally express HIV-Tat protein (1-86 aminoacids) in astrocytes, driven by GFAP promoter, thus Tat is expressed in a CNS targeted manner. Brain Infiltrating Leukocytes (BILs) were isolated and quantitated as a marker for BBB integrity in Tat+ vs Tat- mice using flow cytometry. Further, brain tissues were processed to detect the expression levels of Shh signaling components, tight junction proteins and adhesion molecules by RT-qPCR and immunoblotting. Human astrocytes and brain endothelial cells were treated with purified HIV-Tat protein (100nM) and SAG (500nM), alone or in combination for various time points and cell lysates were used in immunoblotting assays.

Results: Results indicate significantly increased number of brain-infiltrating leukocytes (BILs) in Tat+ mice with concomitant downregulation of Shh signaling as compared to Tat- mice. Administration of Smoothened agonist (SAG), a Shh mimetic, significantly reduced the number of BILs in Tat+ mice. Further, Tat induced activation of ERK1/2 MAP Kinase and downregulation of tight junction proteins in human brain microvascular endothelial cells was rescued by SAG. Interestingly, Tat protein downregulated Shh expression in astrocytes, which might cause disruption in astrocyte-endothelial cell crosstalk, a crucial factor for maintenance of BBB homeostasis. Overall, this study suggests that HIV-Tat induced adverse effect on CNS resident cells can be rescued by pharmacologic stimulation of Shh signaling.

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Functional and Structural Basis of Genetic Polymorphisms in Human Cytochrome P450 2C9: Insights into the Effect of *2 Variant

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The human cytochrome P450 2C9 (CYP2C9) plays a crucial role in the metabolic clearance of a wide range of clinical therapeutics. The *2 allele is one of the most prevalent genetic variations in CYP2C9 that is found in various ethnic populations. A marked reduction of catalytic activity towards many important drug substrates has been demonstrated by CYP2C9*2, which represents an amino acid variation at position 144 from arginine to cysteine. In the crystal structure of the CYP2C9*2 complexed with losartan, the Arg144Cys variation disrupts the hydrogen bonding interactions that were observed between the side chain of arginine and neighboring residues in the wild-type (WT) ligand-free structure and the losartan complex of CYP2C9. The effect of such local change is transduced to the larger secondary structural elements involving important helices and to the distal active site altering the amino acid architecture and binding of losartan. The crystal structure revealed the orientation of losartan in the active site to be unproductive, however, the computational docking studies indicated that losartan likely reorients within CYP2C9 to a more productive mode. Furthermore, the binding studies using enzymatic assays and surface plasmon resonance illustrated lower activity of the CYP2C9*2 towards losartan compared to the WT. Together, the findings yield valuable insights into the decreased hydroxylation activity of losartan in patients carrying CYP2C9*2 allele, and provide a useful framework to investigate the effect of single nucleotide polymorphism that leads to altered metabolism of diverse drug substrates.

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Caspase Activation and PARP Degradation: Evidence of Francisella Tularensis-Induced Apoptosis in Mice Macrophage

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Francisella tularensis (Ft) is the causative agent of tularemia—also called rabbit fever or deer fly fever. This pathogen is a facultative intracellular bacterium that is capable of inducing inflammation and cell death in host cell macrophages leading to a disease that typically attacks the skin, eyes, lymph nodes and lungs. The aim of this study was to investigate the mechanism by which Francisella encoded factor critically modulates cell death pathway(s) and cytokine production leading to an exaggerated pro-inflammatory phase of infection. We obtained evidence that suggest that fragmentation or cleavage of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) of infected macrophages was induced by activation of caspase 3. Also, there was observed transient (p10 to p20) activation of caspase 1 (previously shown to be suppressed at the early phases of infection)—thus allowing the intracellular replication of Ft live vaccine strain (Ft LVS). All these events were observed within 24 to 48 h post infection, and maximum degradation of caspase 3 and PARP occurred at 48 h. The fragmentation or cleavage of PARP but not caspase-3 resulting from activation of this apoptotic pathway was prevented by the pan caspase inhibitor Z-VAD-FMK. Therefore, Ft associated infection of mice macrophage results to apoptosis possibly through presumptive pathways approximating the intrinsic apoptotic pathway.

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Elucidating the Role of Thioredoxin in the Oxidative Stress Response of Francisella Tularensis

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Background: Francisella tularensis (Ft) is an intracellular Gram-negative bacterial pathogen and is classified as a category A select agent by the CDC owing to its potential to be used as a bioterror agent. Ft encodes a repertoire of antioxidant enzymes to counter oxidative stress by the host. The thioredoxin system plays an important role in maintaining redox-homeostasis within bacterial cells. In this study, we are investigating the mechanisms by which thioredoxin(s) cooperate with other antioxidant enzymes of Ft to resist oxidative stress.

Methods: We generated gene deletion mutants of thioredoxin genes trx and trx1. These strains were characterized to determine if the loss of thioredoxin(s) reduces the ability of Ft to resist oxidative stress and survive and replicate in macrophages. Gene expression studies were performed to understand the mechanisms of regulation of antioxidant enzymes by thioredoxin(s).

Results: The Δtrx, but not Δtrx1, mutant is highly sensitive to oxidative stress as compared to wild type Ft LVS. The Δtrx mutant fails to survive and replicate in cultured macrophages. Gene expression studies demonstrate that loss of trx is associated with significantly downregulated expression of antioxidant genes katG and ahpC, as well as oxidative stress response regulator, oxyR, when exposed to oxidative stress.

Conclusions: Our results demonstrate that the trx gene is required for oxidative stress resistance of Ft. The results demonstrate that the expression of oxyR under oxidative stress is controlled by trx. Studies investigating the mechanisms of this regulation are underway. This study highlights the complex intracellular survival mechanisms of Ft.

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Cellular Compartmentalization During the Hyperglycemic Shift from Apoptosis to Necroptosis

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Eukaryotes undergo two distinct principal modes of cell death, apoptosis and necroptosis. Apoptosis is non-inflammatory whereas necroptosis is highly inflammatory. Previously we showed that there is a shift in cell death mechanisms toward necroptosis during hyperglycemia. In addition, we showed that the hyperglycemic shift to necroptosis exacerbated neonatal hypoxia-ischemia (HI) brain injury in vivo. However, the mechanics behind the compartmentalization of proteins during this shift is not well understood. Cell death proteins serve different roles depending upon the cellular compartment in which they are found. During necroptosis, in particular, factors including RIP1, RIP3, and MLKL have distinct roles when they are found in the membrane vs. the mitochondria vs. the cytoplasm. We will analyze cellular compartmentalization of these factors during the hyperglycemic shift to necroptosis using density gradient ultracentrifugation. While in the mitochondria, these factors have effects on metabolism and ROS production. We will investigate the role of ROS and metabolism using antioxidants as well as inducers of ROS. The role of RIP1 in trafficking the necessary proteins for necroptosis will be studied by utilizing a CRISPr knockout RIP1 cell line, studying the effects on cellular compartmentalization during the hyperglycemic shift to necroptosis.

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The Amino Acid Transport xCT Protects Macrophages from HIV Infection Through Hyper-Activation of SAMHD1

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We have previously shown that sulforaphane (SFN), a natural compound found in cruciferous vegetables, protects macrophages from HIV infection. We further demonstrated that this protection depends on the transcription factor Nrf2. SFN interferes with the constitutive turnover of Nrf2. This interference leads to accumulation of Nrf2 and presumably allows for increased expression of Nrf2 responsive genes. Nrf2 upregulates several genes associated with the antioxidant response. We therefore hypothesized that one or more of these Nrf2 responsive gene products is/are responsible for blocking HIV infection in macrophages. Here we show that the Nrf2 responsive gene product: SLC7A11 (xCT) promote hyperactivation of SAMHD1 through both removal of an inhibitory phosphate and possibly increased tetramer formation. We further demonstrate that SFN may impair HIV-2 Vpx mediated SAMHD1 degradation by dismantling the CRL4 DCAF1 ubiquitin ligase complex.

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Adipsin Promotes Tumor Progression in ESR1 Mutant Breast Cancer Cells

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ESR1 point mutations at ESR1 ligand binding domain are frequently identified in metastatic tumor, cfDNA (Cell free DNA), ctDNA (Circulating tumor DNA) and ptDNA (plasma tumor DNA) derived from ESR1 positive breast cancer patients treated SERMs, SERDs and AIs. ESR1 mutations enhance ESR1 transcriptional activity in the absence of estrogen and induce estradiol independent growth as well as increase resistance to SERM or SERD in ESR1 mutant overexpressed cells. ESR1 mutations are found more frequently in cfDNA and tumor DNA from patients with metastatic disease compared to patients with primary tumor, Although SERM, SERD, AI and CDK4/6 inhibitor therapies have demonstrated preclinical and clinical benefits for breast cancer with ESR1 mutations, the development of resistance remains a significant challenge and the detailed mechanisms and potential therapeutic targets in metastatic breast cancer with ESR1 point mutations is yet to be revealed.

Tumor and organ microenvironments are crucial for cancer progression and metastasis. Crosstalk between multiple non-malignant cell types in the microenvironments such as blood and lymphatic endothelial cells and cancer cells promotes tumor growth and metastasis. Possibly the anti-angiogenic and anti-lymphangiogenic therapies can be combined with each other for improved outcomes for patients.

In this study, we identified secretion of Adipsin from two different genome-edited MCF-7 harboring Y537S and D538G ESR1 cells compared to wild-type cells using the Human Cytokine Array Q440 to quantitatively detect 440 human inflammatory factors, growth factors, chemokines, receptors, and cytokines simultaneously. We validated that the expression of Adipsin was highly upregulated in MCF-7-Y537S and D538G ESR1 mutant cells by real time qRT-PCR and ELISA.

Interestingly, we observed that when cells were cultured in estrogen deprivation, the mRNA expression of the Adipsin was significantly increased in MCF-7-ESR1 mutant cells while the treatment of tamoxifen and fulvestrant abrogated the upregulation of Adipsin. These results suggest that Adipsin is an ER target gene. Since the cleavage of factor B by Adipsin results in the conversion of C3 to C3a, which binds to its cell surface receptor (C3aR), our data showed that the C3a production was significantly increased in ESR1 mutant cells compared to WT by ELISA. In addition, we found that C3aR expression was increased in ESR1 mutant cells. To elucidate that C3aR signaling pathway promotes the proliferation of MCF-7-ESR1 mutant cells, we

examined the ESR1 mutant cell viability using SB290157, C3aR inhibitor, and found that ESR1 mutant cell growth was decreased in the treatment of SB290157. Furthermore, we found that the apoptosis was significantly induced in ESR1 mutant cells compared to WT by Annexin V assay.

These findings implicate Adipsin signaling as a critical event in breast cancer metastasis with ESR1 mutations. Further, these studies suggest that Adipsin act as key regulators orchestrating breast cancer with ESR1 mutations. Therefore, we have provided evidence that supports the hypothesis that functional inhibition of the Adipsin signaling pathway has the potential to circumvent breast cancer metastasis.

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Crystal Structure of the Human Cytochrome P450 2C9*8 Genetic Variant

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Cytochrome P450 (CYP) enzymes are one of the major xenobiotic metabolizing enzymes with increasing importance in pharmacogenetics. The human CYP2C9 enzyme that metabolizes over 15% of clinical drugs including warfarin, losartan, tolbutamide, etc. is highly polymorphic with more than eighty genetic variations identified thus far. Many of these variants have demonstrated significantly reduced activity compared with the wild-type (WT) enzyme. The CYP2C9*8 allele, prevalent among different ethnic population and predominantly found in African-American population with a frequency of around 0.06, is associated with altered clearance of several drug substrates of CYP2C9. The *8 represents an amino acid variation from arginine to histidine at position 150 (R150H). The R150H variant was generated using CYP2C9 WT construct by site-directed mutagenesis, and the enzyme was expressed in E. coli followed by protein purification and crystallization. The CYP2C9*8 was crystallized in the presence of the drug substrate losartan and the structure was determined using X-ray crystallography at 2.3 Å resolution. The R150H, found on the surface of the protein on D-helix that is distal from the active site, illustrates minimal effect on the overall conformation of the protein compared to the WT. Despite subtle changes in the structure itself, there were clear differences in the binding of losartan compared to the previously solved CYP2C9 WT complex. One molecule of losartan was bound in the active site and one on the surface, consistent to that observed in the WT complex. However, unlike the WT complex, the losartan in the access channel was not observed in the *8 complex. The region near the access channel was more compact than the WT enzyme. Furthermore, the losartan turn-over rates measured using enzymatic assays differed significantly, with the variant demonstrating marked reduction in activity than the WT enzyme. Together, our findings from multiple techniques suggest an alternate mechanism may be involved in reduced activity of this variant located on the surface that leads to differences in binding of losartan near the active site. The results yield insight into the role of simultaneous binding of multiple substrate molecules, orientation of important amino acid side chains and altered hydroxylation profile of losartan with this variant.

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Prospective Observational Cohort Evaluation of Turnaround Time and Outcomes Associated with a Rapid Diagnostic Test for Nucleic Acidemia in Critically Ill Patients with Sepsis

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Introduction: Blood cultures (BC) are considered the standard of care for diagnosing bloodstream pathogens in sepsis. However, BC are limited because they require viable organisms and larger inoculum sizes as compared to culture-free technology. Despite the potential advantage of culture-free technologies, the clinical utility for specific devices such as T2Biosystems is unknown. This study aimed to evaluate the clinical utility of and outcomes associated with nucleic acidemia for critically ill patients with sepsis.

Methods: Prospective observational cohort study of adult patients (age > 18), who were admitted to the intensive care unit with suspected or confirmed sepsis. Those with neutropenia or pregnancy were excluded. The primary endpoint was specificity, sensitivity, and accuracy of the T2Bacteria test as compared to the nearest blood culture prior to the test (gold standard). Secondary endpoint included turnaround time for T2Bacteria result. Inpatient mortality, hospital length of stay and ICU length of stay were compared between subjects with positive and negative T2Bacteria tests. The primary outcome was analyzed using binomial proportions and 95% confidence intervals using the Clopper-Pearson method. Sensitivity = True Positives/ (True Positive + False Negatives), Specificity = True Negative / (True Negative + False Positive), Accuracy = (True Positive + True Negatives)/ All Results. Nonparametric continuous data were analyzed using Mann-Whitney U tests. Categorical data were evaluated using chi-squared tests or Fisher's Exact Test as appropriate.

Results: 164 patients were screened, 23 patients were enrolled, 19 patients were tested. Four patients had positive T2 tests (2 E. coli, 1 K. pneumoniae, 1 P. aeruginosa). The median (IQR) age was 63 (60 – 75), the median (IQR) Charlson Comorbidity Index was 3 (2-4), 7 (36.8%) were male, and 17 (89.5%) were white. The specificity (95% CI) and accuracy (95% CI) of the T2Bacteria test were each 78.9% (54.4% - 93.9%). Sensitivity was incalculable due to lack of positive blood cultures. The median (IQR) time from blood culture collection to results was 16.8 hours (12.4 – 23.1). This included a median (IQR) test time of 3.5 hours (3.5 – 3.6). Inpatient mortality was statistically significantly higher in patients with positive T2Bacteria tests (75% vs. 6.7%, p = 0.01). There were no statistically significant differences in hospital length of stay (8 vs. 7 days, p = 0.47) or ICU length of stay (3 vs. 5 days, p = 0.47).

Conclusions: T2Bacteria may be useful in the diagnosis of nucleic acidemia in patients with critical illness due to sepsis. A pre-screening algorithm, such as the one utilized in this study, may help improve the implementation of the diagnostic test within the intensive care unit. Nucleic acidemia in the setting of negative blood cultures may be associated with increased risk of mortality. Further research is needed to optimize the utilization of this test within the intensive care unit and to determine the impact of nucleic acidemia on patient outcomes.

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