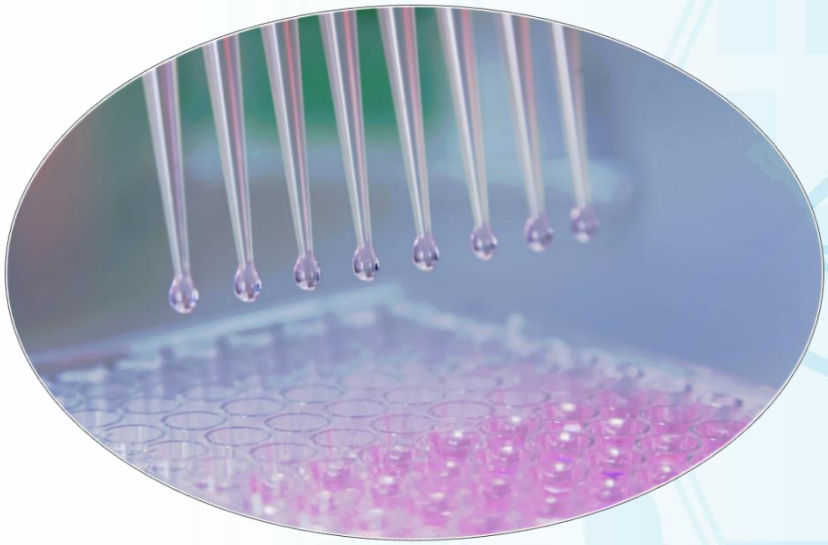
9th Annual AAPS-NDSU Pharmaceutical Research Symposium 2025



7:30 AM-4:00 PM, Thursday, April 17th 2025
Oceti Sakowin Ballroom, Memorial Union, NDSU

NDSU COLLEGE OF HEALTH AND HUMAN SCIENCES

aaps American Association of Pharmaceutical Scientists

American Association of Pharmaceutical Scientists (AAPS) NDSU Student Chapter

The American Association of Pharmaceutical Scientists (AAPS) North Dakota State University (NDSU) Student Chapter was established in the spring of 2005 to foster innovation, collaboration, and excellence in pharmaceutical sciences research. Our chapter is a dynamic platform for graduate students and postdoctoral researchers working in diverse fields such as drug delivery, drug development, pharmacology, pharmaceutical biotechnology, and medicinal chemistry. By promoting the AAPS mission, we strive to advance pharmaceutical science and contribute to discovering novel therapies that enhance global health.

A core initiative of our chapter is the Annual AAPS-NDSU Pharmaceutical Research Symposium, which provides a premier forum for students, researchers, faculty, and industry professionals to exchange ideas, present cutting-edge research, and explore emerging directions in biomedical sciences. This symposium emphasizes the importance of interdisciplinary collaboration by bringing together experts from pharmaceutical sciences, engineering, biochemistry, microbiology, polymer sciences, and animal and plant sciences. The overwhelming response to our call for poster presentations reflects a growing enthusiasm for scientific discourse. The selected abstracts address many pressing health challenges, including cancer, Alzheimer’s disease, asthma, and diabetes and much more. We hope this event will serve as a catalyst for groundbreaking research collaborations and impactful scientific advancements.

The Ninth Annual AAPS-NDSU Pharmaceutical Research Symposium 2025 is a testament to our organizing team's dedication and hard work. This event would not have been possible without the unwavering support of our faculty advisors and student leaders. Dr. Jagdish Singh, Chair of the Department of Pharmaceutical Sciences, has provided invaluable guidance throughout the planning process, ensuring the symposium aligns with our mission of fostering high-quality research.

Under the leadership of Chinenye Edith Muolokwu, Chair of the AAPS-NDSU Student Chapter, our team has worked tirelessly to bring this event to life. Shubhashri Ambhore, Chair-Elect, and her committee managed the event logistics, including speaker coordination, awards, and honoraria. Benjamin Tagoe, Media and Public Relations Coordinator led outreach efforts, ensuring broad participation from the university and beyond. Babita Lamsal, AAPS Student Chapter Coordinator, led the abstract submission and review process, organizing a structured and engaging poster session. Seyedsaber Mirabdali, AAPS Student Chapter Coordinator, and his team provided essential technical support to ensure a seamless

experience for all attendees. We extend our heartfelt gratitude to Diana Kowalski for her steadfast support in making this symposium a resounding success.

We thank Kasen Anderson and Onna Scheuer for their dedication to overseeing the smooth execution of the event. We also appreciate the faculty members who devoted their time and expertise to refining every aspect of this symposium. On behalf of the AAPS-NDSU Student Chapter, we welcome you to the Ninth Annual AAPS-NDSU Pharmaceutical Research Symposium 2025. We hope this event inspires you, fosters meaningful discussions, and opens new avenues for collaboration in the pharmaceutical sciences.

Chinenye Edith Muolokwu
Chair

Shubhashri Ambhore
Chair elect

Ying Kang
Vice Chair

Henryata Rozario
Secretary

Chukwuebuka Ozoude
Treasurer

Benjamin Tagoe
Media & Public Relations Coordinator

Babita Lamsal
AAPS Student Chapter Coordinator

Seyedsaber Mirabdali
Voluntary Committee Coordinator

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Symposium Schedule

Registration **Breakfast** **Poster Set Up**	**Legacy Lounge** **Prairie Rose** **Oceti Sakowin Ballroom**	**7:30 – 8:30AM**
Introductions	**Oceti Sakowin Ballroom**	**8:30 – 9:00AM**
Welcome Address	Teresa Conner Dean- College of Health and Human Sciences	
Introduction	Chinenye Edith Muolokwu Chair – AAPS-NDSU Student Chapter	
Introduction	Dr. Jagdish Singh Chair- Dept. of Pharmaceutical Sciences	
Guest Speaker	**Oceti Sakowin Ballroom**	**9:00 – 10:00AM**
Dr. Casim Sarkar	Topic: Analysis and engineering of multivalent protein interactions	
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Dr. Viera Lukacova	Topic: Utility of PBPK in drug and drug product development process	
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Awards/Closing Remarks	**Oceti Sakowin Ballroom**	**3:00 – 4:00PM**

Sarkar, Casim A, Ph.D.
Professor
Department of Biomedical Engineering
University of Minnesota

Dr. Casim A. Sarkar is a Professor of Biomedical Engineering at the University of Minnesota, specializing in biomolecular engineering, synthetic biology, and systems biology. He earned his B.S. in Chemical Engineering from the University of Texas at Austin, followed by a Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology. He completed postdoctoral training in Biochemistry at the University of Zurich.

Dr. Sarkar's research focuses on understanding cellular decision-making principles and designing molecular and cellular therapeutics. His multidisciplinary approach integrates experimental techniques, computational modeling, and engineering analysis to develop predictive frameworks for biological processes. His lab has published in high-impact journals such as *Cell*, *PNAS*, and *Nature Communications*, and he has secured funding from the NIH, NSF, American Heart Association, and American Diabetes Association.

Beyond research, Dr. Sarkar is an active contributor to the scientific community. He has served as a standing member of NIH study sections, a Fellow of the American Institute for Medical and Biological Engineering (AIMBE), and a member of AIMBE's Diversity and Inclusion Committee. As Director of Graduate Studies (2020–2024), he implemented initiatives to enhance student mental health and professional development.

His ongoing research projects include designing multivalent protein interactions, engineering cell signaling mechanisms, developing plant immune receptor technologies, and advancing receptor-mediated transcytosis for drug delivery. Dr. Sarkar's contributions to biomolecular engineering continue to influence therapeutic innovations and the broader field of biomedical science.

Viera Lukacova, Ph.D.
Chief Scientist
PBPK / Cheminformatics Solutions
Simulations Plus, Inc (NASDAQ: SLP)

Dr. Viera Lukacova is the Chief Scientist at Simulations Plus, Inc., where she has led pioneering research in mechanistic modeling for 20 years. She has played a critical role in advancing industry-renowned software packages, such as GastroPlus®, DDDPlus™, and MembranePlus™, which have transformed drug development, formulation, and PBPK modeling. Her expertise has been instrumental in driving multiple industry collaborations, serving as Principal Investigator (PI) on five projects focused on PBPK modeling, drug-drug interactions, and oral absorption studies. Additionally, she has contributed as a co-investigator and advisor on four projects, enhancing modeling approaches for ocular, intranasal-pulmonary, transdermal, and intraoral drug delivery.

Dr. Lukacova has also led two FDA-funded projects as a PI, focusing on PBPK simulations for long-acting injectable microspheres, and has co-investigated five additional FDA-funded studies on PBPK/PD modeling for ophthalmic and dermal drug products. Dr. Lukacova regularly participates in meetings and events organized by various scientific organizations and institutions (AAPS, ISSX, CRS, ACCP, ACoP, FDA, CRCG, and others) where over the last 20 years she presented or coauthored over 80 poster presentations, delivered over 30 invited speaker presentations, and organized, co-organized, or chaired five symposia.

Academically, she holds an M.S. in Biochemistry and Biotechnology from the Slovak University of Technology and a Ph.D. in Pharmaceutical Sciences from North Dakota State University, followed by a postdoctoral fellowship. With 60 peer-reviewed publications, two book chapters, and extensive manuscript review experience, she continues to shape the field of PBPK modeling and drug development.

Abstract #001

Targeting Tumor Survival: A Selective Approach to Pancreatic Cancer Therapy Targeting COPZ1

Allana C. F. Martins, Roberto Gomes

Department of Pharmaceutical Sciences, College of Health and Human Sciences, North Dakota State University, Fargo, ND, 58078, United States.

Purpose:
Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies due to its late-stage diagnosis and lack of effective treatment options. This study aims to explore COPZ1, a component of the coatomer protein complex (COPI), as a novel therapeutic target for PDAC. COPZ1 is critical for cellular processes and PDAC cells exhibit dependency on COPZ1 for survival, while normal cells rely on its paralogous COPZ2. We hypothesize that targeting COPZ1 function will disrupt COPI vesicle formation, leading to disorganization of the endoplasmic reticulum (ER) and Golgi complex, and subsequent programmed cell death in PDAC cells.

Methods:
In this study, we identified and synthesized small-molecule COPZ1 inhibitors, evaluated their selectivity and efficacy in vitro, and investigated the underlying molecular mechanisms driving tumor cell death, including Golgi stress and ferroptosis.

Results:
Molecular docking identified potential COPZ1 inhibitors from 84 compounds, including 4-aminoantipyrine and enedione fragments. Four lead compounds were selected based on binding interactions. RT-qPCR confirmed similar COPZ1 and COPZ2 expression in normal cells, but we observed COPZ2 downregulation in tumor cells. MTT assays showed that compounds inhibited growth in PDAC cells (MIA PaCa-2, AsPC-1), but not in normal cells (HPNE). The compounds induced lipid droplet formation and oxidative stress in cancer cells, evidenced by increased lipid peroxidation.

Conclusion:
This research could establish COPZ1 as a promising target and pave the way for targeted therapies that selectively suppress PDAC cells, ultimately improving treatment outcomes and patient survival rates.

Abstract #002

Synthesis of Dextran conjugates

Biswas Anik[1], Quadir Mohiuddin[2*], Mathew Sijo[3*]

[1]Materials and Nanotechnology, North Dakota State University, Fargo, USA
[2]Department of Coatings & Polymeric Materials, North Dakota State University, Fargo, USA
[3]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, USA

Purpose:
Dextran, a biocompatible polysaccharide, is widely utilized for conjugating bioactive molecules in biomedical applications. This study investigates the synthesis of dextran conjugates, employing chemical strategies such as carbodiimide coupling to attach functional ligands to dextran's hydroxyl backbone.

Methods and Results:
The resulting conjugates were characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM) to assess size and morphology, alongside Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) to confirm structural modifications. Ultraviolet-visible spectroscopy (UV) quantified the degree of labeling (DOL) and degree of conjugation (DOC), ensuring successful functionalization.

Conclusion:
In conclusion, these dextran conjugates, with their enhanced structural and functional properties, may offer a robust platform for advancing targeted drug delivery systems in biomedical research and therapeutic applications.

Abstract #003

Identifying ligand-specific RAGE antagonists based on monoclonal antibodies

Anupom Deb Nath[1], Stefan Vetter, Ph.D.[1]

[1]*Dept. of Pharmaceutical Sciences, North Dakota State University*

Purpose:
The receptor for advanced glycation end products (RAGE) is a cell surface receptor belonging to the immunoglobulin superfamily that has functions on normal physiology and got upregulated in diseased conditions such as diabetes and cancer. The full-length RAGE protein comprises a variable-like (V) domain, constant type 1 (C1) and constant type 2 (C2) domains, a transmembrane segment, and a cytoplasmic tail. RAGE has multiple ligands such as AGEs, HMGB1, S100 proteins, amyloid β peptides, MAC-1, and nucleic acids. Targeting RAGE to block specific ligand interactions holds therapeutic potential across multiple diseases. This study aims to identify ligand-specific RAGE antagonists using a live-cell NanoLuciferase system.

Methods:
The NanoLuciferase complementation system, NanoBiT, was utilized to investigate RAGE-ligand interactions. For this purpose, HEK293 cells were stably transfected with an LgBiT-tagged human RAGE (hRAGE) receptor. To complement the LgBiT, SmBiT-tagged S100B and HMGB1 were employed, as both ligands are known to bind RAGE. Following complementation of LgBiT and SmBiT, luciferase activity was measured upon the addition of furimazine substrate. Changes in luminescence signals were assessed to identify ligand displacement by anti-RAGE antibody binding.

Results & Conclusion:
The nLuc/LgBiT_hRAGE plasmid was successfully designed, expressed, and used to generate stable HEK293 cell lines. These stable cell lines expressed the target receptor and exhibited luciferase activity. SmBiT-tagged S100B and HMGB1 proteins were successfully constructed, expressed, and purified. The interaction between LgBiT_hRAGE and SmBiT-tagged proteins effectively reconstituted the NanoLuciferase enzyme. Furthermore, the NanoBiT system demonstrated the ability to detect competition with untagged S100B, confirming its utility in studying RAGE-ligand interactions.

Abstract #004

Mesenchymal Stem Cell (MSC) Derived Exosomes Loaded with Paclitaxel for the Management of Pancreatic Ductal Adenocarcinoma (PDAC).

Anurag Banerjee[1], Dr. Buddhadev Layek[1]

[1]*Department of Pharmaceutical Sciences, North Dakota State University*

Purpose:
Exosomes are a type of extracellular vesicles ranging from 50-200nm in size. Exosomes derived from mesenchymal stem cells (MSCs) are promising drug delivery vehicles for transporting therapeutic molecules like drugs, proteins and nucleic acids to tumor sites, potentially improving treatment efficacy and reducing adverse effects. Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with a low survival rate. This study aims to explore the isolation, characterization, and drug-loading efficiency of MSC-derived exosomes for delivering paclitaxel (PTX) to pancreatic cancer cells.

Method:
Exosomes were isolated from MSC-conditioned media using a tangential flow filtration (TFF) system, yielding several fold greater exosomes than traditional ultracentrifugation. Characterization was performed using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and zeta potential measurements. Flow cytometry confirmed exosome identity using markers CD63 and CD81, while CD90 validated their MSC origin. Protein quantification was done using a bicinchoninic acid (BCA) assay. Exosomes were loaded with PTX via ultrasonication. Cytocompatibility of blank exosomes was tested on pancreatic cell lines, and IC50 values of free PTX and PTX-loaded exosomes were determined using Panc-1 and BXPC-3 cell lines.

Results:
PTX-loaded exosomes exhibited lower IC50 values compared to free PTX solutions in pancreatic cancer cell lines, indicating enhanced cellular uptake and optimal drug release. These findings suggest that MSC-derived exosomes hold significant potential as a drug delivery platform for pancreatic cancer treatment, warranting further investigation.

Abstract #005

A novel liposome co-delivery adavosertib and paclitaxel for the treatment of ovarian cancer

Arpita Ghosal, Buddhadev Layek

Department of Pharmaceutical Science, North Dakota State University

Purpose:
Ovarian cancer is the most lethal gynecological cancers with 5-year survival rate is only 30%. Chemotherapy is non-specific, have unwanted side effects, fails to reach optimum cytotoxic concentration in tumor site, development of chemoresistance. Radiotherapy unsuccessful due to location of the organ. Thus, to increase effective concentration of the drug at tumor site, reduction of the side effects and prevent chemoresistance our lab has developed nano-vesicle based formulation for delivery of two drugs, targeting different pathways, at tumor site utilizing enhanced permeability and retention effect (EPR). In this study, we designed a novel liposome-based therapy where the liposome is loaded with two drugs adavosertib (ADA) a Wee1 inhibitor and paclitaxel (PTX) taxane based chemotherapy which blocks cell division by inhibiting microtubule.

Method:
The liposomal formulation was prepared using thin film hydration method. Encapsulation efficiency was measured using High Performance Liquid Chromatography (HPLC). Stability study was done for 5 weeks storing the formulations at 4°C. Cytotoxicity assay was performed using SKOV-3 ovarian cancer cell line.

Result:
The encapsulation efficiency was 41.79% for ADA and 60.44% for PTX. The mean particle size, polydispersity index (PDI) and charge of liposome were 100.15 nm 100.15 nm ± 0.39 and 0.158 ± 0.011, -20.85 ± 0.44 respectively. The IC50 for ADA and PTX were 61.87 nM and 74.44 nM respectively. Liposome co-delivering adavosertib and paclitaxel for the treatment of ovarian cancer was prepared and physicochemical characteristics were studied. Cytotoxicity assay was performed.

Abstract #006

Non-Invasive Intranasal delivery of CBD and pApoE2 to the brain using engineered PLGA nanoparticles for the treatment of AD

Arun Kumar Mahanta[1], Avinash Gothwal, Jagdish Singh[1]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, College of Health and Human Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

Purpose:
Minimizing Aβ plaques and the resulting inflammation may help slow down Alzheimer's Disease (AD) advancement. Cannabidiol (CBD) is widely recognized for its neuroprotective, antioxidant, and anti-inflammatory properties, while ApoE2 plays a crucial role in binding and clearing Aβ plaques in the brain. This study focuses on developing a non-viral delivery system capable of transporting both drugs (CBD) and genes (pApoE2) to the brain through a non-invasive intranasal route.

Method:
CBD loaded mPEG-PLGA nanoparticles were coated with mannose, a brain-targeting ligand, to facilitate the delivery of CBD and pApoE2. The in vitro CBD release was performed in PBS (pH~7.4) at 37ºC. ApoE2 expression level was checked in the brain of C57BL6/J mice after intranasal administration of mPLGA-CBD-MC/pApoE2.

Results:
The designed nanoparticles showed an average particles size of 179.3 ± 4.57 nm and a zeta potential of 30.3 ± 6.45 mV. The coated nanoparticles prolonged the release rate of CBD. Cytotoxicity assay confined that the nanoparticles were nontoxic in nature. Intranasal administration of pApoE2 loaded nanoparticles in C57BL6/J mice significantly ($P<0.0001$) increased the ApoE2 expression in the brain than pApoE2 treated alone.

Conclusion:
The developed nanoparticles can serve as a non-viral delivery system for transporting both drugs and genes to the brain via the intranasal route for Alzheimer's disease management.

Acknowledgment:
This research was supported by the National Institutes of Health (NIH) Grants R01 AG051574, RF1 AG068034 and R01 AG083981

Abstract #007

Altered expression and function of Transient Receptor Potential Vanilloid type 4 in the hypothalamic neurons contributes in salt-sensitive hypertension

Arya Poudel, Chengwen Sun

Department of Pharmaceutical Sciences, North Dakota State University

Purpose:
TRPV4, a hypotonic-activated cationic channel, is expressed in the central nervous system. Sodium concentration in the cerebrospinal fluid is elevated in salt-sensitive hypertension. This study aimed to investigate the role of the TRPV4 channel in the hypothalamus in the development of salt-sensitive hypertension.

Methods:
TRPV4 expression and localization were examined using immunocytochemistry. The effect of TRPV4 activation on blood pressure was evaluated by microinjecting the TRPV4 agonist GSK1016790A into the paraventricular nucleus (PVN) of Dahl salt-sensitive (DS) and control rats. Blood pressure and heart rate were measured via femoral artery catheterization in these rats.

Results:
Immunocytochemistry demonstrated that TRPV4 channels were predominantly localized on neurons in the hypothalamus. Microinjection of GSK1016790A into the PVN significantly reduced mean arterial pressure and heart rate in these rats. Interestingly, the TRPV4-mediated depressor response was more pronounced in DS rats treated with a high-salt diet compared to other groups of rats.

Conclusion:
TRPV4 channels are expressed in hypothalamic neurons, and stimulation of these channels in the PVN region of the hypothalamus decreases blood pressure and heart rate. The TRPV4 agonist-induced depressor response is enhanced in a salt-sensitive hypertension animal model. Future studies will explore the regulatory effects of TRPV4 on neuronal activity using patch-clamp techniques to investigate the cellular mechanisms involved.

Abstract #008

Role of CYPs in Estrogen Metabolite activity in Asthma

Ashish Kumar, MS Pharm[1*], Nilesh Sudhakar Ambhore, M.Pharm, PhD[1], Christina M Pabelick, MD[2,3], YS Prakash, MD, PhD[2,3], Venkatachalem Sathish, PhD[1].

[1]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND, USA. [2]Department of Anesthesiology and Perioperative Medicine, [3]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA.

Purpose:
Studies from autoimmune, and oncologic diseases suggest differential physiological effects of estrogen metabolites that result from the action of cytochrome P450 (CYP) enzymes. CYP1A1 convert E_2 to 2-hydroxyestradiol (2-HE), while CYP3A4 converts it to estriol (16α-hydroxyestradiol; 16αHE_2). In other cell systems, 16αHE_2 promotes proliferation while 2-HE is anti-proliferative. Whether or not local estrogen metabolism plays a role in asthma remains largely unknown.

Method:
Primary ASM cells were isolated from human lung tissue. The expression and activity of ASM CYPs with/without cytokines were evaluated using Western, RT-qPCR, and Glo-luminescence assays. To assess the role of CYPs in regulating the effects of estrogen metabolites on ASM proliferation, cells were treated with Rhapontigenin and PF49 prior to E_2 exposure with/without PDGF.

Results:
We observed higher expression and activity of CYP3A4 in asthmatic ASM. E_2 treatment with a CYP3A4 inhibitor, inhibited PDGF-induced ASM cell proliferation, possibly due to decreased CYP3A4 mediated local production of E_2 metabolite 16αHE_2. Interestingly, E_2 treatment with CYP1A1 inhibitor showed a significant increase in ASM cell proliferation.

Conclusion:
These data show an alteration of ASM CYPs regulates the estrogen metabolites activity and thereby dictating the net effect of E_2 in regulating asthma.

Abstract #009

Multifunctionalized chitosan polymeric micelles for intranasal delivery of pApoE2 to PS19 tauopathy AD mice

Avinash Gothwal, Jagdish Singh

[1]Department of Pharmaceutical Sciences, School of Pharmacy, College of Health and Human Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

Purpose:
The present study aims to develop multi-functionalized chitosan polymeric micelles that can effectively deliver pApoE2 to the brain through the intranasal route for the management of Alzheimer's disease.

Methods:
Chitosan (CS, 50kDa) was conjugated with caproic acid (Cap), followed by penetratin (PEN) and mannose (MAN) conjugation. The synthesized conjugates were subjected to physical characterization, including N/P ratio optimization, pDNase protection, and biocompatibility. In-vitro transfection efficiency of Cap-g-CS-PEN-MAN/pApoE2 polyplex was investigated in primary neurons and astrocytes. The therapeutic effect of the polyplexes was investigated in transgenic PS19 tauopathy mice.

Results:
The Cap-g-CS-PEN-MAN graft polymer formed cationic nano-micelles in an aqueous medium and polyplexed with pApoE2 at an N/P ratio of 15:1. Qualitative in vitro transfection efficiency of Cap-g-CS-PEN-MAN/pApoE2 polyplex was investigated in primary neurons and astrocyte cells. Total tau and phosphorylated tau (p181) levels were analyzed in PS19 mice after intranasal administration for 7 days. Total tau and phosphorylated tau (p181) levels in the PS19 mice' brains after intranasal administration for 7 days were 6067.97 ± 858.33 pg/mg and 157.91 ± 18.14 pg/mg of protein.

Conclusions:
The functionalized polymers formed cationic and uniformly sized polymeric nano-micelles. In-vitro transfection potential of Cap-g-CS-PEN-MAN/pApoE2 polyplex was significantly higher ($p \leq 0.01$) over unfunctionalized nano-micelle-pApoE2 polyplexes. Furthermore, total tau and phosphorylated tau levels were significantly reduced in the brain of PS19 tauopathy mice with Cap-g-CS-PEN-MAN/pApoE2 polyplex. These findings suggest that multi-functionalized polymeric micelles are an efficient non-viral gene delivery vector for brain-targeted gene delivery through the intranasal route.

Acknowledgment:
This research was supported by the National Institutes of Health (NIH) Grants R01 AG051574, RF1 AG068034 and R01 AG083981.

Abstract #010

Triple Functionalized Liposomes for Improved Penetration and Targeting of VGF Gene Across BBB in Alzheimer's disease.

Babita Lamsal, Jagdish Singh

Department of Pharmaceutical Sciences, College of Health and Human Sciences, North Dakota State University, Fargo, ND

Purpose:
VGF, a neuroprotective-neuropeptide precursor, is a potential therapeutic target for neurodegenerative disorders like Alzheimer's disease (AD). In AD patients, VGF mRNA and protein levels are markedly reduced in the hippocampus and prefrontal cortex compared to healthy controls, correlating with cognitive decline and amyloid-beta plaque burden. This suggests VGF deficiency contributes to AD progression. However, delivering VGF across the blood-brain-barrier (BBB) poses a significant challenge, limiting its therapeutic use. To overcome this, we developed multi-functionalized liposomes for VGF gene delivery, incorporating cell-penetrating peptides (CPPs) and brain-targeting ligands to enhance BBB-permeation and brain targeting.

Method:
Liposomes were prepared via the thin-film hydration method with a composition of DOPE(45%):DOTAP(45%):DSPE-PEG(8%):cholesterol(2%). CPPs and targeting ligands were conjugated to DSPE-PEG_{2000}-NHS(4%) for functionalization. VGF gene-chitosan polyplexes were post-inserted and characterized for size, zeta potential, PDI, and gene entrapment efficiency. Their biocompatibility and uptake in brain cells were assessed in vitro. Transfection efficiency was evaluated using reporter genes and VGF protein expression was quantified with ELISA.

Results:
Conjugates achieved >80% coupling efficiency. VGF gene-loaded liposomes had a particle size <200 nm, PDI <0.3, positive zeta potential, and >80% gene encapsulation efficiency. At 100 nmoles, formulations showed >80% cell viability, with peak cellular uptake at 4 hours. Tri-functionalized liposomes exhibited significantly higher transfection efficiency than mono- or dual-functionalized ones, with VGF protein expression ~8-fold higher in treated brain cells versus untreated controls.

Conclusion:
Tri-functionalized liposomes demonstrate efficient gene targeting and expression in-vitro, suggesting potential to cross the BBB and elevate brain VGF levels, offering a promising approach for AD.

Acknowledgement:
This research was supported by the National Institute of Aging (NIA) grants R01 AG083981, R01 AG068034.

Abstract #011

Brain-Targeted Delivery of Plasmid VGF through Trifunctionalized Liposomes for the Management of Alzheimer's Disease

Benjamin Tagoe, Jagdish Singh

Department of Pharmaceutical Sciences, North Dakota State University

Purpose:
Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive decline and synaptic dysfunction. VGF, a neurotrophin-responsive protein crucial for synaptic plasticity, is significantly reduced in AD brains, highlighting its therapeutic potential. However, effective VGF delivery across the blood-brain barrier (BBB) remains a challenge. This study investigates trifunctionalized liposomes incorporating two targeting ligands (Mannose and Transferrin) and a cell-penetrating peptide (Penetratin or Tetanus toxin fragment C) to enhance BBB penetration and neuronal uptake.

Methods:
Liposomes were synthesized using the lipid film hydration method with DOPE: DOTAP: DSPE-MPEG (2000) and cholesterol (45:45:8:2 molar ratio). Functionalization with DSPE-PEG-Mannose, DSPE-PEG-Penetratin, and DSPE-PEG-TTC was performed at 4% molar ratios. The formulations were characterized for size, PDI, zeta potential, encapsulation efficiency and stability against nuclease degradation. Furthermore, in-vitro biocompatibility, cellular uptake of rhodamine-labeled liposomes, and transfection efficiency were assessed in different brain cell lines.

Results:
The synthesized liposomes had sizes <200 nm, PDIs <0.3, and positive zeta potentials. High plasmid VGF entrapment efficiency and protection against enzymatic degradation were observed. Trifunctionalized liposomes exhibited improved cellular uptake, VGF expression levels in brain endothelial (bEnd.3) cells and primary astrocytes, and were non-cytotoxic at 100 nM phospholipid concentration.

Conclusion:
Overall, our findings suggest that trifunctionalized liposomes show considerable potential for effective blood-brain barrier (BBB) penetration, enhanced transfection efficiency, and biocompatibility in bEnd.3 cells and primary astrocytes at therapeutic concentrations. Future studies are expected to demonstrate improved uptake and increased VGF expression in neurons.

Acknowledgement:
This research was supported by the National Institute of Aging (NIA) grants R01 AG083981, R01 AG068034 and R01 AG05157.

Abstract #012

Effect of liposomal delivery of cannabidiol and pBDNF against transgenic models of Alzheimer's Disease (AD)

Bivek Chaulagain, Avinash Gothwal, Arun Mahanta, Jagdish Singh

Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND, USA

Purpose:
Neuroinflammation, in coordination with the amyloid plaques and neurofibrillary tangles, the distinguishing pathological features, expedites AD progression. The hippocampal decline in brain-derived neurotrophic factor (BDNF) levels in AD patients further fuels cognitive deterioration and contributes to the aggravation of pathology. Therefore, this study aims to deliver cannabidiol, an anti-inflammatory phytoconstituent, to counteract neuroinflammation, supplement the BDNF levels in transgenic mice models via brain-specific liposomes, and study its efficacy in managing AD.

Methods:
Mannose and Penetratin functionalized liposomes encapsulating cannabidiol, and pBDNF was prepared using the lipid film hydration method. The formulation was administered intravenously weekly for a month and assessed for alteration in pathological symptoms using ELISA and immunohistochemistry and cognitive functions by Y maze test.

Results and Conclusion:
Liposomes delivered 2.15 % of the injected CBD to the brain within 24 hours (p-value < 0.0001), which is significantly higher than the unmodified controls. The efficacy studies showed a 2 to 3-fold reduction in amyloid levels in the 5XFAD model (p-value < 0.0001) and a 3 to 4-fold reduction in phosphorylated tau in the PS19 mouse model (p-value < 0.0001). It also decreased the pro-inflammatory cytokines TNF-alpha and IL-1 beta by 2 to 3-fold (p-value < 0.0001) in both the 5XFAD and PS19 mouse models while enhancing cognitive function in the Y maze test by 1 to 2-fold (p-value < 0.0001) in both models. These suggest penetratin and mannose-modified liposomes encapsulating CBD and pBDNF could be an excellent strategy for AD management.

Acknowledgment:
This research was supported by the National Institutes of Health (NIH) Grants R01 AG051574, RF1 AG068034 and R01 AG083981.

Abstract #013

Hypoxia-Responsive Polymersomes for Drug Delivery to Pancreatic Ductal Adenocarcinoma (PDAC) Cells

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Department of Pharmaceutical Sciences, South Dakota State University, Brookings, SD 57007

Purpose:
Pancreatic ductal adenocarcinoma (PDAC) is characterized by an aggressive tumor microenvironment and a high degree of chemoresistance, largely driven by hypoxia-induced tumor stemness and metastasis. Targeting hypoxic PDAC cells using a synergistic drug combination strategy may enhance treatment efficacy. This study investigates the use of hypoxia-responsive polymersomes (HRPs) to co-deliver gemcitabine (GEM) with either the CXCR4 antagonist (AMD3100) or the STAT3 inhibitor (Narciclasine), aiming to disrupt tumor stemness and potentiate GEM-mediated cytotoxicity.

Methods:
A hypoxia-responsive amphiphilic polymer, polyethylene glycol-diazobenzene-polylactide was synthesized and characterized. HRPs encapsulating GEM, AMD3100, or Narciclasine were formulated via passive encapsulation, and their physicochemical properties, including particle size and encapsulation efficiency, were evaluated using dynamic light scattering and high-performance liquid chromatography (HPLC), respectively. The therapeutic efficacy of the encapsulated drug combinations was assessed in 2D cell cultures and 3D PDAC spheroid models.

Results:
The HRPs exhibited nanoscale properties, with particle sizes of 206 ± 2 nm (AMD3100), 216 ± 1 nm (GEM), and 163 ± 2 nm (Narciclasine). In vitro studies demonstrated that co-delivery of AMD3100 and GEM resulted in a statistically significant ($p < 0.05$) reduction in PDAC spheroid volume compared to monotherapy, indicating enhanced therapeutic efficacy.

Conclusion:
Hypoxia-responsive polymersomes represent a promising nanocarrier system for targeted drug delivery in PDAC. The synergistic drug combinations improved anticancer activity in 2D assay and 3D cultures of PDAC cells. HRP-mediated co-delivery could enhance drug penetration and therapeutic outcomes in pancreatic cancer.

Abstract #014

Narciclasine and Doxorubicin Encapsulated Hypoxia-Responsive Polymersomes to Reduce Stemness and Migration of Triple-Negative Breast Cancer Cells

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Purpose:
Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype, lacking the estrogen receptor, progesterone receptor, and HER2 protein. The growth of the tumor outpaces the rate at which blood vessels can deliver blood to the inner regions of the tumor. These regions have insufficient oxygen and become hypoxic. Within these hypoxic regions, cells are more resistant to treatment, are more likely to metastasize and invade, and obtain a stem cell-like phenotype. These stem cell-like cells exhibit self-renewal and pluripotency, allowing them to proliferate continuously. These cells are more aggressive, form tumors, are resistant to drugs, and can quickly spread. Thus, to target these regions and the cancer stem cells, hypoxia-sensitive nanocarriers encapsulating anticancer drugs were employed.

Methods:
To target hypoxia, a nanocarrier composed of a block copolymer made of PEG and PLA, linked with a hypoxia-sensitive azobenzene linker, self-assembles to form polymersomes. To enhance targeting, the iRGD peptide was conjugated to the surface of the polymersomes. Narciclasine and Doxorubicin were encapsulated to synergistically treat hypoxic TNBC, reduce stemness, and inhibit migration.

Results:
Nar and Dox displayed high degrees of synergy, were successfully encapsulated, and exhibited anti-stemness and anti-migration properties, thereby reducing the volume of the spheroids. In vivo, the iRGD-targeted polymersomes demonstrated increased selectivity at the tumor site compared to untargeted polymersomes.

Conclusions:
The targeted polymersomes showed higher efficacy, increased selectivity, and penetration than untargeted. The combination decreased stemness and migration, thereby demonstrating potential in the management of TNBC.

Abstract #015

Age dependent changes in the expression of Mas related G-Protein Coupled Receptor D (MrgD) in the mouse tissues: An exploratory study

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Purpose:
Alamandine and its receptor, MrgD are members of the renin angiotensin system, dysregulation of which contributes to aging. The physiological functions of MrgD and its role in aging of are not fully understood. In a preliminary study, we observed that the genetic ablation of MrgD resulted in accelerated aging phenotype in mice based on frailty index. Aging-associated frailty is at least in part due to chronic inflammation and oxidative stress, which impacts organ functions however tissue-specific expression of MrgD and changes with aging are unknown. This study explored MrgD expression in different tissues in the aging mice.

Methods:
The following tissues were collected from 2 - 3 (Young) or 22 - 23-month-old (Old) male or female mice: heart, skeletal muscle (gastrocnemius), colon, and bone marrow stem/progenitor cells. MrgD expression was evaluated by western blotting. In colon and skeletal muscle, localization of MrgD was also evaluated by immunohistochemistry.

Results:
MrgD expression was decreased in the myocardium ($P < 0.05$, n=6) in Old females but unchanged in males compared to the Young. Expression was lower in the male hearts compared to females. In colon, the expression was higher in the Old males ($P < 0.01$, n=6) but unchanged in females. MrgD expression showed a decreasing trend in the Old skeletal muscle. No change in the expression was observed in the stem/progenitor cells between the Young and Old groups. In the colon, MrgD expression was colocalized with Lgr5, a marker for the intestinal stem cells. In the skeletal muscle, colocalization with Pax7, a marker for satellite cells was observed.

Conclusion:
MrgD expression is altered in the aging tissues in sex and tissue-dependent manner. Future investigations are needed to address if MrgD plays a protective or detrimental role in the myogenesis and vasculogenesis with aging.

Abstract #016

Data-independent acquisition (DIA) analysis shows specific regulation of TGF-β1 on airway smooth muscle cell extracellular matrix (ECM) protein interactions

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Purpose:
In this study, we investigated the specific alterations that occur in the ECM of airway smooth muscle cells (ASM) cells, treated with TGF-β1. ASM cells are one of the major structural cells that regular airway remodeling and are the primary target for treating asthma. TGF-β1 induced changes in the extracellular matrix (ECM) lead to the pathogenesis of fibrosis, a characteristic feature of airway remodeling. Due to this, lung functionality decreases as the lung becomes less elastic and airway resistance increases.

Methods:
Cells were treated with TGF-β1 (2 ng/mL) in serum free media. Cells were harvested after 48 hours and 200 µg of protein lysate was prepared. Data-independent acquisition (DIA) mass spectrometry was performed for Quantitative Proteomics. Fold changes for ECM class proteins were collected from DIA results. Pathway analysis was then done using Qiagen ingenuity pathway analysis. Furthermore, expression patterns of select proteins were confirmed via western blotting.

Results:
TGF-β1 exposure upregulated LAMA4, LAMB2, and LAMC1 which is likely to increase the prevalence of Laminins-221 and -421. TGF-β1 augmented integrin signaling by upregulating ITGA7, ITGA11, ITGA1, ITGB1 and downregulating ITGA2 and ITGB8. TGF-β1 increased expression of TSP1, TSP2, TSP3, TNS-C, FB, Elastin and Versican while downregulating TNS-X. Several interactions between ECM proteins and their receptors were highlighted for their enrichment.

Conclusion:
The DIA analysis shows an upregulation of proteins involved in airway remodeling by TGF-β1 in ASM cells. These findings substantiate our previous results as well as provide insights into a more targeted approach in studying ECM-Cell interactions.

Abstract #017

Kp/KISS1R Signaling Mitigates TGF-β1-Driven Airway Remodeling in Lung Fibroblast

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Purpose:
Fibroblasts (FB) drive airway remodeling in asthma by promoting inflammation, ECM alterations, and myofibroblast differentiation, leading to airway thickening and stiffness. Current therapies fail to address this remodeling effectively in asthma. We previously identified a role for Kp/KISS1R in the asthmatic lung. This study explores Kp/KISS1R expression and function in FB under inflammatory conditions in the context of airway remodeling in asthma.

Methods:
Primary human lung FB cells isolated from surgical lung resections and were cultured in DMEM medium (Mayo IRB approved). Cells were serum-deprived and treated with TGF-β1 (10 ng/mL) and Kp-10 (1 μM). Kp/KISS1R expression were analyzed by western blot and immunofluorescence from human FB (non-asthmatic vs. asthmatic). Cell migration was analyzed by Transwell migration assay. MMP-2/9 activity was measured using gelatin zymogram
.
Results:
We found the ubiquitous expression of kisspeptin and KISS1R in human FB. Interestingly, we observed that KISS1R expression was found to be significantly reduced in asthmatic FB compared to non-asthmatic FB. Functionally, Kp-10 was found to reduce TGF-β1-induced FB migration. In addition, Kp-10 significantly reduced the pro- and total MMP-2 and -9 expression and activity in TGF-β1-induced FB.

Conclusion:
This study highlights the role of Kp/KISS1R signaling in mitigating TGF-β1-induced airway remodeling in lung fibroblast. These findings suggest that targeting KISS1R represents a promising therapeutic strategy to address airway remodeling.

Abstract #018

Brain-Targeted ApoE2 Gene Delivery Across the Blood-Brain Barrier Using Dual-Functionalized Liposomal Nanoparticles for Alzheimer's Disease Therapy

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Purpose:
Alzheimer's disease (AD) is a progressive neurodegenerative disorder with no effective cure, primarily influenced by the apolipoprotein E (ApoE) variant. The ApoE4 variant exacerbates AD pathology, whereas the ApoE2 variant is neuroprotective. This study aims to develop a dual-functionalized liposomal system for delivering plasmid ApoE2 (pApoE2) across the blood-brain barrier (BBB) using transferrin and cell-penetrating peptides like RDP and Cgn for a more effective therapeutic approach.

Methods:
Liposomal formulations were prepared using thin-film hydration with 45mol% DOTAP, 45mol% DOPE, 8mol% DSPE-PEG, and 2mol% cholesterol. Cell-penetrating peptides and transferrin were pegylated using DSPE-PEG(2000)-NHS. The liposomes' physicochemical properties, including size, zeta potential, polydispersity index and ApoE2 encapsulation efficiency, were characterized. Biocompatibility and biodistribution were assessed in 3-month-old C57Bl/6 mice, while transfection efficiency was evaluated in C57Bl/6 and ApoE-knockout mice.

Results:
The liposomal formulations exhibited a cationic charge, uniform size distribution (<200 nm), low polydispersity index (<0.3), and an encapsulation efficiency of over 85%. Biocompatibility studies showed no toxicity. Dual-functionalized liposomes (RDP-Tf and Cgn-Tf) demonstrated significantly higher brain accumulation ($p < 0.0001$) compared to single-functionalized or plain liposomes. In C57Bl/6 and ApoE-KO mice, dual-functionalized liposomes significantly elevated ApoE2 expression ($p < 0.0001$) compared to single-functionalized formulations, plain liposomes, or naked pDNA.

Conclusion:
The developed dual-functionalized liposomes effectively delivered the pApoE2 gene across the BBB, increasing ApoE2 levels in the brain. This approach shows potential for treating AD by elevating protective ApoE2 expression in neurons. Further studies will assess the effect of this gene therapy in AD transgenic mice.

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Abstract #019

Synthesis of (+)-Catechin derivatives with Enhanced Bioavailability

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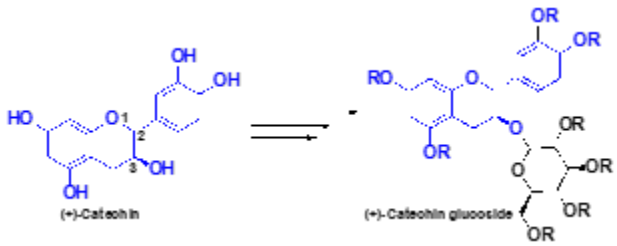
Purpose:
Catechins, a key subgroup of polyphenols found in plants, play crucial roles in biological processes in human metabolism. They are present in different dietary sources, with cacao predominantly biosynthesizing the (+)-catechin enantiomer, while species such as *Centaurea maculosa* produce a racemic mixture of enantiomers. Extracting and purifying these compounds from natural sources is challenging due to their tendency to oligomerize. To overcome these limitations, we are exploring structural modifications, including the conjugation of a glucuronide group at the 3' position, to improve therapeutic potential.



Methods:
The optimized procedure enabled the synthesis of (+)-catechin glucosides from (+)-catechin and *D*-glucose. To prevent unwanted side reactions, each starting material was individually protected, the phenolic groups from catechin were selectively modified using benzyl bromide, while *D*-glucose was protected via acetylation followed by a-bromination. Then, the benzylated catechin reacted with the glycosyl bromide, resulting in glucosylation at the 3′ position. Our next steps include the deprotection reactions to yield the desired (+)-catechin glucosides, which will be evaluated *in vitro* and *ex vivo* for their potential to inhibit activated protein kinase (AMPK).

Results:
Benzylated catechin, acetylated glycosyl, and glycosyl bromide were synthesized in moderate to high yields (70%, 82%, and 100%, respectively). All structures were confirmed by ^{1}H and ^{13}C-NMR spectroscopy. Our current efforts focus on optimizing the coupling reaction between benzylated catechin and brominated sugar.

Conclusion:
The synthetic modifications described herein yield (+)-catechin glucosides, representing promising candidates for further biological evaluation in hypertensive mouse model.

Abstract #020

Regulation of Piezo ion channels by Apelin/APJ signaling in hypertension

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Rationale:
Apelin regulates vasocontractility and vascular remodeling through APJ receptors in vascular smooth muscle cells (VSMCs), however the underlying mechanisms at the cellular level remain unclear. Piezo channels are mechanosensitive cation channels that contribute to vasoconstriction. The aim of this study was to investigate the role of Apelin and Piezo channel in VSMCs in the development of hypertension.

Methods:
The localization of Piezo channels in mesenteric arteries were examined in Wistar Kyoto (WKY) and Spontaneously Hypertensive (SHR) Rats using immunohistochemistry techniques. The vascular contractility was examined in isolated mesenteric arteries of SHR and WKY rats using multi wired myography. VSMCs were dissociated from mesenteric arteries by enzymatic digestion approach.

Results:
Immunohistochemistry study using specific antibodies has demonstrated that Piezo1 and Piezo 2 channels are dominantly expressed in VSMCs. APJ receptors are also expressed in the VSMCs of these arteries and colocalized with α-SMA. In VSMCs, Piezo1 was localized on cell membrane, in contrast, Piezo2 localized in the nucleus. Myograph study using denuded mesenteric arteries showed Apelin-13 induced a dose-dependent vasoconstriction in arteries from both SHR and WKY rats. However, the Apelin-induced vasoconstriction is enhanced in SHR arteries. More interestingly, the vascular response to Apelin-13 was mimicked by Yoda-1, an agonist of Piezo 1 channel.

Conclusion:
Apelin receptor APJ and Piezo channels are localized in VSMCs of mesenteric arteries. Stimulation of Piezo channel or APJ receptor induces vasoconstriction; this vascular response is enhanced in SHR. The effect of Apelin on Piezo channel opening will be examined in the future study.

Abstract #021

L- and D-BAIBA Modulating Bioenergetics in Human Brain Microvascular Endothelial Cells

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Background:
Mas-related G-protein coupled receptor D (MrgD) is expressed in endothelial cells, playing a crucial role in vascular protection and metabolic regulation. Its activation contributes to endothelial homeostasis. β-Aminoisobutyric acid(BAIBA), a non-protein amino acid existing in L- and D-enantiomers, is an agonist for MrgD. While BAIBA exerts anti-atherogenic effects in vascular endothelial cells, its impact on human brain microvascular endothelial cells(hBMECs) remains unexplored. This study investigates whether L-BAIBA(LB) and D-BAIBA(DB) modulate cellular bioenergetics via MrgD in hBMECs.

Methods:
Primary hBMECs were cultured as young (passages7–9) or aged (passages20–22) cells, confirmed by β-galactosidase staining and p21 expression. Age-related changes in MrgD expression were assessed via western blotting. Cells were treated with LB/DB, with or without the MrgD antagonist MU6840. Mitochondrial oxidative phosphorylation(OxPhos) and glycolysis were analyzed using the Seahorse Bioanalyzer.

Results:
β-Galactosidase staining and p21 expression confirmed cellular senescence with aging($P<0.01$). Aged cells exhibited reduced MrgD expression($P<0.05$) and exhibited increased energy consumption via OxPhos compared to young cells (maximal respiration($P<0.05$) and reserve capacity($P<0.01$)). However, aged cells demonstrated reduced glycolytic function in the glycolytic rate assay, with significant decreases in basal glycolysis($P<0.01$),basal proton efflux rate($P < 0.01$), and compensatory glycolysis($P<0.01$). In P8, both LB and DB treatments enhanced basal($P<0.05$) and maximal respiration($P<0.001$) for LB, $P<0.05$ for DB, compared to controls. Reserve capacity($P<0.05$) and ATP-linked respiration($P<0.01$) was also significantly elevated following LB treatment. The effects were abolished in the presence of MU6840.

Conclusion:
LB&DB enhanced mitochondrial function in hBMECs via MrgD activation, highlighting their potential role in endothelial bioenergetics with aging.

Abstract #022

Aryl hydrocarbon receptor activation in airway smooth muscle inhibits DRP1-induced mitochondrial fission during inflammation and asthma

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Purpose:
Airway smooth muscle (ASM) is a major structural cell in the airway that contributes to airway inflammation and remodeling in asthma. Inflammation promotes mitochondrial fission in ASM, thereby promotes asthma. The aryl hydrocarbon receptor (AhR) is a transcription factor that regulates cellular homeostasis. Recently, we found upregulation of AhR in response to inflammation and asthma in ASM. Here we aimed to unveil the role of AhR in regulating mitochondrial dynamics in human ASM during inflammation and asthma.

Methods:
Primary non-asthmatic and asthmatic human ASM cells were cultured in DMEM-F12 medium and treated with AhR agonist FICZ (10 nM) and antagonist CH223191 (10 μM), with/or without TNFα (20 ng/mL). Mitochondrial bioenergetics was determined by Seahorse. Mitochondrial morphology was visualized using mitotracker. Mechanisms of AhR regulation of DRP1 was determined by molecular techniques.

Results:
AhR activation alleviated TNFα-induced disrupted mitochondrial bioenergetics in non-asthmatic and asthmatic ASM cells. TNFα-induced mitochondrial fission and DRP1 expression was restored by AhR activation. AhR inhibition by CH223191 and knockdown promoted mitochondrial fission in non-asthmatic ASM cells. Interestingly, AhR overexpression and activation by FICZ restored the mitochondrial fusion altered by inflammation and asthma. Chromatin-immunoprecipitation-qPCR showed transcriptional binding of AhR to the DRP1 promoter region. Luciferase showed inhibition of DRP1 promoter activity with AhR activation.

Conclusion:
Collectively, AhR activation abolishes mitochondrial fission induced by inflammation and asthma in human ASM. Therefore, AhR is a potential therapeutic target to mitigate altered mitochondrial dynamics in asthma.

Acknowledgments:
NIH grants R01-HL171245 (Venkatachalem and Britt), R01-HL146705 (Venkatachalem), R01-HL155095 (Britt), R01-HL142061 (Pabelick, Prakash).

Abstract #023

Kisspeptin Reduces Inflammatory Cytokine Exacerbation of Airway Smooth Muscle Contractility and Calcium Signaling

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Introduction:
Calcium is a regulator for many pathways within airway smooth muscle cells (ASM). In ASM, calcium is primarily involved in inducing and regulating muscle contractions. Within the airways of asthmatics, ASM become prone to hyperresponsiveness by reacting towards lower threshold of stimuli. This phenomenon is termed Airway Hyperresponsiveness (AHR). Kisspeptins have been shown to reduce [Ca2+]i within inflamed cells, however the exact mechanism is undetermined. In this study we investigated the effects of kisspeptins on ASM contractility.

Methods:
Primary ASM were used for experiments. [Ca2+]i was measured via FURA-2AM (4 μM) labeling on an Olympus Fluoview 300 microscope. Inflammatory cytokines (TNFα 20 ng/ml, IFNγ 25 ng/mL, IL-13 50 ng/mL) and asthmatic therapeutics (Salbutamol 1 μM, Fluticasone Propionate 100 nM) were used to model asthmatic conditions. [Ca2+]i was stimulated with 10 μM Histamine following treatment with cytokines and/or Kp-10 (1 μM) treatments.

Results:
Kisspeptin-10 stimulation reduces [Ca2+]i observed within inflamed cells. Kisspeptin and Fluticasone Propionate showed greater effect in reducing [Ca2+]i in response to histamine when treated together within TNFα/IFNγ treated ASM. Kisspeptins reduced [Ca2+]i within chronic salbutamol treated cells, suggesting KISS1R signaling mechanics are independent or unique from β2-AR signaling.

Conclusion:
Kisspeptin-10 works as a regulator of airway smooth muscle contractility by augmenting signaling pathways. These effects appear to work through mechanisms independent of the current medications prescribed for symptomatic relief and have the potential as a daily symptom therapeutic.

Absract #024

Development of a 3-D Printed *in vitro* Bioreactor to Simulate Tumor Metastasis

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Purpose:
Tumor metastasis is a very difficult process to replicate *in vitro*. The purpose of this study is to replicate the mechanism of tumor metastasis in a dynamic flow environment.

Methods:
PVC tubing connects a 3-D printed bioreactor with a peristaltic pump. This pump is connected to four 3-D printed chambers. Each chamber consists of a primary and secondary chamber. The primary chamber houses a collagen plug seeded with cancer cells. The peristaltic pump pushes cell media at a physiological flow rate into the chamber, through the seeded material, into a secondary chamber. The secondary chamber is identical to the first, except the cell seeding material is blank (unseeded). The secondary chamber is used as an endpoint for measuring metastasis. Directly below the secondary chamber is a central reservoir of cell media. This media is filtered through a 0.22 μm PES filter before being recirculated into the bioreactor using the peristaltic pump.

Results:
3-D printed design minimizes leakages and successfully implanted PANC-1 cells inside collagen dental plugs. Parts printed with ultraclear resin circulate cell media without leakage for over 48 hours. Collagen plugs seeded with PANC-1 cells can be fixed, sliced, and stained via histological techniques.

Conclusion:
While the current resin design provides adequate visualization of the chambers, a autoclavable design consisting of 3D printed polypropylene or a milled design with stainless steel would minimize the sterility and improve reusability of the bioreactor.

Abstract #025

Size-Dependent Effect of Polystyrene Model Microplastics on Human Serum Albumin

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Purpose:
The aim of this study is to investigate the size-dependent interactions of model polystyrene nano- and microplastics (NMPs) with human serum albumin (HSA), focusing on their impact on protein structure and function, which is essential for understanding the health risks of microplastic pollution.

Method:
Polystyrene latex particles with controlled size distributions were synthesized via surfactant-free emulsion polymerization. The interactions between these model NMPs and HSA were studied using fluorescence quenching and circular dichroism (CD) spectroscopy. The size-dependent effects of NMPs on HSA were analyzed at different concentrations and temperatures.

Results:
The study found that smaller polystyrene particles (124 nm) induce more significant fluorescence quenching and structural changes in HSA compared to larger particles (822 nm). The fluorescence data revealed that the quenching effect followed the order of 124 nm > 397 nm > 822 nm, indicating a size-dependent interaction. CD spectra further supported these findings, showing more substantial conformational changes in HSA when exposed to smaller particles.

Conclusion:
Our findings highlight that the size of nano- and microplastics (NMPs) plays a crucial role in determining their interaction with human serum albumin (HSA). Smaller polystyrene particles have a more pronounced impact on HSA, which suggests potential systemic toxicity associated with smaller microplastics. The models presented in this study provide a valuable approach for understanding NMP-protein interactions, offering insights into the broader health implications of microplastic exposure.

Abstract #026

Analysis of Pancreatic Tumors Treated with Gemcitabine and RAGE Inhibitors by Western Blotting: Challenges and Troubleshooting

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Purpose:
Pancreatic cancer is one of the most lethal types of cancer, with a current 5-year survival of only 13%. Because the pancreas sits deep within the abdomen, pancreatic tumors are difficult to detect and are frequently detected at later stages of the disease. One of the current standards of care for treating pancreatic cancer is gemcitabine. However, the overall median survival is only 2 years for these patients and improved treatment options are thus urgently needed. The Receptor for Advanced Glycation End products (RAGE) plays a role in facilitating the growth of pancreatic tumors. In addition, our laboratory previously showed, in an orthotopic mouse model of pancreatic cancer, that blocking RAGE during the treatment with gemcitabine resulted into small tumors that the treatment with gemcitabine alone. The purpose of this study is to determine the signaling pathways affected by RAGE inhibition in gemcitabine treated tumors.

Methods:
We use Western blotting analysis of tumor lysates to determine the signaling pathways affected by RAGE inhibition.

Results:
We are currently investigating if RAGE inhibition affects changes in autophagy, apoptosis and cell survival by comparing the levels of the respective markers p62 and LC3I/II, PARP and caspase 3 (cleaved and non-cleaved) and ERK (P-ERK and TOT-ERK). Western blot analysis of tumor extracts can be challenging, and many optimization steps were performed.

Conclusion:
Our study is ongoing. Our findings would lead us one step further into understanding how to effectively target and kill pancreatic cancer cells.

Abstract #027

How Bergamot Byproduct Prevents Obesity-Induced Cardiovascular Diseases

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[1]North Dakota State University (NDSU), North Dakota, United States. [2]Sao Paulo State University (UNESP), Sao Paulo, Brazil. [3]Federal University of Santa Catarina (UFSC), Santa Catarina, Brazil. [4]National Center for Energy and Materials Research (CNPEM), Sao Paulo, Brazil. [5]University of Milan (UNIMI), Italy

Purpose:
Obesity affects one-third of the global population, promoting metabolic dysfunction, oxidative stress, and inflammation, which contribute to cardiovascular disease (CVD), the leading cause of mortality worldwide. The hydrophilic portion of bergamot (*Citrus bergamia*), often discarded, contains antioxidant and anti-inflammatory bioactive compounds that may serve as a valuable byproduct (BB). This study evaluates the effects of BB on obesity-induced CVD.

Methods:
Wistar rats (n=30) were divided into three groups: control, obese, and obese+BB. Obesity and CVD were induced through a Western diet. BB (250mg/Kg) was administered daily via gavage for 20 weeks. Body weight, adiposity index, Doppler echocardiography, and systolic blood pressure were assessed. Heart tissue analyses included high-performance liquid chromatography for biogenic amines, gas chromatography for fatty acids, nuclear magnetic resonance for metabolomics, and measurement of inflammatory (interleukin-10, tumor necrosis factor-alpha) and oxidative stress markers (malondialdehyde, protein carbonylation, ferric reducing antioxidant power). Data were analyzed using one-way ANOVA and principal component analysis (PCA).

Results:
The Western diet induced obesity (p<0.001), inflammation (p=0.009), oxidative stress (p<0.001), and CVD (p<0.001). BB prevented CVD by reducing inflammatory cytokines (p=0.008) and oxidative stress (p<0.001), modulating biogenic amines (spermine, spermidine, and putrescine), fatty acids (decreasing C18:0 while increasing C17:1, C18:3n6, and C20:4n6), enhancing carbohydrate and amino acid influx (sucrose, glucose, glucose-6-phosphate, total glucose, isoleucine, leucine, threonine, and histidine) and increasing cardioprotective metabolite concentrations (uridine, glycerol, inosine, hypoxanthine, malate, and malonate). PCA distinguished all groups.

Conclusion:
BB prevents obesity-induced CVD by reducing inflammation and oxidative stress while modulating cardiac metabolism, highlighting its therapeutic potential.

Abstract #028

Mesenchymal stem cell-based delivery of Paclitaxel and Entinostat for effective lung cancer management

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Purpose:
Lung cancer is the third most common type of cancer, with the highest death estimated in 2024. The lack of efficient strategies to achieve cytotoxic concentrations of chemotherapeutics in the tumor tissue and the tumor's innate resistance to chemotherapy are significant barriers to improving treatment outcomes for lung cancer. Here, we developed a new therapeutic approach for lung cancer treatment that involves the mesenchymal stem cell (MSC)-mediated tumor-targeted delivery of a paclitaxel (PTX) and entinostat (ENT) combination.

Methods:
The synergistic potential of the PTX and ENT combination against A549 and H1299 cells was determined via Combenefit software from the cytotoxicity data. PTX and ENT-loaded DBCO-functionalized PLGA nanoparticles were prepared separately and characterized for size, charge, drug loading, and release profiles. Nanoparticle-loaded MSCs (nano-MSCs) were generated by incubating azide-labelled MSCs with nanoparticles for 4 h at 37 °C. In vitro, cytotoxicity of nano-MSCs was evaluated against A549 and H1299 cells. Finally, the antitumor efficacy of the PTX nano-MSC and ENT nano-MSCs combination was performed in a PDX lung cancer mouse model.

Results:
We observed that the combination treatment attenuated cell growth more than either drug used in isolation in A549 cells at multiple combinations of concentrations. The nanoparticles of PTX (size: 284.8 nm, ZP: -14.4 mV, drug loading: 25.5%) and ENT (size: 279.2 nm, ZP: -11.7 mV, and drug loading 12.6%) exhibited sustained drug release profiles. The nano-MSCs exhibited efficient cytotoxicity against A549 cells. Most importantly, nano-MSCs mediated PTX and ENT combination exhibited enhanced antitumor efficacy than other controls.

Conclusion:
MSCs-based combination therapy can serve as an effective alternative strategy for lung cancer management.

Abstract #029

P2Y1 or P2Y12 receptor blockade prevents platelet sequestration and intestinal damage in female mice in intra-abdominal sepsis

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Purpose:
Intra-abdominal sepsis, a severe intestinal infection with high mortality, disrupts the immune response and compromises the intestinal barrier, resulting in excessive inflammation and tissue damage. Preserving barrier integrity is essential for restoring immune balance and improving outcomes. Platelets, as key mediators of inflammation, contribute to sepsis by releasing cytokines and modulating immune responses. Our prior research showed that blocking platelet P2Y1 or P2Y12 receptors reduced cytokine secretion and enhanced bacterial clearance in a sex-dependent manner. However, the role of platelets in intestinal inflammation and barrier disruption during sepsis has not been thoroughly investigated. To address this, we examined sex-specific sepsis treatments in female C57BL/6J mice.

Methods and results:
Sepsis was induced using cecal ligation and puncture (CLP) or sham surgery, with platelet secretion inhibited via P2Y1 or P2Y12 antagonists. Analysis of intestinal tissue samples 24 hours post-surgery revealed increased damage in CLP mice, assessed through H&E staining, fluorescence microscopy, and western blot analysis. Blocking P2Y1 or P2Y12 partially mitigated tissue damage, platelet sequestration, and altered protein levels.

Conclusion:
These findings suggest that MRS2279 or Ticagrelor, a P2Y1 or P2Y12 antagonist respectively may offer a localized therapeutic strategy to ameliorate intestinal inflammation in septic females.

Abstract #030

A Synthetic Biomolecular Condensate from Plant Proteins with Controlled Colloidal Properties

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Purpose:
Synthetic biomolecular condensates (sBCs) are membrane-less organelles that enable the spatial and temporal organization of specific biomolecules into distinct compartments. These sBCs have emerged as a promising platform to mimic the natural biomolecular condensate (nBCs) to gain knowledge about their colloidal and soft-materials properties. From therapeutic and biotechnological applications, protein-based sBCs can act as platforms to understand the formation, function, and stability of nBCs, and their role in health and diseases. A synthetic biomolecular condensate (sBC) has been developed using a prolamin-rich plant protein, zein, by using liquid-liquid phase separation method.

Method and Result:
In order to form stable colloidal condensates dual chemical modification of the protein has been done. The protein was chemically modified either via quaternization with glycidyl trimethyl ammonium chloride (GTMAC) or PEGylated to enhance colloidal stability by tuning surface charge and inter-particle interactions. By varying the composition of these modified proteins, the size, charge, and stability of the condensates can be precisely controlled. Different stoichiometric interactions of these proteins electrostatically and thermodynamically stabilized the sBCs. These engineered condensates exhibit dynamic small-molecule exchange and compartmentalization of guest molecules. Furthermore, they demonstrate cellular internalization, highlighting their potential as functional condensates.

Conclusion:
This work presents a scalable approach to designing stable biomimetic condensates, offering insights into the organization and behavior of natural condensates.

Abstract #031

House dust mite exposure increases amyloid β expression and disrupt epithelial integrity *in-vitro*

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Purpose:
Our recent study reported a bidirectional relationship between asthma and Alzheimer's disease. Particularly, house dust mite (HDM) induced asthma exacerbates Alzheimer's disease changes by promoting amyloid β (Aβ) plaque load in APP^{NL-G-F} mice brain. Notably, amyloid precursor protein (APP) and its cleaved product amyloid β is predominantly expressed in brain and regulate Alzheimer's disease progression. However, the amyloid β role in asthma is not explored. Thus, we aim to explore the role of amyloid β in airway epithelial cells in the context of asthma and/or inflammation.

Methods:
Primary epithelial cells were cultured in submerged and air liquid interface (ALI) condition. Cells were treated with HDM, and the expression of APP was determined by immunoblotting and RT-PCR. Additionally, the expression of Aβ was determined by immunofluorescence. Separately, transepithelial electrical resistance (TEER) was assessed in HDM and exogenous Aβ treated cells in ALI in absence/ presence of beta secretase 1 (BACE1) inhibitor (verubecestat).

Results:
Cells treated with HDM upregulated protein and mRNA expression of APP, along with the expression of Aβ. In ALI, HDM decreased TEER in epithelial cells. Additionally, the exogenous Aβ also demonstrated reduction in epithelial cells TEER, which was reversed by BACE1 inhibitor. The effect of HDM on TEER was inhibited in the presence BACE1 inhibitor, suggesting the role of Aβ regulation in epithelial inflammatory changes.

Conclusion:
HDM induces Aβ in epithelial cells and regulates the development and progression of asthmatic changes by disrupting the epithelial barrier *in-vitro.*

Abstract #032

Investigating the Drug Delivery and Release Properties of Differing Hypoxia-Responsive Polymersomes for the Treatment of Triple-Negative Breast Cancer

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Purpose:
Hypoxia in solid tumors is associated with poor patient prognosis and increased difficulty in treatment. In triple-negative breast cancer, hypoxia instigates an increase in invasive and metastatic phenotypes, thereby necessitating a targeted drug delivery system that can also limit the release of anti-cancer drugs that would otherwise express harmful side effects in conventional treatments. Polymersomes, nanoparticles capable of encapsulating drugs, can be a controllable vehicle for potential drug delivery. This study will investigate the stability and release characteristics of polymersomes with different hypoxia-responsive linkers with alternative electron induction properties. Additionally, this study will investigate the usage of Lycorine and Sulfasalazine as adjuvants alongside Doxorubicin for targeted drug delivery.

Methods:
The polymersomes are conjugated with iRGD on the outer surface and encapsulate doxorubicin. These polymersomes were characterized by encapsulation efficiency, size, and integrity. Synthesized hypoxia linkers and peptides were characterized via proton NMR and mass spectrometry.

Results:
Initial imaging studies with triple-negative breast cancer cell line MDA-MB 231, patient derived cell lines and breast cancer cell lines depict the overexpression of the receptor SLC7A11 and cancer stemness in hypoxia conditions. Treatment and synergy were established to assess effectiveness of adjuvant treatments.

Conclusion:
The cytotoxic levels of polymersomes will be assessed against cell line MDA-MB-231 and PDX cells, both in monolayer and spheroid growth settings.

Abstract #033

Sex-dependent Protective Effects of Alamandine in Cardiac Aging

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Introduction:
The renin-angiotensin system (RAS) is critical in regulating cardiac function and also in the pathogenesis of cardiac disease. Alamandine (Ala), a member of the non-classical RAS is produced from the catalytic hydrolysis of Angiotensin A by ACE2 or by the decarboxylation of Angiotensin 1-7. Previous studies showed that Alamandine, a ligand of the Mas-related G-protein coupled receptors member D (MrgD), is cardioprotective. D-pro-(Angiotensin 1-7) [D-Pro] is a Mas receptor and MrgD antagonist. In this study we investigated whether Alamandine is protective in the aging heart.

Method:
Mice aged 2-3 months (Young) or 22–23 months (Old) were treated with a vehicle (saline), Alamandine (1 mg/Kg/min, s.c), or D-pro (1 mg/Kg/min, s.c) for 4 weeks. At the end of treatment, echocardiography was performed before cardiac tissue was collected and probed for protein expression.

Results:
MrgD had a higher expression in young vs. old females, and no difference was noted in MrgD expression in young vs. old males. Interestingly, Alamandine increased MrgD expression in males while no significant change in females. An age-dependent decline in systolic function and worsened cardiac hypertrophy was observed in the females, and this was attenuated by Alamandine. However, while there was an age-dependent increase in cardiac hypertrophy, systolic function did not differ between young and old male mice. Interestingly, Alamandine exacerbated age-dependent cardiac hypertrophy in males.

Conclusion:
Our data suggests that in aging, the beneficial effects of Alamandine are sex-dependent. Additionally,if MrgD activity is not tightly regulated, cardiac function in the aged is impaired.

Abstract #034

Selective Inhibition of HDAC 2 & 6 using acPEG-PLKC Nanoparticles HEK-293T Cells

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Introduction:
Histone deacetylases (HDACs) are critical regulators of gene expression and have been implicated in various diseases, including cancer. Selective inhibition of specific HDAC isoforms presents a promising therapeutic strategy to minimize off-target effects. In this study, we investigated the potential of acPEG-PLKC nanoparticles to selectively inhibit HDAC 6 while assessing their effect on HDAC 2 expression in HEK-293T cells.

Methods:
We synthesized HDAC-responsive nanoparticles using a block copolymer composed of poly(ethylene glycol) and poly(L-acetylated lysine). HEK-293T cells were cultured in DMEM with 10% FBS and transduced with lentiviral vectors to overexpress HDAC 2 and HDAC 6. Western blot analysis confirmed successful overexpression, while immunofluorescence imaging assessed the subcellular localization of the nanoparticles. Cells were treated with 50 µg/mL of acPEG-PLKC nanoparticles, and changes in HDAC expression were analyzed via Western blot.

Results:
Characterization studies demonstrated that PEG-acPLKC nanoparticles formed stable particles with a hydrodynamic diameter of 120-150 nm and a negatively charged surface, preventing nonspecific cytotoxicity. Western blot analysis confirmed that treatment with acPEG-PLKC nanoparticles led to a significant inhibition of HDAC 6 expression and non-significant inhibition of HDAC 2. These findings highlight the selective targeting capability of the nanoparticle system.

Conclusion:
Our results demonstrate that acPEG-PLKC nanoparticles selectively inhibit HDAC 2 & HDAC 6 in HEK-293T cells. This selective inhibition provides a strong foundation for further molecular studies and potential therapeutic applications in HDAC-targeted drug delivery.

Abstract #035

Cardiac E-C Coupling Protein Junctophilin-2 as a Double-Stranded RNA Sensor: A Novel Role in Cardiomyocyte Innate immunity

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Purpose:
Cardiomyocytes' high mitochondrial content raises immunogenic risk by leaking dsRNA into the cytosol. How cardiomyocytes sequester baseline dsRNA immunogenicity remains underexplored. Our study revealed a novel role of JPH2 as a dsRNA-sequestering protein that regulates dsRNA-induced innate immune responses in cardiomyocytes.

Methods:
JPH2 knockout mouse model and overexpression was achieved using a Cas9-AAV system and adenovirus. Gene silencing realized by siRNAs. Cellular protein-protein/ protein-dsRNA interactions were assessed by PLA and co-IP. Innate immune response was assessed by qPCR of IFNβ1, IFIT1, and CXCL10. JPH2's dsRNA-binding capability was characterized by EMSA. RNase-III protection assays and filter trapping were employed to evaluate JPH2's role in dsRNA sequestration.

Results:
Elevated cytosolic dsRNA levels enhance JPH2's interaction with MDA5 while reducing its association with the L-type Ca^{2+} channel. Overexpressing JPH2 diminishes MDA5-dsRNA binding, whereas JPH2 deficiency increases MDA5-dsRNA interaction, MAVS-TBK1 complex formation, and spontaneous IFNβ1 production. JPH2 knockout mouse hearts show an upregulation of dsRNA-induced innate immune responses. Mechanistically, JPH2 binds long dsRNA sequences, including poly(I:C) and mitochondrial dsRNA, undergoing condensation and aggregation with reduced mobility and solvent insolubility. RNase-III protection assays indicate JPH2 sequesters dsRNA within condensates. These aggregates are reversible upon RNase-III digestion. Domain-mapping studies reveal that JPH2's C-terminal half mediates dsRNA binding, while the N-terminal region does not contribute to this function.

Conclusion:
Our findings uncover a previously unrecognized role of JPH2 as a dsRNA-binding protein. On one hand, this discovery highlights JPH2 as a critical regulator in preventing spontaneous activation of innate immune responses in the heart. On the other hand, JPH2's phase transition upon dsRNA binding suggests that elevated cytosolic dsRNA may modulate JPH2's function in electrophysiology and contraction, offering novel insights into the clinical occurrence of arrhythmias and contractile abnormalities in myocardial infarction and viral myocarditis.

Abstract #036

iRGD-functionalized Polymersomes with Doxorubicin and NAPA to Reduce Stemness of Triple-Negative Breast Cancer

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Purpose:
This study aims to develop a more effective treatment strategy for aggressive forms of cancer. Triple-negative breast cancer (TNBC) is challenging to treat due to the absence of estrogen and progesterone receptors and HER2. We focus on targeting breast cancer stem cells (BCSCs), which play a crucial role in tumor initiation, metastasis, and treatment resistance. By functionalizing polymersomes with the iRGD peptide, we aim to enhance the delivery of doxorubicin (Dox) and napabucasin (NAPA). This combination therapy aims to enhance the cytotoxic effects on BCSCs, overcome chemoresistance caused by hypoxia, and increase therapeutic efficacy while reducing systemic toxicity in the treatment of TNBC.

Methods:
We conducted experiments using patient-derived breast cancer stem cells to investigate the effects of combining NAPA and Dox on cell growth under normoxic (21% oxygen) and hypoxic (2% oxygen) conditions. We assessed the expression of the stemness marker through immunofluorescence imaging and flow cytometry analysis. Additionally, we created iRGD-functionalized polymersomes containing Dox and NAPA to perform migration and invasion assays on patient-derived breast cancer stem cells. We also evaluated the impact of these polymersomes in spheroid studies.

Results:
Overall, iRGD-functionalized polymersomes containing Dox and NAPA exhibited potent antitumor activity in both monolayer and spheroid models of breast cancer stem cells (BCSCs). Additionally, the treatment significantly decreased the expression of key cancer stemness markers, further highlighting the therapeutic potential of this approach in targeting BCSC-driven tumor growth and resistance.

Conclusion:
This study demonstrates the potential of iRGD-functionalized polymersomes to enhance the targeted delivery of doxorubicin and napabucasin to breast cancer stem cells, improving therapeutic outcomes in triple-negative breast cancer. By overcoming challenges like hypoxia-induced resistance, this approach offers a promising strategy for more effective and safer cancer treatment.

Abstract #037

Integrin promotes angiotensin II signaling and extracellular matrix dynamics

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Purpose:
Hypertension is one of the significant risk factors for chronic kidney disease, where angiotensin II (Ang II) promotes renal cellular injury and fibrosis. In experimental models of kidney injury, Ang II infusion induced fibrosis in the kidney. Since earlier studies have shown that Ang II signaling pathways are primarily mediated by the receptors AGTR1 and AGTR2, angiotensin II receptor blockers (ARBs) were developed to prevent hypertension and renal fibrosis. AGTR2 mediates antiproliferative and antifibrotic signaling in renal epithelial cells. However, the effect of fibrosis on AGTR2 signaling has never been investigated. Thus, this study addressed this knowledge gap.

Method:
Signaling from the basement membrane is principally mediated by cell surface receptors. Among these, the integrin superfamily is the primary receptor that mediates collagen homeostasis in the basement membrane. Furthermore, integrin activation is regulated by its binding to the adaptor protein talin-1. In this study, integrin β1 and talin-1 protein levels were downregulated in renal epithelial cells with shRNA. Using in vitro assays, the significance of integrin β1 and talin-1 on extracellular matrix dynamics was investigated.

Results:
Our results indicate that the disruption of matrix signaling increased AGTR2 expression in the cells.

Conclusion:
Higher AGTR2 levels resulting from decreased matrix signaling inhibited cell proliferation. In the future, studies will determine the significance of aging on integrins, talin-1, and Ang II signaling.

Abstract #038

Dietary Fiber Intake over Two Years Following Metabolic and Bariatric Surgery is Associated with Changes in the Composition of the Gut Microbiota

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Purpose:
Studies have shown changes in gut microbiota post metabolic and bariatric surgery (MBS) but large prospective longitudinal studies relating these changes to dietary fiber intake over time are lacking. The aim of this study was to describe the changes in the gut microbiota longitudinally over time following MBS and relate these changes to dietary fiber intake over two years following the surgery.

Methods:
We hypothesized that increased dietary fiber intake will support the growth of some beneficial bacteria which will in turn increase microbial metabolites required for maintaining a healthy gut. Adults planning to undergo Roux-en-Y Gastric Bypass or Sleeve Gastrectomy were recruited and assessed at baseline and at 1, 6-, 12-, 18-, and 24-months post-MBS. A 24h dietary recall method was used to record dietary intake at each time point. Fecal samples were collected at each time point and analyzed using 16S rRNA sequencing.

Results:
Dietary fiber from the ASA-24 recorded at baseline was (13.29±6.91) g/day, with the lowest recorded one-month post-MBS (4.91±3.70) g/day. Dietary fiber intake increased from 6 months (7.78±4.97) g/day to 24 months (9.36 ±5.11) g/day post-MBS. Degraders of dietary fiber including *Lactobacillus, Akkermansia, Roseburia, Bifidobacterium, Ruminococcus* and *Faecalibacterium* increased in abundance after MBS.

Conclusion:
Although the dietary fiber intake was below the recommended intake, microbes responsible for their breakdown were abundant. In future analyses, short chain fatty acids produced from dietary fiber breakdown will be analyzed and their concentrations correlated to the amounts of dietary fiber consumed and the abundance of dietary fiber degraders.

Abstract #039

Machine Learning-Based Drug Discovery: QSAR Model to Predict Anticancer Activity

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Purpose:
According to the American Cancer Society, cancer is a second leading cause of death in the United States. Breast cancer remains the most common cancer among women worldwide, accounting for **25% of all cancer cases**, and is the **second leading cause of cancer-related deaths in women** after lung cancer. While significant progress has been made in early diagnosis and treatment, there remains a critical need for more effective drugs—both natural and synthetic—with minimal side effects.

Method:
This study compiled a **database 621 compounds** with demonstrated anticancer activity against **MSF-7 cancer cell lines**. We clustered the compounds to explore structure-activity relationships further to identify promising chemical classes regarding activity and toxicity. Additionally**, SHapley Additive exPlanations (SHAP)** analysis was applied to identify and quantify the most influential molecular descriptors in our QSAR model.

Results:
We developed a QSAR model with high predictive accuracy, where the training and test sets achieved R^2 values of 0.8805 and 0.8526, respectively. The **SpDiam_A** descriptor emerged as the most critical feature, with a mean absolute SHAP value of **0.12.** The **SpMin1_Bh(p)** descriptor was the second most influential descriptor **(SHAP value = 0.06).** Further**, SpDiam_A (0.922)** and **SpMax1_Bh(p) (0.986) negatively** impacted a specific prediction**,** while **SpMin1_Bh(p) (0.689)** and **MDEC-33 (0.477)** contributed positively.

Conclusion:
Our study developed a highly predictive QSAR model (R^2: 0.8805 training, 0.8526 test) for anticancer activity against MCF-7 cells, identifying SpDiam_A and SpMin1_Bh(p) as key molecular descriptors influencing activity.

Abstract #040

Blocking $P2Y_{12}$ and PD-1 in Tumor-Associated Macrophages reduces pancreatic cancer cell growth and migration through the TGF-β1/Smad2 pathway

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Purpose:
Pancreatic ductal adenocarcinoma is the third primary cause of cancer-related mortality globally. The immunosuppressive tumor microenvironment (TME), including tumor-associated macrophages (TAMs), significantly restricts the effectiveness of immunotherapies. Blockade of the programmed cell death protein 1 (PD-1)/PD-1 ligand 1 (PD-L1) axis, on TAMs, reduces pancreatic tumor growth in mice, but not consistently. Therefore, blocking other immune-regulatory pathways could serve a complementary role to enhance the efficacy of PD-1/PD-L1 blockers. $P2Y_{12}$ is an ADP receptor expressed in macrophages, and $P2Y_{12}$ antagonists promote phagocytosis. We aim to investigate a co-treatment that blocks PD-1/PD-L1 and $P2Y_{12}$ on TAMs.

Methods:
TAM-like cells were generated *in vitro* by culturing THP-1 monocytes with PMA, followed by incubation with cancer cell-conditioned media for 48 hours. We used PANC-1, BxPC-3, and patient-derived human pancreatic cancer cells (HPCCs). We used BMS-1 to block PD-1/PD-L1, cemiplimab to block PD-1, and ticagrelor to block $P2Y_{12}$, alone or in combination. We investigated TAM phagocytosis and cancer cell proliferation and migration in the TAM-cancer cell co-culture. ELISA and western blot were performed to investigate the underlying mechanisms.

Results:
Co-treatment of ticagrelor and cemiplimab promotes TAMs' phagocytic ability of PANC-1 and BxPC-3 and inhibits the TAM-induced growth and migration. Co-treatment of ticagrelor and cemiplimab synergistically increases TAMs' phagocytic ability of HPCCs and decreases the TAM-induced migration. The combination of ticagrelor and cemiplimab prevents TGF-β1 release from TAMs and Smad2 phosphorylation in cancer cells.

Conclusion:
Co-treatment with ticagrelor and cemiplimab inhibits pancreatic cancer cell growth and migration by inhibiting the TGF-β1/Smad2 signaling pathway.

Abstract #041

Novel synthesized KpSAT peptides regulates airway smooth muscle remodeling

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Background:
Kisspeptins (Kp) are a group of hypothalamic peptides that aid in regulating pubertal development and sex hormone signaling. Our recent studies showed the protective role of Kp-10 (Kp peptide) and its receptor KISS1R signaling in regulating the airway smooth muscle (ASM) structure and function in the context of asthma. Studies have shown that specific modifications to the amino acids of Kp peptides can result in increased binding efficiency and receptor activation. Accordingly, we developed three novel Kp peptides, named KpSATs (13A, 13B, and 13C), and tested the potential effects/efficiency on ASM cell remodeling.

Methods:
KpSATs were synthesized by GenScript with purity of ≥97%. ASM Cells were treated with PDGF (2ng/mL) and/or TGF-β (2ng/mL) with/without treatments of KpSATs and Kp-10. ASM cell proliferation was measured using MTT. Extracellular matrix (ECM) deposition (fibronectin and collagen-I/III) studies were performed using In-Cell Western. The mechanistic signaling pathway (phospho-PKC-δ) were measured using Western blot analysis.

Results:
The MTT assay indicated a reduction in PDGF-induced ASM cell proliferation upon exposure to KpSATs. Furthermore, ECM deposition studies showed a significant reduction of TGF-β1-induced collagen-I/III and fibronectin in ASM cells exposed to KpSATs. In addition, we observed significant inhibition of PDGF-induced phospho-PKC-δ by KpSATs.

Conclusion:
Overall, these findings highlight that modification in amino acid sequence in endogenous Kp-13 sequence significantly increases the potency of synthesized novel KpSATs on ASM cells. In addition, KpSATs showed better inhibitory effects compared to Kp-10 and could be considered as a promising therapeutic strategy to mitigate asthma.

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Abstract #042

Effect of RAGE inhibition on vemurafenib resistance in melanoma cells

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Purpose:
BRAF mutations are present in over half of melanoma cases, making vemurafenib a targeted treatment option for BRAFV600E mutant melanomas. However, resistance to vemurafenib often develops within seven months after the treatment, leading to tumor recurrence and drug-resistant melanoma. This poses a significant clinical challenge, with various molecular pathways identified as drivers of BRAF inhibitor resistance. The Receptor for Advanced Glycation End-products (RAGE) has been implicated in therapy resistance through various mechanisms, including regulation of autophagy and apoptosis. We hypothesize that RAGE inhibition enhances the sensitivity of melanoma cells to BRAF inhibitor vemurafenib by reducing chronic ER stress-induced autophagy and increasing apoptosis.

Method:
We investigated the synergistic effects of vemurafenib in combination with the RAGE inhibitor FPS-ZM1 in the WM115 human melanoma cell line. Cell viability was assessed using Alamar Blue, and drug synergy was analyzed using the Combenefit software. Changes in the levels of the autophagic markers LC3-I/-II and p62, the ER stress marker SAPK/JNK and cell proliferative markers Erk 1/2, Akt are currently being evaluated by Western blot analysis.

Results:
Our data suggest that RAGE inhibition sensitize BRAF mutated melanoma cells to vemurafenib, and we observed the strongest synergistic effects with 30µM vemurafenib and 10µM FPS-ZM1. Our initial Western blot results suggest that the drug combination reduces the autophagic flux. We are currently investigating the changes in the levels of the ER stress markers and proliferative markers.

Conclusion:
Our findings suggest that RAGE inhibition sensitizes BRAF mutated melanoma cells to vemurafenib.

Abstract #043

Deep Learning-Based Automated Detection of Ca^{2+} Sparks Using Synthetic Training Images

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Purpose:
Calcium (Ca^{2+}) sparks are fundamental signaling events essential for cell excitability. Ca^{2+} sparks are usually recorded by confocal line-scan imaging. Automated Ca^{2+} spark image analysis approach is highly demanded. Deep learning has revolutionized bioimage analysis, but a major challenge is the need for large, annotated training datasets. Due to the labor consuming nature of Ca^{2+} imaging experiments, it is difficult to obtain large training datasets for Ca^{2+} spark detection. This study is to solve this limitation by using synthetic training data.

Methods:
Synthetic images were generated by simulating Ca^{2+} spark kinetics, ensuring well-annotated and diverse training datasets. The math model is:

$$f(t,x) = A\left(e^{-\frac{(t-t_0)}{\tau_{decay}}} - e^{-\frac{(t-t_0)}{\tau_{rise}}}\right)e^{\left(\frac{-(x-x_0)^2}{2\left(\sigma+\alpha\,(t-t_0)\right)^2}\right)}$$

Deep learning segmentation models, including U-Net and transformer-based architectures, were trained using the PyTorch framework with the Adam optimizer and CrossEntropyLoss. Model performance was evaluated on both synthetic and experimental images.

Results:
The use of synthetic data enabled precise ground-truth labels and robust training, reducing dependency on experimental datasets. The trained models accurately detected and segmented Ca^{2+} sparks, achieving a mean IoU of 0.93 on the validation dataset. The models also robustly identified sparks in experimental images, demonstrating high generalizability and robustness.

Conclusion:
This study provides a powerful, automated tool for Ca^{2+} signaling research, enabling high-throughput and standardized analysis of Ca^{2+} dynamics. The use of simulated training data ensures reproducibility, scalability, and eliminates the need for extensive manual annotation. Furthermore, this strategy can be generalized to other events and phenomena that can be mathematically modeled, expanding the applicability of deep learning in biomedical image analysis and beyond.

Abstract #044

Unraveling the Black Box of the Cell Apoptosis Regulation by MAP kinase Synthesis of Isoquercetin and Analogues, Biological Assays and Structure-Activity Relationship.

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North Dakota State University School of Pharmacy

Purpose:
The development of new strategies in organic chemistry have allowed for innovative approaches in drug discovery, especially in oncology. Breast cancer and malignant melanoma are currently the most common and aggressive form of cancer, and existing treatments present severe side effects, emergence of resistance over time, and DNA damage. Thus, there is a need for alternative strategies. Flavonoids, a group of natural compounds, have shown several biological activities, including vasoactive, antioxidant, anti-inflammatory, and antitumor effects. Isoquercetin stands out due to its cardioprotective effects and antioxidant activity, since it mitigates reactive oxygen species. These reports suggest that flavonoids are promising candidates for oncology treatments. Therefore, total synthesis of isoquercetin and analogues, studies regarding structure-activity, and synthesis of tagged probes may determine the best optimized structure for improved outcomes.

Methods:
This study aims to establish an understanding of isoquercetin, to facilitate the preparation of new products. Total synthesis of isoquercetin and analogues will happen through two different routes, and biological activity of the compounds will be assessed. Moreover, structure-activity of these compounds will be studied to determine the best biological targets and mechanisms. Lastly, through synthesis of tagged probes, map and manipulate molecular interaction sites in the global proteome of live cells.

Results:
Expected results are to determine the most promising isoquercetin analogue that will allow for best outcomes in cancer treatment.

Conclusion:
Understanding the mechanism of action and structure-activity of natural compounds may allow for a new pathway in cancer treatments, that may present less side effects and better outcomes.