The 22nd Annual Department of Pathology Research Retreat

featuring the

Pathology 33rd Annual Research Day

Wednesday, May 17, 2023

University of Pittsburgh School of Medicine
Department of Pathology

Department of Pathology

2023 Research Day and Retreat

University of Pittsburgh

UPMC
LIFE CHANGING MEDICINE

Program Contents

Retreat Planning Committee

Faculty	Andrew Duncan, PhD, *Chair* Sameer Agnihotri, PhD Aaron Bell, PhD Yuan Chang, MD Marie DeFrances, MD, PhD Roy Frye, MD Wendy Mars, PhD Tim Oury, MD, PhD Octavia Palmer, PhD Alex Soto, MD PhD
Graduate Students	Joud Mulla, PhD Candidate Sierra Wilson, PhD Candidate
Coordinator	Shanning Wan

Department of Pathology
2023 Research Day and Retreat

May 17, 2023

University of Pittsburgh. UPMC LIFE CHANGING MEDICINE

Keynote Presentation (lunch provided)

Location: S100A BST & https://pitt.zoom.us/j/95297523813

12:00 – 1:00 pm	**Robin G. Lorenz, MD, PhD** Executive Director of Research Pathology, Genentech	*Research Pathology: From Academics to Biotech*

Faculty & Trainee Presentations

Location: S100A BST & https://pitt.zoom.us/j/95297523813

1:15 pm	**Opening Remarks** Andrew Duncan, PhD Associate Professor of Pathology	Liron Pantanowitz, MD Professor and Chair of Pathology

Session 1: Graduate Student Research

Moderator, Rithika Behera, BS, PhD Candidate

1:30 – 1:50 pm	**Sameer Agnihotri, PhD** Assistant Professor of Neurosurgery	*Childhood Brain Tumors: Molecular Insights and Future Directions*
1:50 – 2:00 pm	**Gabrielle Gilmer, BS** PhD Candidate, CMP, MSTP	*Characterizing the Trajectory of Murine Knee Osteoarthritis Across Menopause*
2:00 – 2:10 pm	**Sierra Wilson, BA** PhD Candidate, CMP, CATER	*Compensatory Regeneration After Acetaminophen-Induced Acute Liver Injury Is Driven by Diploid Hepatocytes*
2:10– 2:20 pm	**Joe Maggiore, BS** PhD Candidate, ISB, MSTP, CATER	*Genetically Engineered Endothelial Niche Induces Mature Cell Populations in Human Kidney Organoids*

Session 2: Basic Research

Moderator, Hannah Butterfield, BA, PhD Candidate

2:30 – 2:50 pm	**Smita Iyer, PhD** Associate Professor of Pathology	*Understanding Principles of the Neuro-T Cell Unit*
2:50 – 3:00 pm	**Yue (Yolanda) Wang, MD** Research Instructor	*Role of BCL2L14-ETV6 Gene Fusions in Triple-Negative Breast Cancer Immune Evasion*
3:00 – 3:10 pm	**Sudrishti Chaudhary, PhD** Postdoctoral Fellow	*Western Diet Dampens T Regulatory Cell Function to Fuel Hepatic Inflammation in Nonalcoholic Fatty Liver Disease*
3:10 – 3:20 pm	**Gavin Schmidt** Undergraduate Researcher	*Fusions Involving the Thyroglobulin Gene as a Novel Mechanism of Thyroid Carcinogenesis*

Session 3: Clinical Research

Moderator, Pranav Patwardhan, MD, Pathology Resident

3:30 – 3:50 pm	**Octavia Peck Palmer, PhD** Associate Professor of Pathology	*Health is Wealth: Designing Equitable Clinical Laboratories for Thriving Communities*
3:50 – 4:00 pm	**Dimitrios Korentzelos, MD** Pathology Resident	*Integrated Clinicopathologic and Gene Expression Analysis to Profile Immune Prognostic Indicators in Uterine and Non-Uterine Leiomyosarcoma (LMS)*
4:00 – 4:10 pm	**Zeliha Cetin, MD** Research Fellow	*Contribution of MAFLD-Associated Genetic Variants to the Progression of End-Stage Liver Disease Due to NASH or ALD*
4:10 – 4:20 pm	**Simmi Patel, MD** Pathology Resident	*The Detection of Alternative Lengthening of Telomeres using a Novel Chromogenic in situ Hybridization Assay is a Poor Prognostic Biomarker for Patients with Pancreatic Neuroendocrine Tumors*

Poster Session & Reception

University Club, Ballroom B

5:00 – 7:00 pm — Join your colleagues at the Pitt University Club for an interactive poster session, hors d'oeuvres, and drinks. Say hello to old friends and make some new ones.

Notes

Participation by all individuals is encouraged. Advance notification of any special needs will help us provide better service. Please notify us of your needs at least two weeks before the program by emailing Shanning Wan at shw126@pitt.edu.

All individuals in a position to control the content of this education activity are required to disclose all relevant financial relationships with any entity producing, marketing, re-selling, or distributing health care goods or services, used on, or consumed by, patients.

Photographic Permission Release Announcement: Photographs will be taken at this event. Inform the photographer(s) if you do not want your photograph taken. Consent is granted for the unrestricted worldwide use by the University of Pittsburgh ("University") of your photograph, picture, image, or portrait ("Image") in perpetuity for use in publications, University Websites, or other promotional materials. You further agree that the Image and all of its elements belong entirely and exclusively to the University, so that the University and its licensees and assignees may use the Image, or any portion thereof, without limitation or restriction. You authorize use of your Image for University marketing purposes. This announcement releases the University and its licensees and assigns from any liability arising from the use of your Image.

CME credit is provided for Dr. Lorenz's seminar. Record your attendance by SMS text. To enable the SMS texting feature, log in to your account at http://cce.upmc.com. Click the "Mobile" tab to add your ten-digit mobile phone. Receive credit instantly by texting the following code to 412-312-4424: FUNKUC

The University of Pittsburgh is an affirmative action, equal opportunity institution.

May 17, 2023, 5:00 – 7:00 pm
Ballroom B, University Club
University of Pittsburgh

Presentation Schedule
5:00 – 6:00 pm
Odd-numbered posters

6:00 – 7:00 pm
Even-numbered posters

Department of Pathology
2023 Research Day and Retreat

Poster Session

University of Pittsburgh. UPMC LIFE CHANGING MEDICINE

CMP & CATER Graduate Students

1. **Ibrahim Ahmed**, *Long non-coding RNA, CASC15, regulates smooth muscle cell size, proliferation, and migration*
2. **Shruthi Balasubramaniyan**, *The VCP(T262A) mutation is linked to autophagic and ER-Golgi secretory defects*
3. **Shruthi Balasubramaniyan**, *Investigating the Role of VCP in Clearing Neuronal Nuclear TDP-43 Aggregates*
4. **Yu Bian**, *The role of Tox in regulatory T cells in the tumor*
5. **Emily Brown**, *Investigating the role of the mSWI/SNF (BAF) nucleosome remodeling complex in regulating neural cell differentiation*
6. **Hannah Butterfield**, *Evaluating MAL1 as a therapeutic target in H3K27M-mutant diffuse midline glioma*
7. **Margaret Champion**, *Investigating the Role of Alternative Splicing in Germline Aging*
8. **Mona Chatrizeh**, *Plant-based enteral nutrition is superior to artificial nutrition in recovering antibiotic-induced immune suppression*
9. **Anu Balogun**, *Beta-catenin inhibition as a novel therapeutic strategy for Porphyria*
10. **Yu-Wei Cheng**, *TFEB is required for lysosomal biogenesis and cell survival during cellular senescence*
11. **Andrea Cruz**, *Uncovering therapeutic targets in the tumor microenvironment of H3K27-mutant diffuse midline gliomas*
12. **Ian Eder**, *Profilin-1/CCL2 is a novel signaling axis of tumor cell-directed migration of immune cells*
13. **Sonny Elizaldi**, *a4 integrin+ CCR5+ CD4 T cells mediate acute SIV CNS seeding in Rhesus Macaques*
14. **Taylor Gatesman**, *Inhibiting insulin signaling reverses resistance to PI3K-mTOR inhibitors in aggressive pediatric high-grade gliomas*
15. **Shohini Ghosh-Choudhary**, *Using CRISPR Screening to Find Senolytic Druggable Targets*
16. **Maria Beecher**, *MALT1 is activated by doxorubicin and mediates therapy resistance in triple-negative breast cancer*
17. **Gabrielle Gilmer**, *Characterizing the trajectory of murine knee osteoarthritis across menopause*
18. **Matthew Halbert**, *H3K27M Histone mutant gliomas are sensitive to methionine loss and MAT2A inhibition through a novel feedback mechanisms between AMD1 and METTL16*
19. **Shea Heilman**, *scRNA-seq reveals TET-dependent gene expression events that occur during retinogenesis*
20. **Dana Julian**, *Quantitative Digital Image Analysis of Whole Slide Images to Investigate White Matter Rarefaction in Alzheimer's Disease*
21. **Joshua Hislop**, *An Engineered Human Model of Post-implantation Extra-Embryonic Niche*
22. **Yekaterina Krutsenko**, *Dual loss of β-catenin and γ-catenin from cholangiocytes causes intrahepatic cholestatic injury in mice*
23. **Rithika Behera**, *Transcriptional regulation of SSc dermal myofibroblasts by FOSL2 and FOXP1*
24. **Ryan LeGraw**, *Understanding and Engineering Human Hematopoiesis using a Genetically Engineered Fetal Liver Niche*
25. **Brandon Lehrich**, *β-Catenin Activation Promotes B-cell Exclusion in the Hepatocellular Carcinoma Microenvironment*
26. **Jack Little**, *MALT1 protease coordinates the expression of an immunosuppressive secretome in GPCR+ triple-negative breast cancer*
27. **Jie Bin Liu**, *Characterizing and Targeting ERBB2 Mutations in Invasive Lobular Carcinoma*
28. **Joe Maggiore**, *Genetically engineered endothelial niche induces mature cell populations in human kidney organoids*
29. **Meagan Makarczyk**, *An Innervated Cartilage Synovial Chip to Study Joint Inflammation*
30. **Isabelle Chickanosky**, *Modeling Endometriosis Angiogenesis in Endometriotic Conditions*
31. **Varun Mandi**, *Environmental circadian desynchronization and metabolic stress drive heart failure with preserved ejection fraction (HFpEF)*
32. **Philip Mannes**, *In vivo molecular imaging of chemokine-like receptor 1 (CMKLR1) to monitor ongoing inflammation in a preclinical bleomycin-induced lung injury model*
33. **Joud Mulla**, *Endothelial Cell Caspase-11 Regulates Il-6-Mediated Inflammation and Organ Damage in Mice Following Severe Injury*
34. **Alexis Nolfi**, *Therapeutic Use of an Interleukin-4 Eye Drop in a Rabbit Model of Dry Eye Disease: A Pilot Study*
35. **Kimberly Ortiz**, *Human hepatocytes from explanted livers of patients with cholestatic liver diseases as a valuable model for pathological mechanism studies*
36. **Matthew Poskus**, *Mathematical Modeling of Fibroblast Mediated Drug Resistance in HER2+ Breast Cancer*
37. **Justin Sui**, *Loss of ANT1 increases fibrosis and senescence in idiopathic pulmonary fibrosis*
38. **Mohammad Naser Taheri**, *A Genetic Surveillance Circuit for Liver Organoid Improvement by Eliminating Abnormal Cell States*
39. **Grace Conway**, *Understanding the mechanisms of ultrasound-targeted microbubble cavitation-mediated blood brain barrier opening*
40. **Rick van der Geest**, *BATF2 enhances pro-inflammatory cytokine responses in macrophages and contributes to the host defense against pulmonary Klebsiella pneumoniae infection*

Odd-numbered posters (5:00 – 6:00 pm) • Even-numbered posters (6:00 – 7:00 pm)

41. **Susannah Waxman**, *Visualizing and Quantifying Individual Astrocyte Morphologies Across the Collagenous Lamina Cribrosa*
42. **Sierra Wilson**, *Compensatory regeneration after acetaminophen-induced acute liver injury is driven by diploid hepatocytes*
43. **Jiazhen Xu**, *The study of Circular RNAs and their regulation of TAR DNA binding protein 43 (TDP-43) pathological aggregation*
44. **Hsuan Yeh**, *Role of β-cell glucocorticoid receptor signaling in pregnancy and gestational diabetes*

Basic Research

45. **Shehnaz Bano**, *Hepatocytes Specific Deletion of Epidermal Growth Factor alters Lipid Metabolism and Fibrosis Signaling in a Murine Fast-Food Diet Model of Nonalcoholic Fatty Liver Disease*
46. **Sudrishti Chaudhary**, *Western diet dampens T regulatory cell function to fuel hepatic inflammation in nonalcoholic fatty liver disease*
47. **Meghana Dodda**, *Development of a human glioblastoma model using humanized DRAG mice for immunotherapy*
48. **Gabriella Fricklas**, *PINK1 moderates dendritic mitochondrial content by regulating somatic to dendritic transitions*
49. **Alex Hill**, *Self-timed Differentiation of Human Liver Organoids via Engineering of Gene Regulatory Networks*
50. **Chhavi Goel**, *Loss of β-catenin attenuates lithocholic acid-induced hepatotoxicity*
51. **Bashir Lawal**, *Functional characterization of EPHA3 exon 4-5 duplications (EPHA3d4-5) in high-grade serous carcinoma progression, and recurrence*
52. **Parisa Lotfinejad**, *Landscape of intragenic rearrangements in triple-negative breast cancer reveals RUNX1 exon aberrations driving tumor immune evasion*
53. **Jiayi Lu**, *The roles of PINK1 in regulating dendritic mitochondrial distribution that contribute to the maintenance and maturation of dendritic arbors and spines*
54. **Vineet Mahajan**, *Decoding CRISPR-Cas9 Gene Manipulation in a Human Liver Microphysiological System: Bridging the Gap*
55. **Tahere Mokhtari**, *Enhanced Zinc-finger based Epigenetic Modulator as Next-generation Immunosuppressant*
56. **Laura Molina**, *Loss of TAZ after YAP deletion severely impairs foregut development and worsens hepatocellular injury due to severe cholestasis*
57. **Ravi Rai**, *C-C motif chemokine receptor 9 ablation worsens whereas therapeutic blocking protects mice from western diet-induced nonalcoholic steatohepatitis*
58. **Gavin Schmidt**, *Fusions Involving the Thyroglobulin Gene as a Novel Mechanism of Thyroid Carcinogenesis*
59. **Rayna Schoenberger**, *Collective Behaviors Drive the Formation of Fetal Liver Organoid Vascular Networks*
60. **Anya Singh-Varma**, *Identification of a novel stellate cell-endothelial cell-hepatocyte circuit that regulates zonation and metabolism-proliferation switch in liver pathophysiology*
61. **Yiyue Sun**, *Development of Lipid Nanoparticles-Based Transcriptional Reprograming Therapies for Cirrhosis with End-Stage Liver Disease*
62. **Anil Verma**, *Tailoring helper profile of HIV-1 vaccine-induced CD4 T follicular helper cells*
63. **Yue (Yolanda) Wang**, *Role of BCL2L14-ETV6 gene fusions in triple-negative breast cancer immune evasion*
64. **Yue Wang**, *The association of tumor associated antigen burden with immune checkpoint blockade benefit in selected tumor entities with low T cell exhaustion and mutation burden*
65. **Liyong Zhang**, *Role of Runt-related Transcription Factor 1 Intragenic Rearrangements in Cancers*
66. **Liyong Zhang**, *Therapeutic targeting of ESR1–CCDC170 Rearrangements in Endocrine Resistant Breast Cancer*

Clinical Research

67. **Zeliha Cetin**, *Contribution of MAFLD-associated genetic variants to the progression of end-stage liver disease due to NASH or ALD*
68. **Travis Stueber**, *The Clinical Significance of Increased Large Cells in Marginal Zone Lymphoma*
69. **Saurav Chopra**, *Application of the International Consensus Classification and World Health Organization 5th Edition Classification to a Series of Myeloid Neoplasms*
70. **Saurav Chopra**, *Prognostic Significance of Different Mutations in Acute Myeloid Leukemia with Myelodysplasia-Related Gene Mutations*
71. **Katelyn Davis**, *Frequency of Clinical Diagnosis of IgG4-Related Disease After Positive Lymph Node Biopsy for Increased IgG4 Positive Plasma Cells*
72. **Catherine Gestrich**, *ALK-rearranged Epithelioid Mesenchymal Neoplasm: Expanding the Spectrum of Tyrosine Kinase Altered Mesenchymal Tumors*
73. **Terri Jones**, *Accurately Making the Diagnosis of Mesothelioma Utilizing Serous Fluid Cytology Specimens: An Institutional Experience*
74. **Dimitrios Korentzelos**, *Integrated Clinicopathologic and Gene Expression Analysis to Profile Immune Prognostic Indicators in Uterine and Non-Uterine Leiomyosarcoma (LMS)*
75. **Mason Marshall**, *Correlation of the Detection Rate of Malignant Cells in Fluid Cytology with FIGO Stage in Primary Ovarian Clear Cell Carcinoma*
76. **Simmi Patel**, *The Detection of Alternative Lengthening of Telomeres using a Novel Chromogenic in situ Hybridization Assay is a Poor Prognostic Biomarker for Patients with Pancreatic Neuroendocrine Tumors*
77. **Pranav Patwardhan**, *Incidental detection of JAK2 V617F mutations in next generation sequencing analysis of solid tumors – a case series*
78. **Pranav Patwardhan**, *High TIM-3 Expression is an Independent Predictor of Improved Post-Radiation Therapy (RT) Clinical Outcomes of Vulvar Squamous Cell Carcinoma (SCCA)*
79. **Louis Samson**, *Aberrant neural crest cells migration leads to melanocyte presence in the umbilical cord*
80. **Kotaro Takeda**, *BAP1 is the most common disrupted gene in VHL mutation-negative advanced clear cell renal cell carcinoma*
81. **Rachel Vanderschelden**, *Implementation of Digital Image Analysis in Assessment of Ki-67 Index in Breast Cancer*
82. **Rachel Vanderschelden**, *Determining novel biomarker candidates associated with chronic drug exposure by exploring existing unused data acquired through urine comprehensive drug screening*
83. **Megan Zilla**, *Loss of Histone H3K27 Trimethylation (H3K27me3) Expression as a Potential Diagnostic Pitfall in Sarcomatoid Carcinoma*

Odd-numbered posters (5:00 – 6:00 pm) • Even-numbered posters (6:00 – 7:00 pm)

CMP & CATER Graduate Students

Author: **Ibrahim Ahmed, BS**
Contact: iba15@pitt.edu
Mentor: Delphine Gomez
Co-Authors: Cristina Espinosa-Diez, Mingjun Liu, Jianxin Wei, Thiago Bruder-Nascimento, Adam C Straub, and Delphine Gomez

Poster Number: 1

Long non-coding RNA, CASC15, regulates smooth muscle cell size, proliferation, and migration

Long non-coding RNAs (LncRNAs) have specific functions and confer diverse cellular regulation such as modulation of chromatin conformation, transcriptional and post transcriptional processing. In smooth muscle cells (SMCs), LncRNAs have been identified as regulators of contractility and plasticity in physiological and pathological conditions. In particular, LncRNAs impact the differentiation state of SMC which can switch from a contractile to a synthetic, proliferative, and migratory state in response to modifications of various environmental cues and vascular injury and disease. Through unbiased transcriptomic studies, we found that the lncRNA Cancer Susceptibility 15, CASC15, is preferentially expressed in SMC in various organs including the vasculature. Although studies have implicated CASC15 in regulating cell proliferation and apoptosis in cancer, the role of CASC15 in SMC has not been investigated. Here, we performed CASC15 loss and gain of function studies to delineate the function of this LncRNA on the SMC. We found that CASC15 expression was markedly decreased by treatment with a known molecular modulator of SMC functions, Angiotensin-II (ANGII) of cultured SMC or WT mice. Similarly, to ANGII treatment, CASC15 knockdown induced SMC hypertrophy and a defect in cell proliferation due to arrest in the G1/S phase leading to an increase in polyploidy and binucleation. Conversely, overexpression of CASC15 increased cell proliferation and migration while decreasing SMC hypertrophic-inducing phenotype of ANGII. Finally, we generate a novel CASC15 KO mouse and found that CASC15 deletion was associated with spontaneous hypertrophy of the aorta and exacerbated constriction of resistance arteries. CASC15 knockdown inhibited SMC proliferation and migration in vivo in a model of vascular injury. Together, our study uncovered a central role of the LncRNA CASC15 in the regulation of SMC morphology, proliferation, and migration.

CMP & CATER Graduate Students

Author: **Shruthi Balasubramaniyan, MS**
Contact: SHB127@Pitt.edu
Mentor: Charleen Chu
Co-Authors: Kent Z.Q. Wang, Aidan Reaver, Sara Sannino, Jeffrey L Brodsky, Julia Kofler, and Charleen T. Chu

Poster Number: 2

The VCP(T262A) mutation is linked to autophagic and ER-Golgi secretory defects

Valosin-containing protein (VCP/p97) is a type II AAA+ ATPase which is ubiquitously expressed in all tissues in multicellular organisms. VCP interacts with various co-factors to regulate multiple cellular processes including chromatin remodeling, Golgi apparatus dynamics, ubiquitin-dependent protein degradation and autophagy. Mutations in VCP cause progressive autosomal dominant adult onset multisystem proteinopathies, such as familial amyotrophic lateral sclerosis (ALS), fronto-temporal dementia (FTD), cardiomyopathy, and inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia (IBMPFD). In this study, we harvested primary fibroblasts from a patient carrying the VCP(T262A) mutation at the time of autopsy. The VCP(T262A) mutant showed significantly higher binding affinity to its co-factors, UFDL1 and p47. We found that this mutation has minimal effect in the ER-associated protein degradation (ERAD) pathway but shows a defect in regulating autophagic activities. Interestingly, the T262A mutation affected ER-Golgi protein trafficking in the secretory pathway. In addition, patient fibroblasts displayed compacted Golgi structure in comparison to the structure in wildtype VCP-expressing cells. Notably, both phenotypes are consistent with other VCP functions. Furthermore, we developed an iPSC line from the patient's fibroblasts, which will form a promising tool for further investigations of molecular mechanisms and potential therapeutic solutions for neurodegenerative diseases caused by the VCP(T262A) mutation.

CMP & CATER Graduate Students

Author: **Shruthi Balasubramaniyan, MS**
Contact: shb127@pitt.edu
Mentor: Charleen Chu
Co-Authors: Christopher J. Donnelly and Charleen T. Chu

Poster Number: 3

Investigating the Role of VCP in Clearing Neuronal Nuclear TDP-43 Aggregates

Frontotemporal dementia (FTD) is one of the leading causes of dementia in the United States. It results in the loss of nerve cells in the frontal and temporal regions of the brain. Major clinical FTD subtypes have been linked to mutations in different genes. FTLD-TDP type D is one of the sub-types of FTD in which neuronal TDP-43 inclusions are found and mutations in Valosin-containing protein (VCP) are often associated with the disease etiology (Neumann, Lee, & Mackenzie, 2022). VCP is a highly conserved protein belonging to the AAA-ATPase family. VCP is involved in a myriad of pathways including endoplasmic reticulum associated degradation, autophagy, mitochondria associated degradation, endosomal trafficking, chromatin associated degradation and aggregate handling (Meyer, Bug, & Bremer, 2012) (Yeo & Yu, 2016). A novel mutation in VCP (T262A) was identified in a familial FTD cohort in Pennsylvania and neuronal nuclear TDP-43 aggregate pathology was noted in the post-mortem tissues (Spina et al., 2014). We hypothesised that along with its proteasome-associated functions in the cytoplasm, wild type VCP might also play a role in nuclear protein homeostasis and protect neurons from TDP-43 mediated toxicity. To model TDP-43 proteinopathy in vitro, we used a previously optimised GFP-tagged-TDP-43 construct with mutations in the RNA binding domain (TDP-43-5FL) to induce aggregate formation in the nucleus (Mann et al., 2019). We used lipofectamine 2000 to co-transfect mouse primary neurons on DIV7 with TDP-43-5FL and Myc-tagged wild-type VCP (VCP-MYC) to assess the effect of VCP on intranuclear aggregates. The cells were fixed on DIV13 and stained with corresponding antibodies. Coverslips were imaged at 60X magnification using the Olympus inverted microscope. Image analysis was done using Fiji (ImageJ) software. Preliminary data indicates a lower average number of induced TDP-43 nuclear aggregates when VCP was overexpressed compared to endogenous VCP expression. Ongoing and future experiments include using optogenetic techniques to induce synchronised aggregate formation in isogenic mutant and wild-type cell lines to study differences in the formation and/or clearance of TDP-43 pathology and neuronal health.

CMP & CATER Graduate Students

Author: **Yu Bian, BS**
Contact: yub29@pitt.edu
Mentor: Dario Vignali
Co-Authors: Lawrence Andrews, Angela Gocher-Demske, Ellen Scott, Vaishali Aggarwal, Creg Workman, and Dario Vignali

Poster Number: 4

The role of Tox in regulatory T cells in the tumor

The transcription factor, TOX, (thymocyte selection-associated HMG BOX) contains the highly conserved high mobility group box (HMG-box) motif and belongs to the high mobility group box (HMG-box) superfamily. TOX plays an important role in the development and formation of immune organs such as lymph nodes and Peyer's patches and cells such as CD4+, CD8+ T cells and natural killer cells (NK cells). Recent studies have shown that TOX promotes CD8+ T cell exhaustion. TOX has been reported to be expressed in regulatory T cells (Tregs) and TOX+ Tregs are significantly increased in both myeloma and lymphoma patients. Our lab found that TOX+ Tregs are also increased in the tumor microenvironment of mice. We have recently generated mice in which TOX is specifically deleted in Tregs (ToxL/LFoxp3Cre-YFP) and found that tumors in mice with Tox deficient Tregs responded better to anti-PD-1 treatment compared to controls. We plan to use Cytek, scRNA-seq and ATAC-Seq, cytokine assays and proliferation assays to study the role of TOX in Treg heterogeneity, function, and homeostasis within the tumor microenvironment. We hypothesize that TOX will facilitate Tregs to maintain its suppressive function and deletion of TOX in Tregs will enhance anti-PD-1 efficacy and tumor clearance.

CMP & CATER Graduate Students

Author: **Emily Brown, BS**
Contact: EEB74@pitt.edu
Mentor: Sarah Hainer
Co-Authors: Sarah Hainer

Poster Number: 5

Investigating the role of the mSWI/SNF (BAF) nucleosome remodeling complex in regulating neural cell differentiation

Stem cells are characterized by their capacity to either self-renew or acquire a specialized cell fate. Differentiation of stem cells into specialized cell-types requires re-organization of the genome to establish accessibility at the necessary protein-coding and regulatory loci for appropriate function of the new cell. This process is governed by an orchestra of regulatory components including nucleosome remodeling complexes, transcription factors (TFs), and histone post-translational modification deposition. However, the role of the non-protein-coding transcriptome in regulating cell fate decisions is not well understood. The mSWI/SNF (BAF) complex is the only nucleosome remodeling complex that is essential at every major stage of development and is combinatorially assembled from 15-17 protein subunits to give rise to cell-type specific BAF complex compositions, which are essential for both maintenance of self-renewal in embryonic stem (ES) cells and cell fate commitment in neurogenesis and cardiomyocyte differentiation. Interestingly, the BAF complex is mutated in approximately 20% of all cancers and is also highly mutated in a range of neurological disorders.

We previously established that the BAF complex assembly present in ES cells, esBAF, functions as a global repressor of non-coding transcription, regulates nucleosome positioning to facilitate open chromatin at critical pluripotency TF binding sites, and is essential for maintenance of pluripotency. Here, I am investigating how the BAF complex regulates differentiation along the neuroectoderm lineage. I am utilizing novel synthetic biology approaches to understand how the non-coding transcriptome, genome-wide accessibility, genome-wide TF localization, and histone modification deposition are impacted when the ATPase activity that confers functionality to the BAF complex is abolished, either via chemical degradation or pharmacological inhibition. This work will uncover novel regulatory functions of cell-type specific BAF complex assemblies and provide insight into the role of the BAF complex in both developmental and disease contexts, ultimately informing potential therapies.

CMP & CATER Graduate Students

Author: **Hannah Butterfield, BA**
Contact: HEB62@pitt.edu
Mentor: Linda McAllister-Lucas & Peter Lucas
Co-Authors: Juliana Hofstatter Azambuja, Saigopalakrishna S. Yerneni, Lisa Maurer, Andrea Cruz, Matthew Halbert, Taylor Gatesman, Sameer Agnihotri, Peter C. Lucas, and Linda M. McAllister-Lucas

Poster Number: 6

Evaluating MAL1 as a therapeutic target in H3K27M-mutant diffuse midline glioma

Background: H3K27M-mutant diffuse midline glioma (DMG) is a devastating pediatric brain tumor that affects 200-300 individuals in the US per year. Median survival is 9-11 months, and there are virtually no long-term survivors. Despite decades of clinical trials, radiation therapy remains standard of care, extending survival by 2-3 months. Development of effective therapies for DMG is a critical unmet need. Recent studies implicate MALT1 as a potential therapeutic target in gliomas. MALT1 is the effector molecule of the CARMA-BCL10-MALT1 (CBM) signalosome, a cytoplasmic protein complex that drives downstream pro-survival NFκB transcriptional activity. MALT1, which possesses scaffolding and protease activities, promotes cell viability, proliferation, and migration/invasion in multiple cancer types. Importantly, several MALT1 protease inhibitors (e.g. JNJ-67856633, Janssen) are already in Phase I clinical trial. We hypothesize that MALT1 promotes DMG cancer cell proliferation and survival and that MALT1 inhibition will abrogate tumor progression.

Methods/Results: We screened a panel of patient-derived DMG cell lines by Western blot and found that CBM complex components are present in all cell lines tested. We next performed a lipid nanoparticle (LNP) screen to determine which LNP formulation allows for most efficient transfection of DMG cells. We found that delivering MALT1 siRNA using an optimal LNP delivery system resulted in efficient MALT1 knockdown and induced cell death in DMG cells. We also demonstrated that treatment with the MALT1 protease inhibitor JNJ-67856633 is selectively toxic to DMG cells but not to healthy control cells. Preliminary transwell assays indicate that MALT1 inhibition also reduces DMG cell migration.

Conclusions/Future Directions: Our findings support the hypothesis that MALT1 promotes DMG cancer cell survival and proliferation. Next, we aim to evaluate the mechanism by which MALT1 knockdown/inhibition causes DMG cell cytotoxicity by assessing apoptosis, cell cycle stalling, and autophagy. We will further assess the effect of MALT1 blockade, using the LNP-siMALT1 system or pharmacologic MALT1 protease inhibition, on DMG tumor growth and animal survival in an orthotopic/xenograft model. Given that radiation remains standard of care in DMG, we will also examine how MALT1 inhibition impacts the efficacy of radiotherapy in future studies. This work evaluates the potential therapeutic value of targeting MALT1 to treat H3K27M-mutant DMG. The use of agents that are approved for clinical trial (JNJ-67856633) or standard clinical use (LNPs) supports the potential for rapid translation of our findings for this disease that currently lacks viable treatment options.

CMP & CATER Graduate Students

Author: **Margaret Champion, BS**
Contact: mac575@pitt.edu
Mentor: Arjumand Ghazi
Co-Authors: Francis Amrit and Arjumand Ghazi

Poster Number: 7

Investigating the Role of Alternative Splicing in Germline Aging

Alternative splicing is a process of mRNA editing that allows for both post-transcriptional regulation and the generation of diverse functional proteins from a single gene. Global dysregulation of alternative splicing is a signature of age, and changes in the splicing patterns of specific genes have been linked to age-related decline. However, the extent to which these changes are casual and not merely indicative of the aging process is not fully understood. Here we investigate the role of alternative splicing in age-related reproductive decline. Our lab has recently shown that the pro-longevity factor TCER-1 is necessary for maintaining reproductive fitness in aging Caenorhabditis elegans. We have also demonstrated that TCER-1 regulates alternative splicing processes. In this project we seek to establish whether age-related changes in splicing patterns and fidelity contribute to reproductive senescence, to discover genes that may be implicated in this decline, and to determine how TCER-1's role as a pro-longevity, pro-fertility factor relates to its role as a regulator of alternative splicing.

CMP & CATER Graduate Students

Author: **Mona Chatrizeh, BS**
Contact: moc28@pitt.edu
Mentor: Michael Morowitz
Co-Authors: Jianmin Tian, Brian Firek, Matthew Rogers, Lauren Cass, Dennis Simon, and Michael Morowitz

Poster Number: 8

Plant-based enteral nutrition is superior to artificial nutrition in recovering antibiotic-induced immune suppression

Immune suppression and bone marrow dysfunction are ubiquitous among critically ill patients. Short term, this places an already vulnerable population at additional risk of life-threatening infections. Long term, immune suppression can persist in the form of chronic critical illness which significantly worsens functional outcomes. Many studies have attempted to rescue immune function early in the care of critically ill patients but have generally failed. This may be in part due to lack of consideration about the integral role of the gut microbiome in regulating hematopoiesis and immune function. Recent murine studies have illustrated antibiotic induced dysbiosis impairs hematopoiesis and suppresses bone marrow function. Clinically, our group completed some of the first genomic studies illustrating microbiota derangements in critically ill patients, likely because of liberal use of antibiotics. In addition to antibiotics, most critically ill patients rely on enteral nutrition which shapes their microbiome. Previously we have shown artificial enteral nutrition (AEN), the default and most commonly used formula for patients requiring enteral nutrition promotes dysbiosis. In contrast, high fiber plant based enteral nutrition (PBEN) is well tolerated, promotes the growth of healthy commensal gut anaerobes, and improves outcomes in murine models. We demonstrate PBEN randomized mice exhibit improved immune recovery with higher lymphocyte and white blood cell counts following antibiotic induced bone marrow suppression. We also provide evidence that critically ill patients randomized to PBEN have higher lymphocyte counts than those that received CEN. Together, these data suggest nutrition can be a clinically relavent strategy to boost immune function in critically ill patients.

CMP & CATER Graduate Students

Author: **Anu Balogun, BS**
Contact: olb25@pitt.edu
Mentor: Kari Nejak-Bowen
Co-Authors: Pamela Cornuet and Kari Nejak-Bowen

Poster Number: 9

Beta-catenin inhibition as a novel therapeutic strategy for Porphyria

Porphyrias are metabolic disorders caused by enzymatic defects in the heme biosynthesis pathway, leading to excessive accumulation of toxic porphyrins in the liver. The patterns of accumulation of toxic porphyrins define clinical features of these diseases such as hallucinations, seizures, and liver damage. These debilitating diseases remain incurable, and there is an unmet need to develop effective therapies to treat them. The xenobiotic toxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) induces hepatic porphyria in mice by inhibiting the terminal enzyme in the heme pathway, which causes the buildup of toxic porphyrins. These porphyrin intermediates cause a milieu of cellular abnormalities including inhibition of autophagy. Evidence supports crosstalk between autophagy and Wnt signaling, an evolutionarily conserved pathway that plays an important role in liver pathophysiology. Therefore, we investigated pharmacological inhibition of Wnt signaling to determine its role in autophagy during DDC-induced injury. Prior studies have unveiled a porphyrin accumulation/deaccumulation cycle that modulates porphyrin-induced protein aggregation in external and internal organs. Our data reveals that mice lacking Wnt signaling have increased induction of autophagy over baseline that contributes to the protection from injury by upregulating the autophagic process to clear accumulated toxic porphyrins. These observations collectively offer a novel opportunity to remedy porphyria by targeting the Wnt/beta-catenin signaling pathway.

CMP & CATER Graduate Students

Author: **Yu-Wei Cheng, BS**
Contact: YUC186@pitt.edu
Mentor: Toren Finkel
Co-Authors: Jie Liu and Toren Finkel

Poster Number: 10

TFEB is required for lysosomal biogenesis and cell survival during cellular senescence

Cellular senescence is a state where the cells undergo growth arrest due to permanently exiting the cell cycle. It is one of the central hallmarks of aging and contributes to a plethora of aging-related diseases including atherosclerosis, diabetes, and Alzheimer's disease. One of the most prominent phenotypes of senescence is the expansion of the lysosome compartment which can be quantified by senescence-associated b-galactosidase activity. Lysosomes are essential organelles that regulate cellular homeostasis through protein degradation and autophagy. However, the role of lysosome function and biogenesis in senescence is poorly understood. In this study, we identify that transcription factor EB (TFEB), which regulates lysosome biogenesis and function translocates to the nucleus during senescence to support the expansion of the lysosome compartment. By knocking out TFEB using CRISPR/ Cas9 in SV40LT-immortalized TRF2FI/FlCreER mouse embryonic fibroblasts (MEFs), we also show that TFEB is required for cell survival during senescence.

CMP & CATER Graduate Students

Author: **Andrea Cruz, BS**
Contact: anc232@pitt.edu
Mentor: Sameer Agnihotri
Co-Authors: Neveen Mansi and Sameer Agnihotri

Poster Number: 11

Uncovering therapeutic targets in the tumor microenvironment of H3K27-mutant diffuse midline gliomas

Introduction: Children with H3K27-altered diffuse midline gliomas (H3K27-DMGs), have a 5-year survival rate of only 2% following diagnosis. While in-depth genomic characterization has been performed in H3K27-DMGs, very few druggable targets have been identified. A common feature of H3K27-DMGs is infiltration of microglia, macrophages, other myeloid cells, collectively referred to as GAMs. Cellular crosstalk between non-tumor and tumor cells in the tumor microenvironment (TME) can both promote and or inhibit tumor growth, thus representing an opportunity in the pursuit of novel therapeutics. We have recently determined that H3K27-DMG tumor cells stimulate microglial secretion of pro-tumor growth factors. Hypothesis: GAMs promote H3K27-DMG tumor growth and progression via paracrine signaling axes and oncogenic activation of receptor tyrosine kinase (RTK) signaling pathways. Methods: I performed bioinformatic analyses and inferred cell-cell communication between GAMs and H3K27-DMG malignant cells from publicly available scRNA-seq of human H3K27-DMG tumors. Preliminary Results: The major incoming and outgoing signaling networks in GAMs and H3K27-DMGs have been identified. Moreover, I have identified several ligand-receptor pairs that mediate the paracrine signaling axis and communication between GAMs and H3K27-DMG malignant cells, such as PDGFB − PDGFRA, LGALS9 − HAVCR2, IGF1 − IGF1R, FGF5 – FGFR1 and 3, and EREG – EGFR and ERBB4. Conclusion: This research reveals cell-cell communication within the H3K27-DMG TME between non-tumor and tumor cells. The ligands and receptors identified as mediators of a paracrine signaling axis between GAMs and H3K27-DMG malignant cells are potentially targetable and could inform future treatment paradigms.

Author: **Ian Eder, BS**
Contact: IAE8@pitt.edu
Mentor: Partha Roy
Co-Authors: Virginia Yu, Marion Joy, Peter Lucas, and Partha Roy

Poster Number: 12

Profilin-1/CCL2 is a novel signaling axis of tumor cell-directed migration of immune cells

Profilin-1 (Pfn1) is an actin-binding protein that is downregulated in human breast cancer (BC). Pfn1 has been previously demonstrated to have tumor-intrinsic roles as a suppressor of tumorigenicity and dissemination in BC; however, whether Pfn1 has extrinsic immunological effects on the tumor microenvironment (TME) is unknown. We sought to investigate the effect of Pfn1 expression on the immune composition of the TME and the chemotaxis of immune cells. We performed multiplexed quantitative immunohistochemistry on a tissue microarray of clinical BC samples which revealed a significant positive correlation between tumor cell-specific Pfn1 expression and the percent of CD8+ T cells in the TME, more prominently in triple-negative breast cancer (TNBC) samples. Bioinformatics analyses of the METABRIC transcriptome dataset confirmed Pfn1 expression to be positively correlated with the CD8+ T cell fraction, in addition to, the pro-inflammatory IFN-gamma gene signature and the M1 to M2 macrophage ratio in TNBC. Co-culture studies demonstrated that elevating Pfn1 expression in TNBC cells enhances chemotactic migration of monocytes in a paracrine fashion. To investigate the underlying mechanism, we performed luminex analyses of the conditioned media of TNBC cells, and identified CCL2, a major chemoattractant of monocytes, to be dramatically upregulated and downregulated upon overexpression and knockdown of Pfn1, respectively. These data were further supported by real-time quantitative PCR-based confirmation of Pfn1-dependent changes in CCL2 transcription in TNBC cells as well as a significant positive association between Pfn1 and CCL2 mRNA expression in the clinical specimens of TNBC. Silencing CCL2 expression in TNBC cells abolished Pfn1-dependent changes in the chemotactic migration of monocytes, suggesting that CCL2 is a key mediator of Pfn1-stimulated migration of monocytes. Collectively, these findings provide the first evidence for extrinsic immunological effects of Pfn1 in BC. Since tumor infiltration of CD8+ T cells is associated with improved prognosis in BC and is a key determinant of immunotherapy success in TNBC, our work lays the conceptual foundation for future studies to explore whether Pfn1 modulation could be a novel strategy to enhance immunotherapy efficacy in BC.

Author: **Sonny Elizaldi, BS**
Contact: srelizaldi@ucdavis.edu
Mentor: Smita Iyer
Co-Authors: Chase Hawes, Anil Verma, Giovanne B. Diniz, Sean Ott, Anil Verma, Alyssa Bonillas, Danielle Beckman, John H. Morrison, and Smita S. Iyer

Poster Number: 13

a4 integrin+ CCR5+ CD4 T cells mediate acute SIV CNS seeding in Rhesus Macaques

CCR5+ CD4 T cells survey the CNS during homeostasis and the majority of their counterparts in circulation express the integrin α4 receptor, important for CNS entry. These data implicate CCR5+ α4+ cells in CNS viral seeding during HIV infection. To determine whether blocking α4 reduces early CNS viral seeding, we treated rhesus macaques with anti-Rh-α4 (25mg/kg, n=4) or IgG (n=4) before and during SIV infection. Rh-α4 resulted in complete receptor coverage leading to profound lymphocytosis prior to and during acute SIVmac251 infection (3-fold; $p<0.05$). Within the CSF, a trend for elevated lymphocyte counts was noted with a surprising increase in frequencies of CCR5+ CD4 T cells prior to infection. Following infection, CSF CD4+ CCR5 frequencies increased in all Rh-α4-treated animals, while only two IgG treated animals displayed a similar trend. Based on a negative correlation between CSF vRNA and CCR5+ CD4 T cells in a previous study ($r = 0.5$, $p< 0.01$), we predicted that increased CCR5+ CD4 T cells at week 1 post SIV was consequent to decreased CNS viral seeding in Rh-α4 treated animals. Consistently, Rh-α4 resulted in significantly lower CSF viral loads at week 1 (median vRNA (copies/ml CSF): Rh-α4, 305; IgG, 12,650). Thus, CCR5+ α4+ CD4 T cells mediate early viral seeding within the CNS. Ongoing studies assessing whether Rh-α4 treatment decreases viral seeding and attenuates SIV induced microglial and T cell activation within the brain parenchyma will provide insights into the role of CD4 T cells in acute CNS viral seeding and subsequent neuroinflammation.

Author: **Taylor Gatesman, BS**
Contact: tag85@pitt.edu
Mentor: Sameer Agnihotri
Co-Authors: Katharine E. Halligan, Matthew E. Halbert, Ann-Catherine J. Stanton, Andrea F. Cruz, Brian J. Golbourn, Ian F. Pollack, Stephen C. Mack, and Sameer Agnihotri

Poster Number: 14

Inhibiting insulin signaling reverses resistance to PI3K-mTOR inhibitors in aggressive pediatric high-grade gliomas

Primary central nervous system (CNS) tumors are now the most common cause of childhood cancer–related deaths. Pediatric high-grade gliomas (pHGGs) are among the most lethal brain tumors with a 5-year survival rate of only 20%. MYCN pHGGs represents one subgroup with an unmet need for therapeutics. MYCN belongs to the family of MYC transcription factors that regulate numerous cancer hallmarks such as proliferation, apoptosis, and metabolism. While no direct inhibitors of MYCN are in clinical trial, current strategies focus on targeting the MYCN mediated transcriptional machinery or cell cycle regulators. Lack of relevant pHGG models for pre-clinical testing contribute to limited therapeutic efficacy. To address these knowledge gaps, we developed a novel mouse model of MYCN pHGG using the FLEx-Cre Recombinase switch system, whereby neural progenitor cells are selectively delivered with MYCN cDNA and shRNA targeting the tumor suppressor genes p53 and Pten and form tumors in vivo. We identified that this model harbors hyper-activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of the rapamycin (mTOR) signalling pathway, a pathway that is of universal interest in cancer biology. We demonstrate that dual PI3K-mTOR blood brain barrier penetrant inhibitors are effective in reducing pHGG growth and MYCN protein levels. Because drug-resistance is a fundamental feature of pHGGs, we developed a novel drug-resistance model of MYCN pHGG as a mechanistic tool to identify relevant resistance mechanisms to re-acquisition of the PI3K-AKT-mTOR pathway. Using transcriptome analysis, we identified the insulin growth factor signaling pathway as our top mechanism of resistance. We hypothesized that MYCN is a critical driver of pHGG and can be effectively targeted via dual inhibition of the PI3K-mTOR and IGF/Insulin signalling pathways. We tested next generation inhibitors of the IGF and PI3K-mTOR pathways and performed genetic and pharmacological assays in our MYCN pHGG gliomas. We also investigated this mechanism in human MYCN pHGG cells. Inhibition of both pathways in our MYCN pHGG model and human MYCN cells were synergistic, leading to significant decreases in cell growth and MYCN signaling.

Author: **Shohini Ghosh-Choudhary, BS**
Contact: skg46@pitt.edu
Mentor: Toren Finkel
Co-Authors: Jie Liu, Bill Chen, Jonathan Alder, and Toren Finkel

Poster Number: 15

Using CRISPR Screening to Find Senolytic Druggable Targets

Senescence prevents the transformation of healthy cells to cancerous cells by preventing damaged cells from proliferating. Therefore, cells with oncogenic mutations, or irreparable DNA damage cannot replicate. However, Senescent cells acquire a hypersecretory phenotype (senescence associated secretory phenotype) that has been implicated in the pathogenesis of many diseases. Current research in this field has applied senolytic therapies to a variety of disease states ranging from atherosclerosis to osteoarthritis. One of the central tenants of this work is finding selective senolytic targets and agents that will kill senescent cells without affecting healthy proliferating cells. To answer this question, we used a CRISPR Whole Genome synthetic lethality screen to look for genes that will include cell death in senescent cells without affecting the growth of healthy cells. We used a Tamoxifen inducible CRE cell line that knocks out TRF2 to induce senescence in mouse embryonic fibroblasts. Control cells without the CRE domain were also treated with Tamoxifen. Chromosomal instability induced by TRF2 knockout induced senescence that was characterized in this cell population. Using this model, we performed a CRISPR whole genome lethality screen to identify targets that would lead to death of the senescent cells. With the results of this CRISPR screen we will be able to distinguish between targets that lead to death in the senescent population that did not induce death or growth arrest in the non-senescent control population. We will then validate these targets in vitro and in vivo.

Author: **Maria Beecher, BS**
Contact: mlb207@pitt.edu
Mentor: Peter Lucas
Co-Authors: Linda Klei, Dong Hu, Jack Little, Linda M. McAllister-Lucas, and Peter C. Lucas

Poster Number: 16

MALT1 is activated by doxorubicin and mediates therapy resistance in triple-negative breast cancer

Background: Breast cancer is the most commonly diagnosed malignancy in American women. The triple-negative breast cancer (TNBC) subtype has among the worst prognosis due to high rates of recurrence and metastasis. Since TNBC lacks targetable receptor proteins, treatment relies upon non-specific chemotherapy, which can become ineffective upon onset of resistance. One potential driver of TNBC treatment resistance is MALT1, the effector component of the CARMA-BCL10-MALT1 signaling complex, which activates NF-kB in multiple cancer cell types, including TNBC. Notably, breast cancer cells demonstrate increased sensitivity to chemotherapies such as doxorubicin and cisplatin when MALT1 is depleted. Additionally, NF-kB activation has been suggested to promote DNA repair. Hence, we hypothesize that MALT1 is a pharmaceutically targetable driver of TNBC treatment resistance. Mechanistically, we hypothesize that MALT1 promotes chemotherapy resistance by enhancing DNA repair.

Methods/Results: We analyzed RNAseq and proteomic data from TCGA and CPTAC, respectively, and found that MALT1 is highly expressed in basal breast cancer (a subtype largely composed of TNBC) and its expression level in this context is associated with reduced pathological complete response and survival. With the purpose of evaluating the effect of MALT1 protease inhibition on chemotherapy sensitivity, we first sought to identify TNBC cell lines that were most resistant to doxorubicin using GDSC and CTRP databases. Results indicated that MDA-MB-231, BT20 and HCC1143 cells were highly resistant. We then performed western blots to assess MALT1 expression and CellTiter-Glo assays to determine the doxorubicin IC50s for these cell lines. Results indicate that MALT1 expression correlates with the degree of doxorubicin resistance among these cell lines. To determine if MALT1 blockade, via siRNA-knockdown or MALT1 protease inhibitor treatment (JNJ-67856633 or MLT-748), increased doxorubicin sensitivity, CellTiter-Glo and Incucyte Caspase-3/7 assays were performed. Findings indicated that MALT1 blockade results in decreased cell viability and increased apoptosis in response to doxorubicin. Mechanistically, MALT1 protease and NF-kB are activated by doxorubicin; interestingly, MALT1 protease activity is partially dependent on the DNA repair protein, ATM.

Conclusions and Future Directions: Initial studies suggest that targeting MALT1 enhances TNBC sensitivity to doxorubicin. We will next assess the effect of MALT1 blockade on DNA repair mechanisms in doxorubicin-treated TNBC cells. Through these studies, we hope to inform new approaches for improving treatment response in TNBC.

Author: **Gabrielle Gilmer, BS**
Contact: ggg15@pitt.edu
Mentor: Fabrisia Ambrosio
Co-Authors: Natalie Jackson, Juliana Bergmann, Allison Bean, Hirotaka Iijima, Zachary Hettinger, Christopher Evans, Rebecca Thurston, and Fabrisia Ambrosio

Poster Number: 17

Characterizing the trajectory of murine knee osteoarthritis across menopause

Purpose: Knee osteoarthritis (KOA) is twice as likely to occur in post-menopausal women than in men. However, female mice spontaneously rejuvenate their ovarian follicles in middle-age. Thus, in the few preclinical studies that include female animals, menopause is typically not considered. As such, the purpose of this study was to characterize the trajectory of KOA (cartilage, synovium, subchondral bone) across perimenopause and menopause.

Methods: Middle-aged (14-16 month) female C57/BL6 mice were randomized to receive intraperitoneal (IP) injections of either vinylcyclohexene diepoxide (VCD) (menopause group) or sesame oil (non-menopause group) daily for 10 days. VCD is a specific ovarian toxin that causes atresia in the primary and primordial follicles. Our previous work using vaginal cytology and lavage has shown that perimenopause and menopause begin 25 and 115 days after the first VCD injection, respectively, while sesame oil injected animals continue to have regular estrus cycles. Knees were collected from mice at the following timepoints: (1) start of perimenopause, (2) mid-perimenopause, (3) start of menopause, (4) mid-menopause, and (5) late menopause. Decalcified knees were prepared in paraffin blocks, sectioned, and stained with Safranin-O/Fast green. OARSI cartilage scoring, synovium grade, and subchondral bone grade were assessed by a blinded scorer across all timepoints.

Results: Cartilage degeneration increased with time in the menopause group but not in the non-menopause group. The menopause group had more severe cartilage degeneration at the start of menopause, mid-menopause, and late menopause than the non-menopause group. Synovium pathology increased over time in both the menopause and non-menopause groups. However, at mid-perimenopause, start of menopause, mid-menopause, and late menopause, the menopause group had more severe synovium pathology than the non-menopause group. We saw no differences in subchondral bone grade across time in the menopause and non-menopause groups. There were also no differences between the menopause and non-menopause groups at each individual time point.

Conclusions: These findings suggest menopausal aging induces progressive cartilage degeneration in mice, while non-menopausal aging does not. Additionally, we found aging alone propagates increases in synovial pathology and menopause worsens these effects.

CMP & CATER Graduate Students

Author: **Matthew Halbert, BS**
Contact: meh237@pitt.edu
Mentor: Sameer Agnihotri
Co-Authors: Brian J. Golbourn, Katharine Halligan, Ann-Catherine Jean Stanton, Abigail L. Locke, Stephanie M. Casillo, Michelle Wassell, Taylor A. Gatesman, Andrea F. Cruz, Ian F. Pollack, and Sameer Agnihotri

Poster Number: 18

H3K27M Histone mutant gliomas are sensitive to methionine loss and MAT2A inhibition through a novel feedback mechanisms between AMD1 and METTL16

Diffuse Midline gliomas (DMGs), defined as grade IV tumors by the World Health Organization, are a rare and aggressive pediatric brain cancer. These tumors are inoperable and resistant to chemo/radiotherapies resulting in a median survival of 8-11 months and a 5-year survival of <2%. DMG is an epigenetic disease characterized by mutations on histone H3.3 K27M resulting in global transcriptional reprogramming. This disease lacks appropriate models to predict disease biology and response to treatment. Therefore, we developed a novel syngeneic H3K27M mouse model using clinically relevant co-alterations in Olig2+ neural progenitor cells (NPCs) to study this disease. Using an unbiased integrated systems biology approach, we identified a reliance of H3K27M but not isogenic controls to the amino acid methionine, and the enzymes methionine adenosyltransferase 2A (MAT2A), and adenosylmethionine decarboxylase 1 (AMD1). MAT2A is a master regulator of methionine metabolism that converts methionine into the universal methyl donor S-adenosylmethionine (SAM,) which is later converted into decarboxylated SAM (dcSAM) by AMD1 for polyamine metabolism. We postulated that targeting methionine regulator MAT2A through genetic/pharmacological abrogation would selectively alter DMG viability by disrupting the methylome. Historically methionine/MAT2A sensitivities were dependent on methylthioadenosine phosphorylase (MTAP) deletions resulting in a synthetic lethality mechanism. We discovered a novel mechanism demonstrating H3K27M cells are sensitive to MAT2A loss independent of MTAP and through AMD1 overexpression. The current paradigm shows that MAT2A protein expression is inversely correlated with cellular SAM concentrations as sensed by splicing complex and m6A reader methyltransferase-like protein 16 (METTL16). To investigate the molecular mechanism by which H3K27M represses MAT2A, we postulated that dcSAM might promote high turnover of METTL16–MAT2A transcript interactions like SAM, thereby diminishing MAT2A transcript and, ultimately protein expression. We found that exogenous dcSAM promoted MAT2A intron retention and lower mature transcript and protein levels. Our findings demonstrate that H3K27M leads to increased AMD1 protein expression resulting in dimenished MAT2A expression. Combinatorial treatments inhibiting MAT2A and AMD1 may presents exploitable therapeutic vulnerabilities in histone mutant gliomas.

CMP & CATER Graduate Students

Author: **Shea Heilman, BS**
Contact: shh136@pitt.edu
Mentor: Jeffrey Gross
Co-Authors: Hannah Schriever, Dennis Kostka, and Jeffrey M Gross

Poster Number: 19

scRNA-seq reveals TET-dependent gene expression events that occur during retinogenesis

Purpose: 5hmC is an epigenetic modification required for normal retinogenesis. It is known that tet2-/-tet3-/- (5hmC-null, DMUT) zebrafish show loss of terminal differentiation markers in several retinal cell types, but the extent of developmental impairment and the gene expression events that underly that impairment in each retinal cell type are not well known. The goals of this study are (1) to probe the extent to which developmental phenotypes occur in each cell type in DMUT retinae, and (2) to identify gene expression events required for cell type-specific development that are lost in DMUT retinae.

Methods: We generated 18 scRNA-seq libraries, including sibling control (CTL) and DMUT cells at four timepoints that span retinogenesis (36, 48,72, 120 hpf). Preprocessing (Bioconductor), batch correction (Batchelor), and cell type identification (AUCell) were done. Computational lineage trajectories were built (URD, Principal Curve), and genes differentially expressed between related CTL and DMUT trajectories were determined (Tradeseq). Genes of interest were tested by injecting target-specific F0 loss-of-function CRISPR complexes into embryos of the Sofa1 transgenic line, in which all major retinal neuron subtypes are labeled. Morphological differences and proportions of different retinal cell types were quantified in F0 crispants.

Results: 146,571 high quality retinal cells were profiled. KS tests performed on lineage-specific pseudotime analyses showed that CTL and DMUT pseudotimes are drawn from significantly different distributions ($p<0.05$), and 120 hpf CTL cells show significantly older pseudotimes in 7 of 8 retinal lineages ($p<2.2e-16$, Wilcoxon rank sum). These data suggest significant developmental divergence in timing of CTL and DMUT retinal differentiation, and also suggest that differentiated CTL cells are more mature than DMUT cells of the same lineages. Gene expression alignment of transcriptomic lineage trajectories predicted testable lineage-specific targets that show differential expression between CTL and DMUT retinae.

Conclusions: Our results suggest that tet2-/-tet3-/- retinae are transcriptionally divergent across all major cell lineages. Tet/5hmC LOF studies that assess divergences in lineage-specific gene expression enable us to further understand the gene expression events that facilitate normal retinogenesis.

Author: **Dana Julian, BS**
Contact: drj21@pitt.edu
Mentor: Julia Kofler
Co-Authors: Jr Jiun Liou, Karl Herrup, Thomas Pearce, Howard Aizenstein, and Julia K. Kofler

Poster Number: 20

Quantitative Digital Image Analysis of Whole Slide Images to Investigate White Matter Rarefaction in Alzheimer's Disease

Alzheimer's Disease (AD) is a relentless progressive neurodegenerative disease leading to severe cognitive decline and affects approximately 26.6 million people worldwide. White matter hyperintensities (WMH), regions of myelin rarefaction, precede cognitive symptoms in AD, are predictive of disease onset and progression, can be seen non-invasively by magnetic resonance imaging (MRI). However, little is known about the composition of WMH due to the difficulty locating these regions postmortem. We have overcome that challenge by aligning premortem MRI, with postmortem MRI and histology. The goal of this study was to develop a quantitative digital image analysis pipeline to analyze the composition of these pathologies, including axonal density, myelin quantity, oligodendrocyte density, vascular markers, and ECM markers in postmortem human AD tissue by immunohistochemistry (IHC) and in situ hybridization (ISH). We stained tissue with antibodies targeting myelin (MAG, MBP, PLP), oligodendrocytes (Olig2), and vimentin. ISH probes were designed to identify glial cells, ECM, and integrin receptors. We developed image analysis algorithms using machine learning algorithms in the QuPath software for automated and high-throughput data collection. These data were processed through algorithms identifying cortical regions, perivascular zones, and subcortical vs. deep white matter. This methodology allows us to further interrogate oligodendrocyte populations specifically vulnerable to demyelination in Alzheimer's Disease. Understanding these subpopulations is critical to understand AD disease pathogenesis and may allow for early intervention in the disease.

CMP & CATER Graduate Students

Author: **Joshua Hislop, BS**
Contact: joshua.hislop@pitt.edu
Mentor: Mo Ebrahimkhani
Co-Authors: Amir Alavi, Qi Song, Rayna Schoenberger, Kamyar Keshavarz F., Ryan LeGraw, Jeremy Velazquez, Tahere Mokhtari, Mohammad Nasser Taheri, Matthew Rytel, Susana M Chuva de Sousa Lopes, Simon Watkins, Donna Stolz, Samira Kiani, Berna Sozen, Ziv Bar-Joseph, and Mo R. Ebrahimkhani

Poster Number: 21

An Engineered Human Model of Post-implantation Extra-Embryonic Niche

Implantation of the human embryo commences a critical developmental stage that comprises profound morphogenetic alteration of embryonic and extra-embryonic tissues, axis formation, and gastrulation events. Our mechanistic knowledge of this window of human life remains limited due to restricted access to in vivo samples for both technical and ethical reasons. Additionally, human stem cell models of early post-implantation development with both embryonic and extra-embryonic tissue morphogenesis are lacking. Here, we present iDiscoid, produced from human induced pluripotent stem cells via an engineered a synthetic gene circuit. iDiscoids exhibit reciprocal co-development of human embryonic tissue and engineered extra-embryonic niche in a model of human post-implantation. They exhibit unanticipated self-organization and tissue boundary formation that recapitulates yolk sac-like tissue specification with extra-embryonic mesoderm and hematopoietic characteristics, the formation of bilaminar disc-like embryonic morphology, the development of an amniotic-like cavity, and acquisition of an anterior-like hypoblast pole and posterior-like axis. iDiscoids offer an easy-to-use, high-throughput, reproducible, and scalable platform to probe multifaceted aspects of human early post-implantation development.

CMP & CATER Graduate Students

Author: **Yekaterina Krutsenko, BS**
Contact: yek14@pitt.edu
Mentor: Satdarshan P Monga
Co-Authors: Minakshi Poddar, Sucha Singh, and Satdarshan P Monga

Poster Number: 22

Dual loss of β-catenin and γ-catenin from cholangiocytes causes intrahepatic cholestatic injury in mice

Background: β-catenin as a key effector of the canonical Wnt signaling, as well as a structural component of adherens junctions (AJs), has been shown to be indispensable for normal liver development, homeostasis, and regeneration. However, functions of β-catenin have primarily been characterized in hepatocyte biology, whereas its role in cholangiocytes remains poorly understood. Previous studies have shown that, in hepatocytes and other epithelial cells, γ-catenin, a highly homologous desmosomal protein, is capable of compensating for the functions of β-catenin at AJs. In the present study we inducibly and specifically delete both β- and γ-catenins from biliary epithelium, in order to avoid the compensatory effects of γ-catenin, and study biological implications of dual elimination.

Methods: We utilized Opn-iCreERT2+/-; Ctnnb1fl/fl; Jup fl/fl mice (DKO), in which both beta-catenin (Ctnnb1 gene) and γ-catenin (Jup) are acutely deleted from biliary epithelium by the Tamoxifen-inducible, cholangiocyte-specific osteopontin-driven expression of Cre-recombinase. 4 doses of Tamoxifen (100mg/kg) were administrated to adult animals intraperitoneally in order to achieve efficient recombination. Tissues were harvested at least 3 weeks after the last injection. Tamoxifen-treated Opn-iCreERT2-/- sex-matched littermates were considered as controls.

Results: Interestingly, DKO mice showed increased morbidity and mortality as compared to the controls. Kaplan-Meier curve showed a significant decrease in survival in double knockouts. DKO mice also manifested decreased body weight and size as well as reduced liver-weight-to-bodyweight ratio. The mice were grossly yellow and lethargic suggesting severe liver injury among the survivors. Indeed, DKO animals showed elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase, in addition to hyperbilirubinemia and high cholesterol. Histological examination revealed multiple biliary infarcts, inflammation, stellate cell activation and portal fibrosis in DKO livers. While there was presence of CK19-positive bile ducts, these structures showed decreased and sometimes absent lumen in the DKOs, but not in Opn-iCreERT2+/-; Ctnnb1fl/fl; Jup het or Opn-iCreERT2+/-; Ctnnb1het; Jup fl/fl animals. Immunohistochemical examination for CEACAM1 did not reveal any notable abnormalities in the apical surface of hepatocytes or biliary canalicular structure, despite evidence of hyperbilirubinemia and cholestasis in the DKOs. Quantitative RT-PCR showed that in DKO livers, expression levels of the canalicular as well as basolateral efflux transporters remained unchanged, while they showed significantly decreased mRNA levels of select basolateral influx transporters (specifically, NTCP and OATP4) suggesting adaptation to cholestasis. Likewise, mRNA expression of the components of bile acid synthesis pathways, namely Cyp27a1 and Hsd3b7, were significantly decreased in DKO livers as well.

Conclusions: Dual loss of beta-catenin and gamma-catenin from cholangiocytes causes serious intrahepatic cholestatic injury which is associated with notable morbidity and mortality. The observed phenotype is histologically reminiscent of cholangiopathies like primary sclerosing cholangitis and primary biliary cholangitis, and detailed characterization of the phenotype may lead to discovery of novel cellular and molecular mechanisms contributing to disease pathogenesis. This model also demonstrates a critical role of AJs in cholangiocytes in maintaining biliary homeostasis.

Author: **Rithika Behera, BS**
Contact: RIB35@PITT.EDU
Mentor: Robert Lafyatis
Co-Authors: Tracy Tabib, Christina Morse, Robert Lafyatis

Poster Number: 23

Transcriptional regulation of SSc dermal myofibroblasts by FOSL2 and FOXP1

Background: Systemic Sclerosis (SSc) is characterized by fibrosis, vasculopathy, and immune dysregulation. Skin fibrosis is the hallmark of SSc and is driven by the contractile action of myofibroblasts. The number of myofibroblasts in the skin correlates with the modified Rodnan skin score, the most widely used clinical measure of skin severity. Using single cell RNA sequencing, we have identified different dermal fibroblast populations and shown that SSc dermal myofibroblasts arise in two steps from SFRP2hi/DPP4 expressing progenitor population. Bioinformatic analyses of the SSc dermal fibroblast transcriptome implicated the role of transcription factors FOSL2 and FOXP1 in the first and second step of SSc myofibroblast differentiation respectively. Our aims are to understand the transcriptional regulation of FOSL2 and FOXP1 in dermal myofibroblast activity and SSc pathogenesis.

Methods: We used si-RNA to knockdown the RNA expression of FOSL2 and FOXP1 in primary dermal fibroblasts from SSc patients. The perturbed transcriptome, signaling pathways, and epigenetic changes were characterized using bulk RNA sequencing, Western blotting, and ATAC sequencing.

Results: We found that knocking down FOSL2 and FOXP1 RNA using si-RNA led to a reduction in fibrotic genes and biomarkers for SSc disease progression such as: COL1A1, alpha-SMA, THBS1, PRSS23, THY1, and FN1. On generating activity modules of the perturbed transcriptome, we found that the genes downregulated by si-RNA activity had a high expression in the SFRP2hi/DPP4 expressing progenitor population.

Conclusion: Our study provides a novel understanding of the transcriptional and epigenetic regulation of SSc dermal myofibroblasts by FOSL2 and FOXP1 and provides evidence of their role in the pathogenesis of SSc. We have identified target genes which are regulated by FOSL2 and FOXP1 and responsible for driving fibrosis in dermal fibroblasts.

CMP & CATER Graduate Students

Author: **Ryan LeGraw, BS**
Contact: ryl17@pitt.edu
Mentor: Mo Ebrahimkhani
Co-Authors: Armin Aryannejad, Kamyar Keshavarz, Joshua Hislop, Matthew R Rytel, Jeremy J Velazquez, Michael Badowski, David T Harris, Ryohichi Sugimura, Moses Turkle Bility, Samira Kiani, and Mo R Ebrahimkhani

Poster Number: 24

Understanding and Engineering Human Hematopoiesis using a Genetically Engineered Fetal Liver Niche

Human fetal liver morphogenesis is poorly understood and difficult to study. The fetal liver is of particular interest for its role in the early hematopoiesis, as it is a site of rapid expansion of hematopoietic stem cells (HSCs), which during adulthood reside in the bone marrow and remain largely quiescent. HSC transplantation remains the gold standard for treatment of numerous blood diseases, and a method for long-term culture and expansion of HSCs has yet to be achieved. Synthetic biology aims to understand complex biological systems and their applications through their re-construction and engineering. Here we employ a "build to understand" approach to gain novel insights in the development of human fetal liver hematopoietic niche while using it for HSC expansion. We took advantage of our previously developed protocol that employs transient 5-day expression of GATA6 in a single population of human induced pluripotent stem cells (hiPSCs) to initiate self-organization and codifferentiation of multiple germ layers similar to the human fetal liver over a course of 14 days. Using this synthetic liver niche as a platform for the ex vivo expansion of HSCs through coculture with cord blood derived CD34+ cells, we observe rapid proliferation of these cells from day 6 to day 8 post-seeding. Flow cytometry reveals that these cells have both a larger CD34+CD38- HSPC population and CD34+CD38-CD90+CD45RA-CD49f+ HSC population compared to traditional culture methods. Functional analysis via colony forming assay further reveals the maintenance of greater quantities of HSPCs of multiple lineages. The fetal liver microenvironment facilities HSC expansion in the presence of minimal exogenous growth factors or serum which are required for HSC expansion using traditional methods, and lentiviral delivery of cytokine transgenes allows for HSC expansion without addition of any exogenous growth factors. We plan to further customize this synthetic niche using epigenetic modification to optimize HSC expansion and generate customized niches for the expansion of other hematopoietic cells of interest, such as T cells.

Author: **Brandon Lehrich, BS**
Contact: brl88@pitt.edu
Mentor: Paul Monga
Co-Authors: Evan R. Delgado, Junyan Tao, Silvia Liu, Aatur D. Singhi, and Satdarshan P. Monga

Poster Number: 25

β-Catenin Activation Promotes B-cell Exclusion in the Hepatocellular Carcinoma Microenvironment

Background: Current immunotherapeutic approaches for hepatocellular carcinoma (HCC) are focused on T-cell specific immune checkpoint inhibitors (ICIs). Work in other cancer models have linked ICI response to B-cell signaling in the tumor microenvironment. Our group and others have demonstrated that β-catenin-mutated HCCs promote resistance to ICIs. Here, we investigated if β-catenin-mutation in HCCs may play a role in B-cell exclusion and impact subsequent response to ICIs.

Methods: Public HCC datasets were assessed for mutations in the β-catenin gene, CTNNB1, and B-cell related gene signatures. Our clinically relevant mouse HCC models of T41A-CTNNB1/G31A-NFE2L2, S45Y-CTNNB1/hMET, and MYC/hMet treated with and without anti-PD-1 therapy, were assessed for the presence or absence of B-cells by immunohistochemistry (IHC). Next, the influence of β-catenin suppression on B-cells was explored using antisense technology in HCC models.

Results: Overall, 26% of HCC cases in The Cancer Genome Atlas (TCGA) showed CTNNB1 mutations. Ingenuity pathway analysis of TCGA RNA-sequencing data demonstrated that multiple pathways in B-cells were significantly altered in CTNNB1 mutated vs non-mutated cases. Similarly, comparing these two cohorts, differentially expressed genes were overlapped with a publicly available B-cell signature which revealed 130 overlapping genes, of which 101 were downregulated, including MS4A1 (which encodes for CD20). In our murine HCC models, we also noticed decreases in CD20+ immune cells on IHC in both β-catenin-driven models compared to MYC/hMET model. Additionally, we noted no differences in B-cell numbers following anti-PD-1 treatment in β-catenin-driven models. Moreover, using an antisense oligonucleotide to suppress β-catenin in HCC models increased CD20+ immune cell infiltration in the tumor microenvironment and simultaneously significantly reduced tumor burden.

Conclusion: β-Catenin-driven HCC may drive a B-cell exclusionary phenotype. Future directed studies aim to elucidate the mechanism of B-cell signaling in ameliorating tumor burden following β-Catenin inhibition in conjunction with anti-PD-1 therapy in CTNNB1-mutated HCC.

Author: **Jack Little, BS**
Contact: jol114@pitt.edu
Mentor: Peter Lucas and Linda McAllister-Lucas
Co-Authors: Dong Hu, Linda Klei, Prasanna Ekambaram, Linda McAllister-Lucas, and Peter Lucas

Poster Number: 26

MALT1 protease coordinates the expression of an immunosuppressive secretome in GPCR+ triple-negative breast cancer

Background: Breast cancer is the most common cancer in women with the second highest mortality rate. Breast cancers are classified by expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. Triple-negative breast cancer (TNBC) represents the case where tumors lack expression of each marker (ER-/PR-/HER2-). This subclass constitutes approximately 15% of breast cancer with over 200,000 new cases diagnosed each year. Unfortunately, there are no well-established targeted therapies available for patients diagnosed with TNBC and treatment relies heavily on non-specific chemotherapeutics. There is a significant clinical need to identify innovative ways to harness the tumor immune microenvironment (TIME) to augment the efficacy of these agents and to enable the effective use of immunotherapeutics. Using preclinical models, our lab ascertained that an intracellular signaling complex composed of CARMA3, BCL10, and MALT1 (the CBM signalosome) is particularly active in a substantial subset of TNBCs and drives tumor progression partially by promoting an immunosuppressive TIME. We speculate that targeting MALT1, the enzymatic effector protein of the CBM signalosome, may be an effective approach to re-invigorating the TIME, thereby enhancing the response to chemotherapy and enabling response to adjunctive immunotherapeutics.

Methods and Results: We analyzed transcriptomic data from CCLE to identify TNBC cell lines with relatively high levels of MALT1 and verified that MALT1 protease was active in these cells using Western blot analysis. We performed an initial screen of MDA-MB-231 conditioned media using an antibody-based cytokine array and identified numerous immunosuppressive cytokines upregulated by MALT1 protease activity including CSF2 and CXCL1. We verified the expression of CSF2 and CXCL1 at the mRNA and protein level using RT-qPCR and ELISA, respectively, in these MALT1-active TNBC cell lines.

Conclusions and Future Directions: Our initial experiments indicate that MALT1 protease increases the expression of numerous immunosuppressive factors as part of a MALT1-dependent secretome. CSF2 and CXCL1 are both factors known to influence the expansion and activation of myeloid-derived suppressor cells (MDSCs). We will next assess the ability of MALT1 to influence MDSC biology both in vitro and in vivo, and test the ability of MALT1 protease inhibition to reverse these effects. Since our initial screen of 105 cytokines suggests that many factors are regulated by MALT1 protease activity, we will perform a comprehensive transcriptomic and proteomic analysis to fully characterize the MALT1-dependent secretome. This large-scale, unbiased approach will enable a deeper understanding of how MALT1 activity within TNBC may affect the broad breast cancer microenvironment.

Author: **Jie Bin Liu, BA**
Contact: jil279@pitt.edu
Mentor: Steffi Oesterreich and Adrian Lee
Co-Authors: Adrian V. Lee and Steffi Oesterreich

Poster Number: 27

Characterizing and Targeting ERBB2 Mutations in Invasive Lobular Carcinoma

Activating mutations in ERBB2 are approximately 10-fold enriched in invasive lobular carcinoma (ILC) with up to 19% in metastatic ILC. ILC is a histologic subtype of breast cancer characterized by loss of E-cadherin (CDH1), suggesting a potential interaction between loss of CDH1 and mutations in ERBB2. Recently, a number of clinical trials demonstrated promising efficacy using anti-HER2 tyrosine kinase inhibitors (TKIs) in patients with ERBB2 mutant ILC, suggesting targetability of ERBB2 mutations in ILC. However, the molecular mechanism by which ERBB2 mutations are enriched in ILC is poorly understood. Additionally, as clinical response to TKI monotherapy is generally transient, further investigation into other available anti-HER2 therapies and combinations is warranted.

To date there are no reported breast cancer cell lines with endogenous ERBB2 mutations. Our lab recently performed WES on ILC cell lines and identified two with activating ERBB2 mutations commonly detected in patients, namely ERBB2 S310F and L755S. We observed marked increases in HER2 signaling activation and sensitivity to neratinib, an anti-HER2 TKI, in these two cell lines (IC50: 0.0019-0.29uM) compared to ILC cell lines with wildtype ERBB2 (IC50: 2.0-6.1uM). To understand if and how E-cadherin modulates HER2 signaling, we generated isogenic cell lines -/+ CDH1 and found elevated HER2 mRNA and protein expression in CDH1 knockout cell lines compared to wild-type cells. Intriguingly, we also observed accelerated HER2 receptor degradation with loss of E-cadherin.

Based on these findings, we hypothesize that loss of E-cadherin provides a permissive environment for elevated HER2 expression and activity, and that hyperactivation of HER2 signaling subsequently leads to selection of ERBB2 mutations that confer growth advantages and therapeutic vulnerabilities. We also hypothesize that ILC has exquisite susceptibility to HER2 antibody-drug conjugates, whose efficacy is dependent on the efficiency of HER2 receptor internalization and degradation to deliver the cytotoxic payload inside tumor cells.

CMP & CATER Graduate Students

Author: **Joe Maggiore, BS**
Contact: jcm161@pitt.edu
Mentor: Neil Hukriede
Co-Authors: Jeremy Velazquez, Aneta Przepiorski, Evan Streeter, Ryan LeGraw, Anne Silva-Barbosa, Jonathan Franks, Joshua Hislop, Alex Hill, Haojia Wu, Katherine Pfister, Sara E. Howden, Christopher Chaney, Melissa Little, Benjamin Humphreys, Simon Watkins, Alan Watson, Donna Stolz, Tom Carroll, Alan Davidson, Ondine Cleaver, Samira Kiani, Sunder Sims-Lucas, Mo R. Ebrahimkhani, and Neil A. Hukriede

Poster Number: 28

Genetically engineered endothelial niche induces mature cell populations in human kidney organoids

Increasing the vascular density in human kidney organoids is critical for mechanistic studies in kidney health and disease. Particularly in the glomerulus, where endothelial cells and podocytes interact in a highly regulated way, which, when dysregulated drives various pathophysiological outcomes. Many methods for vascularizing in vitro kidney models have been taken, however, few have been robust, reliable, high-throughput, and can be used to study podocyte/endothelial morphology and morphological changes in injury settings. Using a combinatorial approach, a doxycycline inducible ETV2 iPSC line was combined with a naïve iPSC line to generate highly vascularized human kidney organoids. Here, we show these organoids generate a robust endothelial cell network that invaginates into podocyte cell clusters, and on electron microscopy form glomerular basement membrane between interdigitated foot processes as well as highly organized fenestrations on endothelial cells. With snRNAseq we demonstrate that vascularized podocytes exhibit enhanced VEGF signaling, basement membrane formation, glomerular development signatures, and endothelial differentiation and migration markers. Lastly, we demonstrate that vascularization of human kidney organoids enables the formation of a functional interstitium with a drug responsive set of renin cells. These renin cells were confirmed to exist within podocyte clusters of vascularized kidney organoids only. Finally, in previous work, our group has identified HDAC8 as a potential target for the regulation of renal health and disease. In our current studies, we demonstrate the HDAC8 pathway is a potential targeted pathway to regulate mesenchymal transition of endothelial cells and podocytes. Here, we demonstrate the upregulation of mesenchymal transition markers in patients with glomerular disease, and show in our kidney organoid injury model that HDAC8 inhibition results in the amelioration of this disease process. Overall, this work demonstrates the generation and validation of a vascularized human kidney organoid model that can be used for mechanistic studies of podocyte and endothelial health and disease.

Author: **Meagan Makarczyk, BS**
Contact: mjm392@pitt.edu
Mentor: Hang Lin
Co-Authors: Matais Preisegger, Zhong Li, Qi Gao, Sophie Hines, Bruce A. Bunnell, Stuart B. Goodman, Douglas Weber, Michael S. Gold, and Hang Lin

Poster Number: 29

An Innervated Cartilage Synovial Chip to Study Joint Inflammation

Osteoarthritis (OA) is the 11th global contributor to disability and is a painful and debilitating disease. However, the correlation between OA and pain is not well understood. To date there are not any disease modifying osteoarthritis drugs that have reached FDA approval leaving pain management to non-steroidal anti-inflammatory drugs or invasive surgeries such as total joint arthroplasty (TJA). Due to the lack of appropriate models to recapitulate the whole-joint disease nature of OA in humans, there is a clinical need to establish safe and effective methods for the treatment OA-associated pain. Here we report the creation of an innervated organ-on-chip model to enable the dynamic interplay between the nervous system and the synovial joint. The objective of this study is to utilize the incorporated neurons to model the relationship between pain and OA in the established Neu-microJoint.

1) Establishing the Neu-microJoint: Primary human chondrocytes were encapsulated in 15% methacrylated gelatin (GelMA) to form cartilage tissue (CT). Human bone marrow derived mesenchymal stem cells (hbMSCs) were expanded and differentiated into fibroblasts. Blood derived monocytes were polarized then combined with hbMSC-derived fibroblasts in 10% GelMA to form the synovial-like construct (SLC). A two-chamber microfluidic tissue chip was two dimensionally seeded with human dorsal root ganglion (DRG) neurites in one chamber and the other was used for the SLC. Single-chamber CT bioreactors were treated with interleukin (IL) 1β (10 ng/mL) for three days prior to connection with the SLC. 2) Induction of “synovitis” in the SLC: Medium from healthy and OA-like CT was collected and shared with SLCs for four days to induce control and inflamed states in the SLC. 3) Neuronal Fluorescence and imaging: Fibrous cells were stained with DiO prior to encapsulation in GelMA with the hopes of generating a green fluorescence. Neurites were treated with DiI and virally with GFP for fluorescence detection.

CTs treated with IL-1β showed robust inflammation compared to the negative control follow qRT-PCR. Treatment of CT medium in SLC showed an increase of pro-inflammatory proteins and enzymes in conditioned medium from treated SLC groups using LUMINEX assay. Fluorescence imaging of rodent DRG neurites showed neurite extension through the microchannels towards the SLC. Live imaging was used to track neuronal activity and electrical stimulation of the neurites evoked calcium transients in the corresponding soma. Importantly, neurons continue to respond to algogenic stimuli including "synovium fluid" from the “osteoarthritic” microJoint.

The relationship between OA and pain is not well understood and can vary throughout the stages of OA, making the relationship between pain and OA complex. Here we aim to engineer a model to elucidate these complexities. Through the microJoint system previously reported, an innervated microfluidic system now allows for the integration of human-cell derived tissues and human DRG neurites. The use of advanced technologies and electrical stimulation provide real-time data of how neurites respond to OA-CT conditioned medium. The end objective of this study is to use the Neu-microJoint to serve as a platform for personalized medicine for the assessment and treatment of OA-patients.

CMP & CATER Graduate Students

Author: **Isabelle Chickanosky, BS**
Contact: isc27@pitt.edu
Mentor: David Vorp
Co-Authors: Nicole M. Donnellan and David A. Vorp

Poster Number: 30

Modeling Endometriosis Angiogenesis in Endometriotic Conditions

Introduction: Endometriosis is gynecological disease associated with chronic pelvic pain and infertility affecting 10% of women worldwide. The main pathogenesis hypothesis, retrograde menstruation, proposes endometrial tissue sheds into the peritoneal cavity via the fallopian tubes. The presence of upregulated estrogen (E2) and a dysregulated immune environment could contribute to disease growth following retrograde menstruation. Current in vitro models of endometriosis use 2D cell culture, 3D cell (co)-culture, and microfluidic devices, but these models are limited in that they only consider the upregulated E2, not immune dysregulation. In this combined 2D and 3D cell culture study, we define endometriotic conditions as the presence of both upregulated E2 and immune dysregulation and test both E2 alone and endometriotic conditions for effects on proliferation and cord formation by endometriosis-relevant cells (endothelial and endometriotic 12Z cells).

Methods: Endometriotic conditions were modeled using THP-1 monocytes exposed to varying elevated quantities of 17-β Estradiol. 12Z cells, immortalized endometriotic cells, were used to understand how an endometriosis lesion might respond to endometriotic conditions. Human coronary artery endothelial cells (HCAECs) were used to study how endometriotic conditions might impact endothelial cell early angiogenic behavior, or cord formation. 2D cell proliferation was measured for E2 exposures of 1.0 ng/mL, 1.5 ng/mL, 2.0 ng/mL, and 4.0 ng/mL in phenol-red free Dulbecco's Modified Eagle Medium (DMEM) using alamarBlue. 3D cell culture in collagen gels were observed via an inverted light microscope.

Results: It was found that in 2D culture of HCAECs and 12Zs, ECs and 12Zs respond differently to the E2-supplemented media compared to endometriotic conditions. Furthermore, 12Z proliferation decreased in endometriotic conditions compared to only estrogenic conditions. In 3D culture, it was found that HCAECs and 12Zs on collagen gel demonstrated preliminary cord formation in endometriotic conditions. These findings will be helpful in developing a more advanced model of endometriosis angiogenesis by demonstrating desirable cell seeding densities and expected cellular performance in different conditions.

Conclusion: This work shows cord formation due to endometriotic conditions and promise in maintaining cell viability in 3D cultures under endometriotic conditions. Future work will include transmigration and invasion studies to understand the interaction between ECs and 12Zs in endometriotic conditions. In the future, a microfluidic model using endometriotic conditions will be a novel way to study endometriosis angiogenesis and pathogenesis. This research contributes a novel and standardized method for creating in vitro endometriotic conditions and will provide a platform for studying disease pathogenesis in vitro.

CMP & CATER Graduate Students

Author: **Varun Mandi, BS**
Contact: vam52@pitt.edu
Mentor: Vijay Yechoor
Co-Authors: Jeongkyung Lee, Rajaganapathi Jagannathan, Ping Yang, Vinny Negi, Feng Li, Yijen Wu, Dhivyaa Rajasundaram, Vijay Yechoor, and Mousumi Moulik

Poster Number: 31

Environmental circadian desynchronization and metabolic stress drive heart failure with preserved ejection fraction (HFpEF)

Background: The pathogenic mechanisms underlying HFpEF (Heart Failure with preserved Ejection Fraction), which accounts for 50% of heart failure, remain unclear. Elevated endoplasmic reticulum (ER) stress has been implicated in HFpEF. Circadian disruption (CD) as seen in shift workers has been associated with an excess risk for chronic age-related disorders, including cardiovascular disease. Currently, the role of CD in HFpEF is not known.

Hypothesis: The dual 'hits' of chronic metabolic stress and circadian disruption lead to increased ER stress, impaired unfolded protein response (UPR), and adverse cardiac remodeling resulting in HFpEF.

Methods: High fat diet (HFD) fed C57BL6 male mice were subjected to chronic shifted light-dark (LD) cycles mimicking shift work (Shift), with appropriate controls. Metabolic (glucose tolerance, insulin sensitivity, body fat) and cardiac function parameters (systolic and diastolic function by ECHO, MRI, and terminal PV-loops) were collected. Cardiac tissue was analyzed using qPCR, western blot, bulk RNA-seq, spatial transcriptomics, proteomics, and histology. For in vitro studies, Bmal1 was deleted in H9C2 cells using CRISPR/Cas9.

Results: ‘HS’ mice (HFD and Shift) had the most insulin resistance, glucose intolerance, and obesity, compared to ‘HR’ (HFD & Regular LD), ‘CS’ (Normal Chow & Shift) and ‘CR’ (Chow and Regular LD) groups. ECHO and MRI revealed preserved EF in all groups, with diastolic impairment in both HS and HR (↑MV E/E’, ↓MV E/A, and ↓LV diastolic strain) groups, HS>HR. Only ‘HS’ mice showed ↓cardiac compliance (↑β in EDPVR) and ↑ lung weight suggestive of HF consistent with HFpEF phenotype. Gene and protein expression revealed dysregulation of numerous UPR pathway molecules (Atf6, Chop, Bip, Perk, and others) in HS mice. Mechanistically, Bmal1 deletion in CMs displayed a similar dysregulation of UPR genes. HS mouse hearts showed increased fibrosis, and spatial transcriptomics displayed enrichment of ATF4-related pathways in cardiomyocytes and activated fibroblasts.

Conclusion: The dual hit of metabolic and circadian stress induces HFpEF, which cannot be recapitulated by only one stressor. Our results identify previously unrecognized roles of UPR perturbation in driving HFpEF under circadian and metabolic stress.

Author: **Philip Mannes, BA**
Contact: pzm2@pitt.edu
Mentor: Sina Tavakoli
Co-Authors: Clayton Barnes, Qin Zhu, Kathryn Day, Joseph Latoche, Jessie Nedrow, Carolyn Anderson, Janet Lee, and Sina Tavakoli

Poster Number: 32

In vivo molecular imaging of chemokine-like receptor 1 (CMKLR1) to monitor ongoing inflammation in a preclinical bleomycin-induced lung injury model

INTRODUCTION: Lung diseases driven by non-resolving inflammation represent a significant burden on the US healthcare system. There are currently few clinical tools to monitor ongoing lung inflammation and stratify patient care using a personalized medicine approach. A promising target is chemokine-like receptor 1 (CMKLR1), a GPCR expressed by select leukocytes that binds to ligand chemerin released during inflammation. The CMKLR1-chemerin axis has been implicated in various lung diseases and modulation of CMKLR1 signaling results in altered inflammatory responses. We evaluated CMKLR1-targeted positron emission tomography (PET) to non-invasively measure ongoing lung inflammation in the context of preclinical bleomycin-induced fibrotic lung injury. We further characterized the kinetics of CMKLR1 expression throughout the course of bleomycin-induced lung injury/remodeling.

METHODS: PET/CT imaging with 64Cu-NODAGA-CG34 was conducted in a bleomycin-induced murine model of lung injury, and the radiotracer uptake in the lungs was quantified by percent injected dose per gram (%ID/g) using Inveon Research Workspace. Specific uptake of the radiotracer was confirmed by co-injection of excess non-radiolabeled NODAGA-CG34. Following imaging studies, radiotracer uptake in individual organs (%ID/g) was measured with ex vivo gamma-counting. Ex vivo autoradiography of the lungs was performed and adjacent lung sections were stained for immunofluorescence microscopy. Cellular patterns of CMKLR1 expression throughout the course of bleomycin-induced lung injury was determined by flow cytometry quantification of 6CF-CG34 uptake by lung leukocytes.

RESULTS: In vivo uptake of 64Cu-NODAGA-CG34 both globally (%ID/gmean) and focally (%ID/gmax) was highest at 1 week and 2 weeks following bleomycin treatment, and decreased nearly to baseline by 4 weeks. 64Cu-NODAGA-CG34 uptake was completely blocked upon co-injection with excess non-radiolabeled NODAGA-CG34. Quantification of 64Cu-NODAGA-CG34 uptake in the lungs by ex vivo gamma-counting strongly correlated with in vivo uptake obtained by PET. Additionally, 64Cu-NODAGA-CG34 uptake measured by in vivo PET and high-resolution ex vivo autoradiography spatially co-localized within regions of lung inflammation and increased CMKLR1 expression determined by histology. Further, we found that the increased CMKLR1 expression in the lungs of mice at 1- and 2-weeks post-bleomycin was mostly driven by interstitial and monocyte-derived macrophages, and matches the observed kinetics of radiotracer uptake determined by in vivo PET.

CONCLUSION: In vivo molecular imaging of CMKLR1 with 64Cu-NODAGA-CG34 measures ongoing inflammation in the context of a preclinical fibrotic lung injury model. PET imaging of CMKLR1 provides a potential strategy to non-invasively quantitate, spatially localize and monitor the dynamics of lung injury.

Author: **Joud Mulla, BS**
Contact: joud.mulla@pitt.edu
Mentor: Melanie Scott
Co-Authors: Nijmeh Alsaadi, Zachary Secunda, and Melanie J. Scott

Poster Number: 33

Endothelial Cell Caspase-11 Regulates Il-6-Mediated Inflammation and Organ Damage in Mice Following Severe Injury

Major traumatic injury is a leading cause of mortality and elicits innate immune dysregulation, immunological dysfunction, coagulopathy and organ damage. The non-canonical inflammasome (caspase-4/11) triggers inflammation and coagulopathy in sepsis, but its role in non-infectious injury, such as trauma, is unknown. In this study we investigated the effect of endothelial caspase-11 on inflammation and coagulation in a murine polytrauma model.

Male C57BL/6J (WT), caspase-11KO (casp11ko), and endothelial cell-specific caspase-11KO (casp11ECko) mice, were subjected to polytrauma (n=3-4/gp). The model consists of blind cardiac puncture (25% total blood volume taken), a liver crush, and bilateral pseudofractures (hindlimb crush injury followed by the injection of crushed bone solution from an age- and weight-matched syngeneic donor). Plasma IL-6 and IL-1B (inflammation markers) were measured at 2 and 6h after polytrauma. ALT levels were measured 6h after polytrauma to assess liver damage. To assess coagulation, plasma tissue factor activity (TF) was measured at both timepoints after trauma. Lastly, baseline hemostasis in mice was assessed using a tail vein transection model (1cm tail tip cut followed by submersion in 20mL of lactated Ringer's solution). Time to the cessation of bleeding was measured in seconds and recorded as bleeding time.

Plasma IL-6 levels increased 6h post-trauma in WT mice, but were significantly lower in casp-11ko mice ($p<0.0016$). Interestingly, IL-6 values in casp-11ECko 6h post-trauma did not increase and remained as low as the baseline measurement, suggesting a role for endothelial caspase-11 in regulation of IL-6 release following trauma. Plasma IL-1B levels increased 2h and 6h post-trauma, but were not different between experimental groups. Casp11ko and casp-11ECko mice were both significantly protected from organ damage compared to WT mice 6h following polytrauma ($p<0.0001$). Trauma increased TF activity in WT mice at 2 and 6h post-trauma, and was significantly higher in casp11ko. Similar to IL-6 levels, TF activity was not increased over baseline in casp11ECko. Bleeding time at baseline was higher in WT and casp11ECko, compared to casp11ko ($p<0.005$), suggesting the better clotting potential exhibited in casp11ko mice is not derived from endothelial caspase-11.

Caspase-11 plays an important role in organ damage and coagulation changes after major trauma. Our data suggest endothelial-derived caspase-11 is a major component in the regulation of inflammation and organ damage. Systemic or targeted Inhibition of caspase-11 early after trauma may improve outcomes in trauma patients.

Author: **Alexis Nolfi, BS**
Contact: ALN24@pitt.edu
Mentor: Bryan Brown
Co-Authors: Mangesh Kulkarni, Vishal Jhanji, Clint Skillen, and Bryan Brown

Poster Number: 34

Therapeutic Use of an Interleukin-4 Eye Drop in a Rabbit Model of Dry Eye Disease: A Pilot Study

Introduction: Dry eye disease (DED) is estimated to affect up to one third of the population, with a spectrum of severity. However, severe, chronic DED can lead to vision-threatening complications, negative psychological consequences, and reduced quality of life. Current treatment options have limited efficacy and either provide temporary symptomatic relief or target limited aspects of the immune system. While the exact etiology of DED may vary patient to patient, two key elements, tear film instability (of which reduction in mucin-producing ocular goblet cells contribute) and ocular inflammation, are well-recognized drivers of DED pathogenesis. The failure of current therapies to target both of these elements concurrently may be a reason for their limited efficacy. Interleukin-4 (IL-4) has been shown to cause the differentiation of epithelium into goblet cells and to induce mucin expression – both key factors in tear film stability. IL-4 is also a potent anti-inflammatory cytokine causing transition of immune cells from a pro-inflammatory to anti-inflammatory phenotype. Thus, we hypothesize that IL-4 may represent an ideal cytokine for the simultaneous treatment of both tear film instability and inflammation in DED.

Methods: After surgical lacrimal gland removal, a rabbit model of DED was allowed to develop for 4 weeks. Two drops of PBS or IL-4 in PBS were administered daily for 14 days. Clinically relevant ocular assessments (e.g., fluorescein staining) were performed at 7 and 14 days post-treatment-initiation. After sacrifice, eyes were prepared for histological assessment, which included a periodic acid-Schiff (PAS) stain for goblet cells, pan-macrophage RAM11 immunolabeling, and H&E stain for general tissue morphology and cellularity.

Results: Fluorescein staining demonstrated significant reductions in corneal damage in IL-4 treated animals, and these improvements were associated with restoration of goblet cell numbers to native values in IL-4 treated animals. H&E staining and RAM11 immunolabeling demonstrated qualitative differences between PBS and IL-4 treated animals, with PBS treated animals showing areas of dense cellular infiltrate and epithelial thinning indicative of ulceration. Interestingly, increased RAM11 labeling was also seen in the epithelial basal layer of PBS treated animals. While RAM11 is primarily considered a macrophage specific antigen, others have shown that it is expressed at low levels in the basal epithelium of multiple tissues in rabbits, with transient increases in expression following epithelial wounding, suggesting increased epithelial damage in PBS treated animals.

Conclusions: These preliminary studies suggest that IL-4 treatment results in improved ocular surface integrity as well as increased number of goblet cells when used in an eye drop in a rabbit DED model. Thus, we hypothesize that IL-4 can provide improvements in DED that are superior to those associated with currently available treatments.

Author: **Kimberly Ortiz, BS**
Contact: kno10@pitt.edu
Mentor: Alejandro Soto-Gutierrez
Co-Authors: Zeliha Cetin, Rodrigo M. Florentino, Yiyue Sun, Zhiping Hu,Takeshi Kurihara, Guvanch Kakamyradov, Edgar N. Tafaleng, Alina Ostrowska, Lanuza A. P. Faccioli, and Alejandro Soto-Gutierrez

Poster Number: 35

Human hepatocytes from explanted livers of patients with cholestatic liver diseases as a valuable model for pathological mechanism studies

Cholestatic liver diseases (CLDs) include many etiologies resulting in reduced bile flow and disruption of enterohepatic circulation, leading to an accumulation of toxic bile acids and hepatocellular damage. Two major types of CLDs are primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). One of the key challenges in conducting research on these diseases is the lack of ideal animal models for both PBC and PSC. Instead, researchers have begun looking to human cells to understand the mechanisms of disease. While much of the literature focuses on the bile ducts, immune system, or gut-liver axis, we aim to elucidate how hepatocytes respond and cope with the cholestatic injury from PBC and PSC. Hepatocytes are the primary site of bile acid (BA) synthesis, metabolism, and transport, which are central to the pathogenesis of CLD. These primary human hepatocytes (PHHs) therefore represent a powerful experimental model for these diseases. Thus, this study focuses on the characterization of PHHs from patients with PBC (n=5) and PSC (n=5). Hepatocytes were isolated from explanted liver specimens obtained from patients receiving orthotopic liver transplantation due to PBC or PSC, using a modified three-step perfusion technique. The yield and viability of the freshly isolated hepatocytes were estimated using Trypan Blue, and the cells were then immediately cultured or cryopreserved. These isolated hepatocytes were characterized using qPCR for hepatocyte-specific gene markers, many of which are essential for cellular response to cholestasis. Additionally, intracellular levels of bile acids were measured by a Bile acid assay kit. To analyze the presence of oxidative stress, an immunostaining for 8-hydroxydeoxyguanosine (8-OHdG) in fixed cells was done. Our initial results demonstrate that these hepatocytes express differing levels of genes vital to the pathogenesis of CLD and exhibit increased levels of oxidative stress, making them valuable targets for in vitro studies investigating how hepatocytes respond to these cholestatic states and what therapeutic strategies can aid their defense.

Author: **Matthew Poskus, BS**
Contact: mdp77@pitt.edu
Mentor: Ioannis Zervantonakis
Co-Authors: Thomas McDonald and Ioannis Zervantonakis

Poster Number: 36

Mathematical Modeling of Fibroblast Mediated Drug Resistance in HER2+ Breast Cancer

Breast cancer is the most diagnosed cancer in women and accounts for an estimated 287,000 diagnoses in 2022. Of those cases, approximately 15% of women will be diagnosed with HER2-overexpressing (HER2+) breast cancer and many patients (38-75%) experience resistance to HER2-targeted therapies such as lapatinib. Stromal fibroblasts found in the tumor microenvironment are linked to poor patient outcomes. As the tumor cell:fibroblast ratio varies between patients, the effect of varying fibroblast density on cancer death remains an open question. We developed an in vitro tumor-fibroblast coculture system to monitor cancer cell growth/death dynamics during lapatinib treatment for several HER2+ breast tumor cell lines. We used these experimental data to calibrate a mathematical model to infer mechanisms of tumor-fibroblast communication, predict therapy response, and examine alternate treatment strategies.

We cultured four fluorescently labeled (H2BGFP) HER2+ breast cancer cell lines (EFM192, BT474, HCC202, and HCC1954) with or without AR22 fibroblasts in a 96-well plate and treated with 0-3μM lapatinib and measured drug response (live/dead cells) over four days using time-lapse microscopy. Cells were incubated with ethidium homodimer to identify dead cells. MATLAB was used to model tumor cell growth/death dynamics and fibroblast growth using ordinary differential equations (ODEs). Model parameters were fitted to cell line-specific growth/death data from coculture experiments. Model agreement with experimental data was assessed using Akaike Information Criterion (AIC).

EFM192 and BT474 cell lines showed greater survival when cocultured with fibroblasts compared to monoculture for all lapatinib doses. However, the response of HCC202 and HCC1954 was similar in monoculture compared to coculture. Using these data, we fit both a unidirectional (fibroblast to cancer cell) and bidirectional (tumor-fibroblast) model and determined that the bidirectional model fit three of four cell lines better than the unidirectional model by comparing AIC. Using the bidirectional model, we generated model predictions for four days of treatment for different fibroblast densities, lapatinib doses, and treatment duration. Our model predicted that extended treatment duration does not improve cancer cell death when protected by fibroblasts.

CMP & CATER Graduate Students

Author: **Justin Sui, BA**
Contact: jus104@pitt.edu
Mentor: Corrine Kliment
Co-Authors: Jennifer c. Boatz, Qianjiang Hu, Yingze Zhang, Melanie Koenigshoff, Jian Shi, Ian Walsh, and Corrine R. Kliment

Poster Number: 37

Loss of ANT1 increases fibrosis and senescence in idiopathic pulmonary fibrosis

Rationale: Epithelial cellular senescence, an age-related permanent state of cell cycle arrest, is a major driver of idiopathic pulmonary fibrosis (IPF) progression. While much work has been conducted to characterize cellular senescence in disease contexts, the underlying cellular dysfunction that leads to senescence remain poorly understood. Changes in mitochondrial structure and function is a hallmark of aging, IPF, and senescence. We utilize loss of adenine nucleotide translocase 1 (ANT1) to study the influence of mitochondrial dysfunction on senescence and IPF. ANT1 is a mitochondrial protein critical for maintaining energy balance of the cell, and epithelial cells that lack ANT1 have reduced mitochondrial metabolism. We hypothesize that loss of ANT1 results in metabolic dysfunction leading to enhanced cellular senescence and pulmonary fibrosis.

Methods: We measured ANT1 and ANT2 expression using bulk RNA-seq and a single cell RNA-seq database of lungs from IPF patients and healthy controls. Gene expression of senescence markers were measured from microarray datasets of whole lung lysates of IPF and healthy patients via the Lung Genome Research Consortium (LGRC). To test the function of Ant1 in IPF and senescence, we utilized the bleomycin-induced injury mouse model of pulmonary fibrosis. Ant1-null and C57/BL6 mice were intratracheally treated with bleomycin alongside saline controls for 28 days. Lung tissue fibrosis scoring and tissue staining for senescent markers was performed. Bronchoalveolar lavage fluid (BALF) was assessed for cytokines.

Results: ANT1 expression is reduced in whole lungs from IPF patients and alveolar type II cells through bulk RNA-seq and single cell RNA-seq, respectively. RNA expression of senescent markers were inversely correlated with ANT1&2 expression. In epithelial cell lines, loss of ANT1 increased protein expression of cellular senescence markers. Loss of ant1 in bleomycin-treated mice resulted in enhanced fibrosis after 28 days and increased p21 staining. Examination BAL revealed an increase in pro-inflammatory cytokines in ant1-null mice compared to wildtype.

Conclusions: Here we demonstrate that ANT1 is reduced in IPF patients compared to healthy controls, and loss of Ant1 in a mouse bleomycin-induced lung injury model of fibrosis led to exaggerated fibrosis. These findings highlight the importance of investigating the relationship between metabolic function and cellular senescence in the context of IPF. Senolytic drugs and drugs that target mitochondrial proteins such as ant1 are promising therapeutic avenues for IPF treatment.

CMP & CATER Graduate Students

Author: **Mohammad Naser Taheri, BS**
Contact: mot23@pitt.edu
Mentor: Mo Ebrahimkhani
Co-Authors: Yuda Xiang, Jennie Bostich, Alex Hill, Jason Lohmueller, Samira Kiani, and Mo R. Ebrahimkhani

Poster Number: 38

A Genetic Surveillance Circuit for Liver Organoid Improvement by Eliminating Abnormal Cell States

Liver organoids are promising models for studying liver development and disorders, drug screening, and likely surrogates for organ transplantation in regenerative medicine. However, current methods based on differentiation of pluripotent stem cells still fall short of generating organoids that perfectly capture liver properties. One main problem is the persistence of unintended cell types including undifferentiated or immature cell types. To address this problem, we developed cell identity detectors and actuators to discriminate between cell types, eliminate unintended cells, and allow the more liver-like cells to survive and found a new population.

We used cell type specific promoters to make cell-identity detectors. To assess the specificity of detectors, we employed state of the art gene assembly to construct fluorescent reporters. Lentiviral transduction was utilized to deliver the fluorescent reporters into the liver organoids. The specificity of detectors was interrogated based on immunofluorescence staining. Then, we used an inducible caspase9 (iC9) for making the actuator layer of the surveillance circuit. To investigate if actuator can specifically target intended populations for elimination, we linked the actuator killing function to hepatocyte and undifferentiated stem cell identity detectors and delivered them to the liver organoid. Finally, qPCR and live apoptosis imaging were used to evaluate the iC9 function.

The best detectors were selected based on exclusive expression of the reporter in the target cells as confirmed by the fluorescence microscopy. We confirmed the specific elimination of target hepatocytes based on a reduction in transcript levels of AAT and HNF4α while there we detected an increase in signatures of fibrosis like CD146. At the same time, live apoptosis imaging confirmed targeted cell death in hepatocytes and undifferentiated cells.

In conclusion, we illustrated that iC9 activity can be linked to identity detectors to bring about specific apoptosis in hepatocytes. Elimination of hepatocytes was found to be accompanied by a change in population composition (i.e., an increase in CD146 fibrotic cells). In addition, we indicated that our proposed approach can be harnessed to eliminate undifferentiated stem cells to increase safety for future transplantation applications. In future works, we will employ this system to make better liver organoids.

Author: **Grace Conway, BS**
Contact: gec36@pitt.edu
Mentor: Flordeliza Villanueva
Co-Authors: Anurag N. Paranjape, Xucai Chen, and Flordeliza S. Villanueva

Poster Number: 39

Understanding the mechanisms of ultrasound-targeted microbubble cavitation-mediated blood brain barrier opening

Ultrasound-targeted microbubble cavitation (UTMC) transiently opens the blood brain barrier (BBB). We previously determined that UTMC induces BBB hyperpermeability through an influx of calcium. As activation of RhoA is a calcium-dependent pathway that causes cytoskeletal reorganization, leading to the breakdown of tight junctions, we tested the hypothesis that UTMC-induced activation of RhoA leads to BBB hyperpermeability. We utilized a transwell model with brain endothelial cells and astrocytes on opposite sides of a support membrance. Ultrasound (1 MHz, 250 kPa, 10 μs pulse duration, 10 ms pulse interval) was applied in the presence of lipid microbubbles for 20s. BBB permeability was assessed using dextran flux and transendothelial electrical resistance (TEER) measured across the membrane. Integrity of tight junctions was evaluated by staining for ZO-1. UTMC reduced TEER ($p<0.05$), confirming reduced barrier integrity. One hour after UTMC, there was a significant decrease in ZO-1 mean pixel intensity ($p<0.05$). Treatment of cells with Rho inhibitor II (Y16) significantly reduced UTMC-induced dextran flux across the BBB ($p<0.05$) and UTMC-induced decrease in TEER. In our co-culture model of the BBB, UTMC induces hyperpermeability through modulation of tight junctions, at least in part through a RhoA-dependent mechanism.

Author: **Rick van der Geest, BS**
Contact: riv13@pitt.edu
Mentor: Janet S. Lee
Co-Authors: Hernán F. Peñaloza, Zeyu Xiong, Shekina Gonzalez-Ferrer, Xiaojing An, Huihua Li, Hongye Fan, Mohammadreza Tabary, S. Mehdi Nouraie, Yanwu Zhao, Yingze Zhang, Kong Chen, William G. Bain, and Janet S. Lee

Poster Number: 40

BATF2 enhances pro-inflammatory cytokine responses in macrophages and contributes to the host defense against pulmonary Klebsiella pneumoniae infection

Basic leucine zipper transcription factor ATF-like 2 (BATF2) is a transcription factor that is emerging as an important regulator of the innate immune system. We previously identified BATF2 among the top upregulated genes in human alveolar macrophages treated with LPS, but the signaling pathways that induce BATF2 expression in response to Gram-negative stimuli are incompletely understood. In addition, the role of BATF2 in the host response to pulmonary infection with a Gram-negative pathogen like Klebsiella pneumoniae (Kp) is not known. We show that induction of Batf2 gene expression in macrophages in response to Kp in vitro requires TRIF and type I interferon (IFN) signaling, but not MyD88 signaling. Analysis of the impact of BATF2-deficiency on macrophage effector functions in vitro showed that BATF2 does not directly impact macrophage phagocytic uptake and intracellular killing of Kp. However, BATF2 markedly enhanced Kp-induced macrophage pro-inflammatory cytokine responses. In vivo, we observed that Batf2 gene expression was elevated in the lung tissue of wild type (WT) mice 24 h after pulmonary Kp infection, and Kp-infected BATF2-deficient (Batf2-/-) mice displayed a higher bacterial burden in the lung, spleen and liver compared to WT mice. WT and Batf2-/- mice showed similar recruitment of leukocytes following infection, but in line with our in vitro observations, pro-inflammatory cytokine levels in the alveolar space were reduced in Batf2-/- mice. Altogether, these results indicate that BATF2 enhances pro-inflammatory cytokine responses in macrophages in response to Kp, and that as such, BATF2 contributes to the host defense against pulmonary Kp infection.

CMP & CATER Graduate Students

Author: **Susannah Waxman, BA**
Contact: suw31@pitt.edu
Mentor: Ian Sigal
Co-Authors: Marissa Quinn, Cara Donahue, Louis Falo Jr., Daniel Sun, Tatjana Jakobs, and Ian A. Sigal

Poster Number: 41

Visualizing and Quantifying Individual Astrocyte Morphologies Across the Collagenous Lamina Cribrosa

Astrocytes in the lamina cribrosa (LC) are key regulators of the physiology necessary for healthy vision. In glaucoma, alterations in LC astrocyte function and morphology have been observed throughout the process of pathogenesis. However, techniques to visualize and evaluate individual collagenous LC astrocyte morphologies in close proximity to one another are greatly limited. To address this, we evaluated the ability of multicolor DiOlistic labeling, a particle-mediated dye transfer approach, to allow visualization and quantitative evaluation of collagenous LC astrocyte morphology.

Gold microparticles were coated with all combinations of three fluorescent lipophilic dyes (DiI, DiD, and DiO) to create 7 different groups of microcarriers. Coronal vibratome sections were obtained through the LC of goat, sheep, and pig eyes (N = 16 eyes) at 150-200 μm thickness. Dye-coated microcarriers were ballistically delivered into sections with a gene gun. Tissues were labeled with GFAP to investigate the identity of DiOlistically labeled cells. Labeled cells were imaged via confocal microscopy. Collagen was imaged with second harmonic generation microscopy. 3D models of 56 dyed astrocytes were created in Imaris from image segmentations. Morphological features of models were quantified for LC astrocyte characterization.

Microcarriers embedded within cells delivered dye that distributed across their respective cell membranes. DiOlistically-labeled cells with astrocyte morphologies were GFAP-positive. Somas and branched processes of astrocytes labeled with all 7 combinations of dyes were visualized. Distinct dye combinations allowed discerning individual astrocytes with processes in close proximity to one another, sharing spatial domains. Average astrocyte branch number, hierarchy, length, thickness, and straightness were 132.0 ± 46.1, 7.4 ± 4.0, 11.2 ± 10.7μm, 1.9 ± 1.2μm, and 0.9 ± 0.1, respectively.

Multicolor DiOlistic labeling in vibratome sections of the collagenous LC is a viable approach to visualize and quantify individual astrocyte morphological features for in-depth analysis. LC astrocytes demonstrated heterogeneous morphologies and physical interactions with neighboring astrocytes and collagen beams. Healthy astrocyte morphologies and their corresponding functions can later be compared with those from glaucomatous LCs to better understand the role of astrocytes in glaucoma pathophysiology.

CMP & CATER Graduate Students

Author: **Sierra Wilson, BA**
Contact: siw40@pitt.edu
Mentor: Andrew Duncan
Co-Authors: Evan R. Delgado, Rosa L. Loewenstein, Frances Alencastro, Madeleine P. Leek, Kero Kamel, Patrick D. Wilkinson, Silvia Liu, Joseph Locker, Bharat Bhushan, and Andrew W. Duncan

Poster Number: 42

Compensatory regeneration after acetaminophen-induced acute liver injury is driven by diploid hepatocytes

The liver contains diploid and polyploid hepatocytes, with polyploids comprising nearly 50% of human and 90% of mouse hepatocytes. The functional differences between diploid and polyploid hepatocytes are poorly understood, but emerging data suggest that each ploidy population promotes regeneration in an injury-specific manner. We hypothesize that diploid hepatocytes drive rapid regeneration in the context of acute liver injury. To study ploidy populations in vivo, we utilized mice with a lifelong liver-specific knockout of E2f7 and E2f8 (LKO) that are functionally normal but are depleted of polyploid hepatocytes (LKO livers are >70% diploid). Acute liver injury was induced by acetaminophen (APAP), a common analgesic that causes liver injury and failure when taken in excess. LKO and control mice were injected intraperitoneally with 300 mg/kg APAP, and livers were harvested over 96 hours. Elevated liver enzymes, necrosis, and DNA fragmentation in hepatocytes revealed that while APAP damaged both treatment groups, LKO livers were less damaged than wild-type (WT) controls. Reduced damage and accelerated liver healing in the LKO model could be caused by gene expression effects associated with E2f7/E2f8 loss or by enrichment of diploid hepatocytes. To discriminate between these possibilities, we first analyzed gene expression by bulk RNA-sequencing after APAP injury. Minimal gene expression differences were observed between LKO and control at baseline, but differences emerged over the APAP time course, which could contribute to APAP sensitivity. Second, to focus on gene expression differences only, we knocked out E2f7/E2f8 in adult livers where ploidy was equivalent in each group. Both groups responded to APAP equivalently. Finally, to evaluate ploidy effects in a WT model, the response to APAP by diploid and polyploid hepatocytes was investigated in vitro. Both populations were equally damaged, but diploid hepatocytes showed enhanced proliferation. Together, the data suggest that the response to APAP overdose in the LKO model is controlled by variations in gene expression and the enrichment of diploid hepatocytes. In conclusion, consistent with previous observations during liver regeneration, diploid hepatocytes are a driver of rapid compensatory regeneration after drug-induced acute liver injury, underscoring a novel role for hepatic ploidy populations.

CMP & CATER Graduate Students

Author: **Jiazhen Xu, BS**
Contact: jiazhen5x@gmail.com
Mentor: Christopher Donnelly
Co-Authors: Cristian Bergmann Muñoz, Marilyn Ngo, William Numro Tennant, Jocelyn Mauna, and Christopher Donnelly

Poster Number: 43

The study of Circular RNAs and their regulation of TAR DNA binding protein 43 (TDP-43) pathological aggregation

TAR DNA binding protein 43 (TDP-43) is a nuclear RNA binding protein (RBP) that is found mislocalized to the cytoplasm and forms insoluble inclusions in the CNS of Amyotrophic Lateral Sclerosis (ALS) patients. Functional TDP-43 exists as a soluble protein that condenses to form dynamic liquid-like droplets and localizes to membraneless organelles through liquid-liquid phase separation (LLPS) under physiological conditions. Recent work indicates that abnormal LLPS, hypothesized to be driven by genetic or environmental factors, initiates the deposition of pathological TDP-43 aggregates observed in neurodegenerative disorders. Work from our lab and others indicates that TDP-43's RNA binding status contributes to the ability of purified and cellular TDP-43 protein to undergo aberrant LLPS and RNA engagement to TDP-43 prevents its pathological condensation. Circular RNAs (circRNAs) are covalently closed circular RNA previously thought of splicing by-products of longer mRNAs transcripts. Recent evidence indicates circRNAs are highly enriched in brain and play a role in neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD) and ALS. While the specific role of circRNAs in neurodegenerative disease pathogenesis remains unknown. Using the circRNA-expression system and a light-Inducible model of TDP-43 proteinopathy, opto-TDP43, we performed functional cellular assays to determine if circRNA biogenesis could affect insolubility and aggregation of TDP-43. Furthermore, our results shows that specific circRNA like circNEFM prevents TDP-43 aggregation and stress granule formation. This work provides foundational understanding of circRNA:RBPs regulation, which could provide new insight into neurodegenerative disease pathogenesis.

Author: **Hsuan Yeh, MD**
Contact: hsy15@pitt.edu
Mentor: Henry Dong
Co-Authors: Henry Dong and Wen Quan Zheng

Poster Number: 44

Role of β-cell glucocorticoid receptor signaling in pregnancy and gestational diabetes

Gestational diabetes mellitus (GDM) is characterized by glucose intolerance in pregnant women without previously diagnosed diabetes. Although GDM usually recovers after birth, GDM imposes a long-lasting adverse impact on the health of mothers and babies. To date, the underlying etiology of GDM is unknown. Pregnancy constitutes a unique physiological state, in which mothers develop insulin resistance with maternal weight gain, especially during the 3rd trimester of pregnancy. This effect serves to spare blood glucose supply for the fetus at the expense of maternal insulin resistance. To overcome insulin resistance, islets of mothers undergo adaptive changes in β-cell mass and function, termed “β-cell compensation” to release more insulin for maintaining euglycemia in the mother. Impaired β-cell compensation for maternal insulin resistance contributes to GDM. Glucocorticoid (GC) is a steroid hormone that is produced from the cortex of adrenal glands. During pregnancy, GC production is markedly upregulated, coinciding with the physiological induction of β-cell compensation in the mother. It is unknown whether the gestational surge of GC production contributes to β-cell compensation for pregnancy. To address this fundamental question, I will generate β-cell conditional glucocorticoid receptor (GR) knockout mice, using the GRLoxP/LoxP and PDX1-Cre-ER mice. The PDX1-Cre-ER mice express a tamoxifen-inducible Cre recombinase from the β-cell-specific PDX1 promoter. This system allows β-cell GR depletion conditionally in mature islets of adult mice, thereby avoiding the confounding factor of GR associated with prenatal β-cell development. I will also examine the effect of GR signaling on β-cell survival and secretory function in vitro. My central hypothesis is that β-cell GR signaling is critical for β-cell compensation for maternal insulin resistance in normal pregnancy. I will delineate β-cell GR signaling to uncover GR-targeted genes in regulating β-cell mass and function. These studies will fill in the gap of knowledge about the role of GR signaling in β-cell compensation and its contribution to GDM.

Basic Research

Author: **Shehnaz Bano, PhD**
Contact: shb208@pitt.edu
Mentor: Bharat Bhushan and George Michalopoulos
Co-Authors: Matt Avery Copeland, John W. Stoops, Anne Orr, William C. Bowen, Silvia Liu, Joseph Locker, Wendy M. Mars, George K. Michalopoulos, and Bharat Bhushan

Poster Number: 45

Hepatocytes Specific Deletion of Epidermal Growth Factor alters Lipid Metabolism and Fibrosis Signaling in a Murine Fast-Food Diet Model of Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder that is linked to an increased risk of developing liver fibrosis and hepatocellular carcinoma. Epidermal growth factor receptor (EGFR) is mostly known to regulate hepatocyte proliferation and regeneration in liver. In our previous study, we found that the EGFR inhibition utilizing Canertinib, reduced steatosis, liver injury, and fibrosis, in a murine fast-food diet (FFD) model, indicating a potential role for EGFR in regulating NAFLD. To establish this novel role of EGFR, we investigated the effect of hepatocyte-specific EGFR deletion in a murine NAFLD model. 8-10 weeks old EGFRflox/flox mice were injected adeno associated virus 8 (AAV-8) expressing Cre recombinase with a thyroxin binding globulin (TBG) promoter (hepatocyte-specific promoter) to knockout EGFR in hepatocytes (EGFRΔhep). EGFRΔhep or wild-type (WT) mice were fed normal chow diet or FFD for 2 months. FFD-fed EGFRΔhep mice displayed significant reduction in serum triglyceride levels with histologically evident lower fat accumulation, specifically in the periportal areas of liver compared to WT mice. At transcriptional level, EGFR deletion significantly reduced expression of SREBF1 (a major transcriptional regulator of fatty acid synthesis) and its downstream fatty acid synthase gene. However, these effects were not observed at protein level. EGFRΔhep mice showed lower protein expression of PPARγ, another important transcriptional regulator of lipid metabolism. Further, our transcriptomic analysis via RNA sequencing and subsequent Ingenuity Pathway Analysis revealed significant alteration of hepatic fibrosis/stellate cell activation pathways along with inhibition of TGFβ1 signaling (a key driver of liver fibrosis) in EGFRΔhep mice. However, the overall effect of hepatocyte-specific EGFR deletion on steatosis and gene signatures associated with NAFLD was much weaker compared to systemic pharmacological inhibition observed in our previous study. Lastly, deletion of EGFR enhanced expression and phosphorylation of the other ErbB family members (HER2 and HER3), indicating a potential compensatory mechanism for the loss of EGFR signaling in hepatocytes. In conclusion, hepatocyte-specific deletion of EGFR alters lipid metabolism and fibrosis signaling in a murine FFD model of NAFLD and is much less effective compared to EGFR pharmacological inhibition.

Basic Research

Author: **Sudrishti Chaudhary, PhD**
Contact: schudhry@pitt.edu
Mentor: Reben Raeman
Co-Authors: Ravi Rai, Pabitra B. Pal, Dana Tedesco, Aatur D. Singhi, Satdarshan P. Monga, Arash Grakoui, Smita S. Iyer, and Reben Raeman

Poster Number: 46

Western diet dampens T regulatory cell function to fuel hepatic inflammation in nonalcoholic fatty liver disease

Background and Aims. The immunosuppressive T regulatory cells (Tregs) regulate immune responses and maintain immune homeostasis, yet their functions in nonalcoholic fatty liver disease (NAFLD) pathogenesis remains controversial.

Methods. Mice were fed a normal diet (ND) or a western diet (WD) for 16 weeks to induce NAFLD. Diphtheria toxin injection to deplete Tregs in Foxp3DTR mice or Treg induction therapy in WT mice to augment Treg numbers was initiated at twelve and eight weeks, respectively. Liver tissues from mice and NASH human subjects were analyzed by histology, confocal imaging, and qRT-PCR.

Results. WD triggered accumulation of adaptive immune cells, including Tregs and effector T cells, within the liver parenchyma. This pattern was also observed in NASH patients, where an increase in intrahepatic Tregs was noted. In the absence of adaptive immune cells in Rag1 KO mice, WD promoted accumulation of intrahepatic neutrophils and macrophages and exacerbated hepatic inflammation and fibrosis. Similarly, targeted Treg depletion exacerbated WD-induced hepatic inflammation and fibrosis. In Treg-depleted mice, hepatic injury was associated with increased accumulation of neutrophils, macrophages, and activated T cells within the liver. Conversely, induction of Tregs using recombinant IL2/aIL2 mAb cocktail reduced hepatic steatosis, inflammation, and fibrosis in WD-fed mice. Analysis of intrahepatic Tregs from WD-fed mice revealed a phenotypic signature of impaired Treg function in NAFLD. Ex vivo functional studies showed that glucose and palmitate, but not fructose, impaired the immunosuppressive ability of Treg cells.

Conclusions. Our findings indicate that the liver microenvironment in NAFLD impairs ability of Tregs to suppress effector immune cell activation, thus perpetuating chronic inflammation and driving NAFLD progression. These data suggest that targeted approaches aimed at restoring Treg function may represent a potential therapeutic strategy for treating NAFLD.

Basic Research

Author: **Meghana Dodda**
Contact: med188@pitt.edu
Mentor: Baoli Hu
Co-Authors: Rashmi Srivastava, Alireza Labani-Motlagh, Apeng Chen, Fan Yang, Danish Ansari, Sahil Patel, Honglong Ji, Scott Trasti, Meghana Dodda, Yash Patel, Han Zou, Guohua Yi, and Baoli Hu

Poster Number: 47

Development of a human glioblastoma model using humanized DRAG mice for immunotherapy

Glioblastoma (GBM) is the most common and lethal primary brain tumor with high mortality rates and a short median survival rate of about 15 months despite intensive multimodal treatment of maximal surgical resection, radiotherapy, and chemotherapy. Although immunotherapies have been successful in the treatment of various cancers, disappointing results from clinical trials for GBM immunotherapy represent our incomplete understanding. The development of alternative humanized mouse models with fully functional human immune cells will potentially accelerate the progress of GBM immunotherapy. In this study, we developed a humanized DRAG (NOD.Rag1KO.IL2RγcKO) mouse model, in which the human hematopoietic stem cells (HSCs) were well-engrafted and subsequently differentiated into a full lineage of immune cells. Using this humanized DRAG mouse model, GBM patient-derived tumorsphere lines were successfully engrafted to form xenografted tumors, which can recapitulate the pathological features and the immune cell composition of human GBM. Importantly, the administration of anti-human PD-1 antibodies in these DRAG mice bearing a GBM patient-derived tumorsphere line resulted in decreasing the major tumor-infiltrating immunosuppressive cell populations, including CD4+PD-1+ and CD8+PD-1+ T cells, CD11b+CD14+HLA-DR+ macrophages, CD11b+CD14+HLA-DR−CD15− and CD11b+CD14− CD15+ myeloid-derived suppressor cells, indicating the humanized DRAG mouse model as a useful model to test the efficacy of immune checkpoint inhibitors in GBM immunotherapy. Together, these results suggest that humanized DRAG mouse models are a reliable preclinical platform for brain cancer immunotherapy and beyond.

Basic Research

Author: **Gabriella Fricklas, BS**
Contact: gaf38@pitt.edu
Mentor: Charleen Chu
Co-Authors: Kent Wang, Anthony Otero, and Charleen Chu

Poster Number: 48

PINK1 moderates dendritic mitochondrial content by regulating somatic to dendritic transitions

Parkinson's disease (PD) is the second most common neurodegenerative disease in the United States. Loss-of-function mutations in the gene PINK1, which codes for the protein PTEN-induced kinase 1, have been identified as the second most common cause of autosomal recessive Parkinson's disease. PINK1 is known to be involved in mitochondrial transport (Weihofen et al. 2009, Liu et al. 2012), though its physiological role in the absence of mitophagy-inducing stimuli remains unclear. Given our published and ongoing work showing that PINK1 increases mitochondrial density in dendrites, we hypothesized that PINK1 promotes the movement of somatic mitochondria into dendrites. We developed an assay using a mitochondrially targeted photoactivatable GFP to track the movements of somatic mitochondria into dendrites over a 10-minute period using live neuron imaging. PINK1 overexpression increased the number of mitochondria that exited the soma. We then studied endogenous PINK1 by repeating the experiment with PINK1 WT and KO mouse primary neurons. Dendritic transport utilizes different molecular machinery than axonal transport. Whereas dynein exclusively regulates retrograde transport in axons towards the soma, it plays an important role in determining whether cargo can enter dendrites (Tas RP et al., 2017; Kapetein et al 2010). We found that PINK1 overexpression promotes dynein phosphorylation at a novel site. S>D mutation to mimic dynein phosphorylation at this site rescued the diminished mitochondrial export in PINK1 KO neurons compared to PINK1 WT neurons. While studies are pending to determine if dynein is a direct target of PINK1, our data indicates that PINK1 regulates dendritic mitochondrial occupancy through effects on dynein phosphorylation.

References:

Liu S, Sawada T, Lee S, Yu W, Silverio G, Alapatt P, Millan I, Shen A, Saxton W, Kanao T, Takahashi R, Hattori N, Imai Y, Lu B. Parkinson's disease-associated kinase PINK1 regulates Miro protein level and axonal transport of mitochondria. PLoS Genet. 2012;8(3):e1002537. doi: 10.1371/journal.pgen.1002537. Epub 2012 Mar 1. PMID: 22396657; PMCID: PMC3291531.

Kapitein LC, Schlager MA, Kuijpers M, Wulf PS, van Spronsen M, MacKintosh FC, Hoogenraad CC. Mixed microtubules steer dynein-driven cargo transport into dendrites. Curr Biol. 2010 Feb 23;20(4):290-9. doi: 10.1016/j.cub.2009.12.052. Epub 2010 Feb 4. PMID: 20137950.

Weihofen A, Thomas KJ, Ostaszewski BL, Cookson MR, Selkoe DJ. Pink1 forms a multiprotein complex with Miro and Milton, linking Pink1 function to mitochondrial trafficking. Biochemistry. 2009 Mar 10;48(9):2045-52. doi: 10.1021/bi8019178. PMID: 19152501; PMCID: PMC2693257.

Tas RP, Chazeau A, Cloin BMC, Lambers MLA, Hoogenraad CC, Kapitein LC. Differentiation between Oppositely Oriented Microtubules Controls Polarized Neuronal Transport. Neuron. 2017 Dec 20;96(6):1264-1271.e5. doi: 10.1016/j.neuron.2017.11.018. Epub 2017 Nov 30. PMID: 29198755; PMCID: PMC5746200

Basic Research

Author: **Alex Hill, BS**
Contact: arh134@pitt.edu
Mentor: Mo Ebrahimkhani
Co-Authors: Alex Hills, Mohammad Taheri, Elena Puccio, Kamyar Keshavarz, Yuda Xiang, Samira Kiani, and Mo R Ebrahimkhani

Poster Number: 49

Self-timed Differentiation of Human Liver Organoids via Engineering of Gene Regulatory Networks

In-vitro organogenesis from human induced pluripotent stem cells is a process that requires controlled direction of differentiation. While some approaches rely on exogenous addition of growth factors to developing cultures, this method can suffer from a lack of control in multiple areas, including multilineage differentiation, batch-to-batch variability, and continued advancement of the organoid past an immature state. Previously, our lab has developed human liver organoids (dubbed DesLO, Designer Liver Organoids) by genetically embedding iPSCs with an engineered gene circuit for inducible expression of GATA6, a transcription factor related to liver organogenesis. Differentiating cultures are dissociated following 5 days of GATA6 induction and a subsequent layer of transgenes are delivered via lentivirus to promote continued maturation and upregulation of hepatic genes via expression of transcription factors PROX1 and ATF5, as well as hepatic enzyme CYP3A4. Subsequently, a portion of the cells are re-seeded onto culture plates and allowed to continue differentiation in basal media. Currently, we aim to integrate our iPSCs with synthetic gene circuits that will allow real-time sensing of cell state in order to activate the secondary layer of transgenes without need for dissociation and lentiviral transduction of the culture. This will reduce cellular stress due to lentiviral infection and variability between organoids caused by differences between lentiviral batches and re-seeded cell populations (bottleneck effect). It could also allow cell-type specific expression of target transgenes for improved maturation of specific cell types. Gene expression modulation based on cell state can be achieved at the level of transcription by usage of cell-specific promoters, such as the AAT promoter (pAAT), which is active in hepatic-like cells. It can also be regulated via detection of factors expressed by first-layer induced transgenes, such as GFP, using GFP-dependent Cre recombinase to activate second-layer circuits initially in an off-state. Integration of these transgenes into iPSCs can be achieved via lentiviral transduction or piggybac transposition. Testing of these sensor components in a HEK293T cell line using fluorescent reporters as output has revealed some functionality for GFP-dependent and hepatic cell-type specific activation of transgene expression, providing a promising level of proof-of-concept for these designs. Continual fine-tuning of sensor mechanics and the amount of transgene payload integrated into iPSCs can likely allow for higher state-specificity and target output of these circuits.

Basic Research

Author: **Chhavi Goel, PhD**
Contact: CHHAVI@pitt.edu
Mentor: Kari Nejak-Bowen
Co-Authors: Rong Zhang, Pamela Cornuet, Xiaochao Ma, and Kari Nejak-Bowen

Poster Number: 50

Loss of β-catenin attenuates lithocholic acid-induced hepatotoxicity

Background & Aim: Lithocholic acid (LCA) is a toxic hydrophobic bile acid associated with hepatotoxicity and adverse clinical outcomes in primary sclerosing cholangitis patients. We previously reported that conditional loss of β-catenin decreases bile acid synthesis and prevents the development of cholestatic liver injury after bile duct ligation, wherein obstructive bile flow is the primary cause of injury. To ascertain if loss of beta-catenin can attenuate injury in models of toxic bile acids, we aimed to investigate the role of beta-catenin inhibition in modulating liver injury against LCA.

Methods: Age-matched wild-type control (Con) and liver-specific β-catenin knockout (KO) mice were fed LCA for a week. Mice were euthanized and liver tissues analyzed by histology, qPCR, RNAseq, immunostaining, Western blot and liquid chromatography-mass spectrometry. Serum biochemistry was measured to assess liver injury parameters.

Results: β-catenin KO livers displayed fewer and smaller bile infarcts as compared to Con livers after LCA administration. Serum biochemistry showed significant decrease in hepatic and biliary injury in KOs compared to Cons. Gene expression analysis revealed decreased bile acid uptake transporters, increased apical and basolateral efflux transporters, and increased expression of detoxifying cytochrome P450 enzymes in the KO group with the net result of decreased accumulation of toxic bile acid in KO liver and hence less hepatic injury. Pan-cytokeratin immunostaining showed increased ductular response in KOs, likely as a defense mechanism for mediating enhanced BA clearance. RNA-seq analysis demonstrated decreased proinflammatory genes IL-33, IL-1β and target receptors IL-1R, and TNFR in the KO livers compared to Con. Immunostaining analysis further confirmed significant activation of IL-33 in the hepatocytes of Con group but not in KOs. Interestingly, BA composition was altered in KO after LCA, with a predominance of TbMCA over TMDCA.

Conclusions: As observed after BDL, loss of beta-catenin offers protection from LCA-induced hepatotoxicity and biliary injury through increased transport and hydroxylation of toxic BA. IL-33 seems to be an important target of β-catenin and its secretion from hepatocytes may contribute to hepatic injury induced by the toxic bile LCA. Detailed mechanistic pathway(s) will be assessed in future studies.

Basic Research

Author: **Bashir Lawal, PhD**
Contact: bal140@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Ling Lin, Li Li, Matthew Wexler, Suet Kee Loo, Binfeng Lu, Adrian Lee, Ronald J Buckanovich , and Xiaosong Wang

Poster Number: 51

Functional characterization of EPHA3 exon 4-5 duplications (EPHA3d4-5) in high-grade serous carcinoma progression, and recurrence

High-grade serous ovarian cancer (HGSC) accounts for 70-80% of ovarian cancer mortality, and overall survival has not been improved for decades. While standard therapy typically induces an initial response, most HGSC patients develop recurrent diseases following chemotherapy. The genetic aberrations that can be targeted to manage chemo-resistance remain ill understood. This suggests a desperate need to identity new therapeutic targets for the management of HGSC. Intragenic rearrangements (IGRs) leading to duplication or deletion of one or more exons have been sporadically reported to be cancer drivers. However, IGRs have not been rigorously studied in HGSC despite their potential significance as genetic drivers. Our analysis of TCGA copy number data revealed that IGRs as a special type of genomic rearrangements may be far more frequent events than realized in HGSC. In addition, this analysis identified a duplication of exons 4-5 in the Eph-Like Receptor Tyrosine Kinase A3 (EPHA3) in 8.3% of HGSC tumors, which we termed EPHA3d4-5, and our recent data suggest that it may be far more frequent in recurrent tumors. Exon 4-5 duplicated EPHA3 transcript encode in an in-frame protein with an extra fibronectin type 3 domain, which we speculate could alter the function of EPHA3 protein. Indeed, specific knockdown of EPHA3d4-5 potently reduced the viability of the EPHA3d4-5 positive HGSC cell line, which is not observed in the EPHA3 wild-type cell line, suggesting that EPHA3d4-5 may drive cancer cell growth in HGSC. Furthermore, transcriptome sequencing of genetic perturbation and ectopic overexpression models revealed that EPHA3d45 appears to activate cell cycle, oncogenic Rho signaling, and MYC pathways, and suppresses apoptosis, P53, TGF-β and interferon signaling. We thus hypothesize that EPHA3 exon 4-5 duplication may play a key role in promoting HGSC progression and recurrence and thus constitute a viable therapeutic target.

Basic Research

Author: **Parisa Lotfinejad, PhD**
Contact: pal140@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Parisa Lotfinejad, Liyong Zhang, Yuehua Zhu, Suet Kee Loo, Binfeng Lu, and Xiaosong Wang

Poster Number: 52

Landscape of intragenic rearrangements in triple-negative breast cancer reveals RUNX1 exon aberrations driving tumor immune evasion

Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype, accounting for 10-20% of breast cancer morbidity. Chemotherapy remained the mainstay of intervention for TNBC due to the lack of well-defined genetic targets, and recent genomic sequencing studies have revealed a paucity of TNBC-specific mutations. In this project, our landscape study of genomic rearrangements in TNBC revealed that recurrent intragenic rearrangements that result in one or more exons being duplicated or deleted may constitute a major TNBC-specific genetic landscape that may contribute to its pathobiology. As a proof of concept, we discovered novel intragenic rearrangements (IGRs) involving RUNX1, a proto-type cancer gene, which dictate an immune contexture in TNBC tumors that lack lymphocyte infiltrates. RUNX1 IGRs are preferentially detected in approximately ~7% of TNBC, which result in in-frame rearranged proteins that disrupt the RHD domain required for DNA binding and interaction with the CBFβ regulatory protein. RUNX1 is a master regulator that plays key roles in hematopoiesis, epithelial cytokine production, and induction of immune response. Our data suggest that RUNX1 rearrangements lead to potent repression of RUNX1 and NFκB target genes, resulting in upregulation of immunosuppressive cytokines such as CCL5 and repression of key proinflammatory cytokines such as CXCL10, suggesting its role in dictating tumor immune contexture. RUNX1 rearranged tumors are more aggressive showing larger tumor sizes, geographic necrosis, relative cold immune microenvironment that lack interferon γ signature and lymphocyte infiltration (especially CD8+ and CD4+ T cells), and a devastating clinical outcome. To date, this is the first report of somatic RUNX1 exon rearrangements in solid tumors, and the first study of their functions on regulating cancer immune landscape.

SIGNIFICANCE: Intragenic rearrangements could be a dark area of TNBC-specific genetic landscape that play a crucial role in its pathobiology. The discovery of RUNX1 exon rearrangements sheds a new light on a dark area of breast cancer genetics underlying immune evasion, which could pave the way to novel immunotherapeutic strategies to tackle this devastating disease. This represents a transformative concept in breast cancer genetics interfacing with immunotherapy that may have significant game-changing potential.

Basic Research

Author: **Jiayi Lu, MD, PhD**
Contact: JIL496@pitt.edu
Mentor: Charleen Chu
Co-Authors: Charleen Chu

Poster Number: 53

The roles of PINK1 in regulating dendritic mitochondrial distribution that contribute to the maintenance and maturation of dendritic arbors and spines

PTEN-induced kinase 1 (PINK1) is a serine/threonine kinase that plays critical roles in mitochondrial turnover, dynamics, mitophagy, and transport. Loss of PINK1 function has been linked to autosomal recessive Parkinson's disease with cognitive and neuropsychiatric comorbidities. However, the role of PINK1 in regulating dendritic mitochondrial distribution, motility, and function that contribute to the maintenance and maturation of dendritic arbors and spines is not well understood. In this study, we investigated the effects of pink1 knockout (KO) on dendritic architecture and mitochondrial distribution in primary mouse cortical neurons. Our findings indicated that pink1 KO neurons exhibited a striking simplified dendritic architecture, reduced spine density, and spine maturation. We analyzed the mitochondrial density and distribution by measuring the percentage of the dendrite length that was occupied by mitochondria. We observed a decrease in mitochondrial density in the dendrites of KO neurons, with a higher degree of variation in mitochondrial occupancy among different types of branches. Moreover, KO neurons had less mitochondrial supply for spines, particularly the mature mushroom and stubby spine, which may contribute to decreased length of their mature spines. Taken together, our results suggest that an increased susceptibility to mitochondrial distribution dysregulation contributes to dendritic injury and spine immaturity in mutant PINK1 pathogenesis.

Basic Research

Author: **Vineet Mahajan, PhD**
Contact: vsm14@pitt.edu
Mentor: Samira Kiani
Co-Authors: Victor Hernandez-Gordillo, Tahere Mokhtari, Farzaneh Moghadam, Jeremy Velasquez, Caleb Casolaro, Saraf Saman, Cheng Xi, Yining Zhang, Ramani Chavan, Jin Park, Michael Casey, Katja Pekun, Mark Kay, Mo Ebrahimkhani, and Samira Kiani

Poster Number: 54

Decoding CRISPR-Cas9 Gene Manipulation in a Human Liver Microphysiological System: Bridging the Gap

Introduction: CRISPR cytotoxicity and immune response present safety concerns for human applications. We developed a liver-on-a-chip system to study gene-editing effects on primary human hepatocytes (PHH) and Kupffer cells (KC), investigating cytotoxic responses and pathway influences of Cas9/gRNA delivery.

Methods: We employed liver chips with PHH monoculture and PHH-KC coculture (4:1 ratio). Liver-on-a-chip wells were seeded with hepatocytes and Kupffer cells, and CRISPR targeted α1-Antitrypsin (A1AT), homeobox (EMX), and Transthyretin (TTR) genes. Cas9 and gRNA were delivered using Adeno-Associated Virus (AAV), and the experiments were terminated on days 3, 5, and 10. Cytokine levels and RNA/DNA were analysed.

Results: CRISPR-Cas9 based gene editing in coculture activated pro-inflammatory immune response pathways and inhibited IL10 due to mere presence of Cas9-sgRNA. Cas9-A1AT14nt (disabled gRNA) showed up-regulation of p53, slowing over 10 days, and elicited an immune response, indicating activation of the immune pathway due to Cas9 and gRNA presence. Treatments with Cas9-A1AT, Cas9-EMX, and Cas9-TTR affected hepatocyte function, clotting factors, and regulation of the complement pathway. Editing TTR resulted in a stronger immune response than EMX and A1AT, which could cause cytokine storms. IL-6 and TNFα were present in the groups that were treated with Cas9-TTR and Cas9-EMX, with IL-6 originating from hepatocytes or Kupffer cells as a significant cytokine. The Cas9-TTR response differed between coculture and monoculture, with Myd88 playing a key role in immune modulation. Myd88 emerged as an upstream regulator in Cas 9-TTR and Cas 9-EMX treated groups, causing downstream effects such as monocyte activation and cytokine release, suggesting its involvement in the immune response. Myd88 repression reduced immune response and may improve cell survival post gene editing in coculture.

Conclusion: Our study elucidates the intricate immune responses triggered by CRISPR-Cas9 gene editing in cocultures, underscoring the activation of pro-inflammatory pathways and IL10 inhibition. A comprehensive analysis of Cas9-disabled gRNA and Cas9-gRNA combinations revealed distinct impacts on immune pathways and hepatocyte functionality in both monoculture and coculture systems. Notably, Cas9A1AT-14nt induced p53 upregulation and immune pathway activation, while TTR editing prompted more robust immune responses compared to EMX and A1AT editing. The presence of IL-6 and TNFα, along with the identification of Myd88 as a pivotal upstream regulator, highlights their critical roles in modulating downstream immune responses. Repression of Myd88 could attenuate immune response and may improve cell survival post-gene editing. These findings hold implications for refining gene editing techniques and understanding potential side effects. Furthermore, the liver-on-a-chip platform offers a promising avenue to bridge the gap between preclinical and human trials, facilitating the evaluation of CRISPR toxicity and delivery vehicle efficacy in human cells.

Basic Research

Author: **Tahere Mokhtari, MS**
Contact: tam190@pitt.edu
Mentor: Samira Kiani
Co-Authors: Vineet Mahajan, Samuel LoPresti, Mohammad Naser Taheri, Kamyar Keshavarz, Ryan LeGraw, Armin Aryannejad, Kathryn A. Whitehead, Mo Ebrahimkhani, and Samira Kiani

Poster Number: 55

Enhanced Zinc-finger based Epigenetic Modulator as Next-generation Immunosuppressant

Temporal transcriptional modulation of genes involved in immunity provides a powerful means for developing a therapeutic modality to treat a wide selection of infectious diseases and inflammatory conditions. Zinc finger (ZF)-based epigenetic engineering offers a safe, efficient, and reversible approach for achieving this goal. Here, we introduce an enhanced ZF-based transcriptional repressor to modulate transcriptional programming controlling immune-related signaling pathways in vivo. In this repressor system, two transcriptional repressors—heterochromatin protein 1 (HP1a) and Krüppel-associated box (KRAB)—are exploited and fused to ZF protein enabling enhanced repression. With our ZF-based supper repressor, we indicate transcriptional repression of Myeloid differentiation primary response 88 (Myd88) gene in vitro and in vivo. Myeloid differentiation primary response 88 (MyD88) is an essential adaptor molecule that plays a major role in innate and adaptive immune responses, such as TLR signaling, response to septicemia , and the development of adaptive immunity. We achieve efficient downregulation of Myd88 expression in lung, blood, liver, and bone marrow of the mice that receive systemic injection of adeno-associated virus (AAV)2/1-carrying Myd88 targeting ZF-HP1a-KRAB. Myd88 downregulation leads to transcriptional repression of the immune-related genes (e.g. TNF-α) that are downstream of Myd88-dependent signaling pathways as well as reduction of immunoglobulin G (IgG) response against AAV2/1. Our results also indicate that our Myd88 targeting ZF repressor can be delivered efficiently in the form of mRNA encapsulated in lipid nanoparticle and act as both prophylactic and therapeutic measure against septicemia in C57BL/6J mice. In addition, Myd88 targeting lipid nanoparticles improves the efficiency of repeated AAV administration by reducing immune response in hosts with pre-existing antibody. Overall, we report that ZF-mediated repression of Myd88 can result in a more effective and robust amelioration of inflammation during the course of AAV-mediated gene therapy and septicemia. Our study has significant translational impact, as we have for the first time developed an enhanced ZF transcriptional repressor and made use of different delivery cargos to achieve transient and long-lasting immunomodulation enabling efficient control of acute and chronic inflammatory conditions.

Basic Research

Author: **Laura Molina, PhD**
Contact: lmm208@pitt.edu
Mentor: Satdarshan Monga
Co-Authors: Adelya Gabdulkhakova, Yekaterina Krutsenko, Junjie Zhu, Silvia Liu, Sungjin Ko, Minakshi Poddar, Sucha Singh, Xiaochao Ma, Kari Nejak-Bowen, and Satdarshan P. S. Monga

Poster Number: 56

Loss of TAZ after YAP deletion severely impairs foregut development and worsens hepatocellular injury due to severe cholestasis

Background: We previously showed that loss of Yes-associated protein 1 (YAP) in early liver development (YAPKO) leads to an Alagille syndrome-like phenotype, with failure of intrahepatic bile duct development, severe cholestasis, and chronic hepatocyte adaptations to reduce liver injury. TAZ, a paralog of YAP, was significantly upregulated in YAPKO hepatocytes and interacted with TEAD transcription factors, suggesting possible compensatory activity.

Methods: We deleted both Yap1 and Wwtr1 (which encodes TAZ) during early liver development using the Foxa3 promoter to drive Cre expression, similar to YAPKO mice, resulting in YAP/TAZ double knock out (DKO) and YAPKO with TAZ heterozygosity (YAPKO TAZHET). We evaluated these mice using immunohistochemistry, serum biochemistry, bile acid profiling, and RNA-sequencing.

Results: DKO mice were embryonic lethal, but their livers were similar to YAPKO, suggesting an extrahepatic cause of death. Male YAPKO TAZHET mice were also embryonic lethal, with insufficient samples to determine the cause. However, YAPKO TAZHET females survived and were phenotypically similar to YAPKO mice, with increased bile acid hydrophilicity and similar global gene expression adaptations but worsened hepatocellular injury. TAZ heterozygosity in YAPKO impacted expression of canonical YAP targets Ctgf and Cyr61, and we found changes in pathways regulating cell division and inflammatory signaling correlating with an increase in hepatocyte cell death, cell cycling, and macrophage recruitment.

Conclusion: YAP loss (with or without TAZ loss) aborts biliary development. YAP and TAZ play a co-dependent critical role in foregut endoderm development outside the liver, but they are not essential for hepatocyte development. TAZ heterozygosity in YAPKO had a subtle impact on cell cycling and inflammatory signaling in the setting of chronic injury, highlighting genes that are especially sensitive to TAZ regulation.

Basic Research

Author: **Ravi Rai, PhD**
Contact: raviprakash.rai@pitt.edu
Mentor: Reben Raeman
Co-Authors: Daniel Rossmiller and Reben Raeman

Poster Number: 57

C-C motif chemokine receptor 9 ablation worsens whereas therapeutic blocking protects mice from western diet-induced nonalcoholic steatohepatitis

Intestinal epithelial permeability, fueled by gut microbial dysbiosis-associated mucosal inflammation, is central to nonalcoholic steatohepatitis (NASH) pathogenesis; however, the mechanisms underlying mucosal inflammation in NASH remain incompletely understood. The gut-trophic chemokine receptor, CC chemokine receptor 9 (CCR9), regulates intestinal immune homeostasis. However, its pathogenic roles in chronic gastrointestinal diseases reveal the complex role of CCR9 on gut health. In this study, we used knockout mice and pharmacologically targeted mice fed a western diet (WD) to elucidate the role of CCR9 in modulating intestinal mucosal inflammation in NASH. Our studies revealed inherent defects in the intestinal epithelial barrier (IEB) and higher intestinal mucosal inflammation in Ccr9-/- mice relative to Ccr9+/+ (WT) mice. Consequently, compared to WT mice, when fed a WD, Ccr9-/- mice developed severe intestinal mucosal inflammation and IEB dysfunction. Heightened mucosal injury in WD-fed Ccr9-/- mice correlated with more severe hepatic inflammation and fibrosis relative to WT mice. In contrast, the CCR9 antagonist, vercirnon-mediated CCR9 blockade improved mucosal inflammation and restored IEB function in NASH mice. This correlated with improved biochemical, histologic, and metabolic parameters associated with NASH. Collectively, these findings emphasize the significance of CCR9 in maintaining gut health and highlight the potential of selective, intermittent CCR9 targeting as a therapeutic strategy for NASH.

Basic Research

Author: **Gavin Schmidt**
Contact: gms122@pitt.edu
Mentor: Alyaksandr Nikitski
Co-Authors: Yuri E. Nikiforov and Alyaksandr V. Nikitski

Poster Number: 58

Fusions Involving the Thyroglobulin Gene as a Novel Mechanism of Thyroid Carcinogenesis

Thyroid cancer (TC) is a common endocrine malignancy, yet its molecular mechanisms are not fully understood. Approximately 15-20% of TC are driven by gene fusions, which activate oncogenes through aberrant expression of their functional domains, through ligand-independent dimerization, or through loss of inhibitory motifs. Using RNA-Seq analysis of TC negative for known driver mutations, we identified 11 cases of fusions involving the thyroglobulin (TG) gene. TG encodes the main precursor to thyroid hormones and is expressed at a very high level in thyroid follicular cells. To date, TG has not been implicated in TC. The aim of this study was to characterize TG-fusion-positive thyroid tumors and to determine if TG-fusions represent a novel mechanism of oncogene activation. RNA-Seq analysis showed that fusion with TG resulted in a >100-fold increase in expression of the 3' partner genes. Based on the type of 3' partner, all discovered TG-fusions could be assigned to one of three groups: (i) receptor tyrosine-kinases (RTK), including four TG(ex47)-FGFR1(ex3), three TG(ex47)-RET(ex11) and one TG(ex1)-NTRK1(ex9); (ii) IGF2-mRNA-binding proteins, with one TG(ex1)-IGF2BP1(ex2); (iii) driving aberrant expression of chromosome 19 microRNA cluster, including TG(ex15)-DPRX(5'UTR) and TG(ex35)-DPRX(5'UTR). Out of eleven TG-fusion-positive thyroid nodules, seven were surgically resected. Histopathological analysis revealed TC in 71% (5/7) and NIFTP in 29% (2/7) of cases. Using immunohistochemistry and western blot, we confirmed the expression of TG-FGFR1 and TG-IGF2BP1 and the activation of MAPK signaling in the fusion-positive tumor samples. To functionally characterize the most prevalent category of TG-fusions, HEK293 and thyroid HTORI-3 cells were transfected with HA-tagged TG-NTRK1, which showed membranous and intracellular/endosomal localization in both cell lines. Using HEK293 cells stably expressing TG-NTRK1, we found ligand-independent homodimerization and phosphorylation of the fusion protein. In these cells, western blot and phospho-RTK assays revealed that TG-NTRK1 induced MAPK and STAT3 signaling as well as the phosphorylation of MET, JAK2, EphA1/2/10, HER4, and ALK. Treatment of TG-NTRK1-expressing HEK293 cells with the NTRK-specific inhibitor Larotrectinib for 72 hours showed a dose-dependent decrease in the phosphorylation of TG-NTRK1, MET, STAT3, and ERK.

In summary, we report that fusions involving thyroglobulin occur in TC, lead to strong overexpression of the 3' partner genes, activate RTKs and downstream signaling pathways, and thus likely represent a novel oncogenic event in TC. Inhibition of TG-NTRK1 by Larotrectinib and the resultant decrease in MAPK, MET, and JAK-STAT3 activation indicates that TG-RTK fusions may serve as a potential therapeutic target for a subset of TC.

Basic Research

Author: **Rayna Schoenberger**
Contact: rls189@pitt.edu
Mentor: Mo Ebrahimkhani
Co-Authors: Joshua Hislop and Mo Ebrahimkhani

Poster Number: 59

Collective Behaviors Drive the Formation of Fetal Liver Organoid Vascular Networks

iPSC-derived organoids have the potential to revolutionize regenerative medicine and developmental biology through the recreation of human tissue, a critical component of which are the vascular networks enabling oxygen, nutrient, and waste transport. When 3D models of fetal liver tissue, i.e. fetal liver organoids (FeLOs), are generated from iPSCs harboring a doxycycline (dox)-inducible GATA6 gene circuit, some cells differentiate and self-organize into complex networks of endothelial cells vascularizing the liver tissue. To investigate how these organized vascular networks spontaneously emerge, this study combined dox-inducible GATA6-EGFP iPSCs with iPSCs containing mKate and dox-inducible ETV2, where ETV2 is a transcription factor that promotes endothelial cell fates. Differentiating these cells in coculture produced FeLOs with red fluorescent endothelial cells, allowing for spatiotemporal tracking of cell morphology and behaviors throughout vascular network development. We observed that ETV2 cells follow a distinct series of collective behaviors when forming vascular networks within FeLOs. The cells will migrate toward each other to form hemangioblast-like aggregates, compress, and re-disperse across the culture before interconnecting to form vascular networks. This reveals the key role of collective behaviors in the formation of self-organized vascular networks in organoids, potentially opening new avenues for the optimization of organoid vascularization in scientific and clinical settings.

Basic Research

Author: **Anya Singh-Varma, BS**
Contact: ans391@pitt.edu
Mentor: Satdarshan P Monga
Co-Authors: Shikai Hu, Leon Min, Brandon Lehrich, Minakshi Poddar, Sucha Singh, Aaron Bell, Michael Jurczak, Silvia Liu, and SP Monga

Poster Number: 60

Identification of a novel stellate cell-endothelial cell-hepatocyte circuit that regulates zonation and metabolism-proliferation switch in liver pathophysiology

Background: The liver, a main regulator of homeostasis, is histologically organized into metabolic zones defined by specific gene expression along the portal-central axis, which allows for division of labor for efficient function. Current understanding of the maintenance of zonation defines endothelial cells (ECs) in pericentral zones as the source of Wnt2 and Wnt9b, which regulate gene expression in hepatocytes in this zone by transactivating ß-catenin. Here, we investigate if there is any role of Wnts from hepatic stellate cells (HSCs), whose function in liver physiology has not been extensively studied, by characterizing mice incapable of secreting Wnts from HSCs.

Methods: Male and female HSC-specific Wntless (Wls) knockout (KO) mice were generated by interbreeding Wls-floxed mice and lecithin retinol acyl-transferase-driven Cre transgenic mice. Single nuclei RNA sequencing (snRNA), single cell spatial transcriptomics (scST), qPCR, and immunohistochemistry (IHC) were performed to identify changes in gene expression and zonation between KO and control mice. Metabolic cage studies and Oroboros were conducted to characterize differences in metabolism. Challenge models included partial hepatectomy and acetaminophen studies.

Results: No difference in ALT, AST, and LW/BW ratio between HSC-Wls KO and control mice is observed at baseline. KO mice are smaller and have a greater lean-to-fat mass body composition than controls ($p<0.05$). Metabolic cage data reveal KO mice are less active, yet they expend more energy ($p<0.01$). Interestingly, Oroboros on liver reveals KO mitochondria possess greater maximum function than controls when fatty acids are the substrate. IPA analysis on snRNA shows significant upregulation of pathways involving cholesterol and bile acid metabolism in KO hepatocytes. scST and IHC reveals expansion in expression of Cyp2e1, Cyp7a1, Cyp1a2, and Oat, from pericentral to periportal zones. Intriguingly, this coincides with altered zonation of sinusoidal ECs which show expanded expression of pericentral markers like Fabp4, c-Kit, and Wnt2 to periportal ECs. Midzonal protein (not RNA) expression of Cyclin D1 is decreased and Ki67 is nearly absent in KO livers. Following hepatectomy, KO mice express Ccnd1 and Ki67 at later timepoints than controls, indicating delay in regeneration. Acetaminophen challenge leads to fewer Cyclin-D1- and Ki67-positive hepatocytes in HSC-Wls KOs along with greater necrosis extending beyond the midzone due to expanded Cyp2e1 expression.

Conclusions: Abrogation of Wnt signaling from HSCs alters zonation of both ECs and hepatocytes, implicating them to be master regulators of zonation and consequently, play a role in maintaining the balance between metabolic and proliferative homeostasis in the liver.

Basic Research

Author: **Yiyue Sun, MD**
Contact: sunyiyue@pitt.edu
Mentor: Alejandro Soto-Gutierrez
Co-Authors: Zhiping Hu, Takeshi Kurihara, Ricardo Diaz-Aragon, Takashi Motomura, Kimberly N. Ortiz, Zeliha Cetin, Guvanch Kakamyradov, Alina Ostrowska, Lanuza A. P. Faccioli, Rodrigo M. Florentino, Edgar N. Tafaleng, and Alejandro Soto-Gutierrez

Poster Number: 61

Development of Lipid Nanoparticles-Based Transcriptional Reprograming Therapies for Cirrhosis with End-Stage Liver Disease

Chronic liver injury leads to cirrhosis which may progress to end-stage liver disease (ESLD), a leading cause of mortality worldwide. Unfortunately, liver transplantation which is the only curative therapy is limited to only a fraction of patients in need due to donor organ availability. Liver-enriched transcription factors, especially HNF4α, are key regulators of xenobiotic metabolism, carbohydrate and fatty acid metabolism, bile acid synthesis, blood coagulation, and ureagenesis in hepatocytes. Previously, we have shown that loss of HNF4α expression is strongly correlated with worsening liver function and, most importantly, have conclusively demonstrated in proof-of-concept studies that upregulating HNF4α expression in hepatocytes using transfection or viral delivery in both in vivo and in vitro models of ESLD improved hepatocyte function and completely reversed cirrhosis and ESLD. Based on these findings, we hypothesize that the use of modified mRNA delivered by lipid nanoparticles (LNPs) can be used to augment HNF4α expression and improve hepatocyte metabolic functions in in vitro and in vivo models. Towards this end, we initially tested various LNP formulations for their ability to effectively target human hepatocytes isolated from explanted cirrhotic livers with ESLD due to alcohol liver disease or non-alcoholic steatohepatitis. We have identified a clinically relevant LNP that outperformed others by effectively targeting >90% of human hepatocytes in vitro. Furthermore, to investigate the therapeutic efficacy (dosage window, liver mass target, and cellular specificity) of LNPs in cirrhotic livers in vivo, we have been developing a rat model of ESLD using thioacetamide (TAA)-induced hepatotoxicity. Following several weeks of TAA treatment, histological examinations of rat livers showed significant evidence of cirrhosis. Moreover, evaluation of parameters included in the calculation of the Child-Pugh score used for clinical assessment of the severity of liver dysfunction, revealed that the TAA rat model recapitulates the abnormal coagulation ability and bilirubin metabolism observed in human ESLD patients. Finally, analysis of rat HNF4α expression showed loss of HNF4α expression concurrent with worsening liver function over time. These findings highlight the relevance and validity of the TAA rat model for studying ESLD. In summary, we have identified a clinically relevant LNP that effectively targets human cirrhotic hepatocytes from patients with ESLD, and we are in the process of developing a rat model of ESLD. This study lays the groundwork for preclinical studies on the delivery of the next generation of nucleotide-based therapeutics that could potentially treat/reverse ESLD in humans.

Basic Research

Author: **Anil Verma, PhD**
Contact: anil12aiims@gmail.com
Mentor: Smita Iyer
Co-Authors: Chase E Hawes, Gabriel Kristian Pedersen, Dennis Christensen, Rama R. Amara, and Smita S. Iyer

Poster Number: 62

Tailoring helper profile of HIV-1 vaccine-induced CD4 T follicular helper cells

Generation of durable antibodies against the HIV-Envelope (Env) glycoprotein hinges on optimal cytokine and co-stimulatory help from CD4 T follicular helper cells (Tfh). We demonstrated that adjuvant-induced stimulation of T helper (h) 1- Tfh cells (Tfh1) enhances magnitude and quality of anti-Env antibody to a Clade C DNA-prime/protein-boost platform relative to one designed to induce Tfh2 responses. Based on the significance of Th17 cells in mucosal immunity, here we asked whether adjuvant-driven modulation of cytokines is an effective strategy to elicit Tfh1/Tfh17 germinal center (GC) response. To simultaneously prime and boost Tfh1/Tfh17 cells, we immunized rhesus macaques with a Clade C DNA plasmid molecularly adjuvanted with interferon protein 10 (IP-10) and interleukin 6 (IL-6). Ex vivo characterization of the DNAIP-10/IL-6 plasmid demonstrated expression of trimeric gp160 with the production of IP-10 and IL-6 in supernatants. Animals were then boosted with a Clade C gp140 Env protein (Pro) adjuvanted with cationic liposome-based formulation (CAF01) resulting in the induction of IP-10 and IL-17 in sera of most animals. We observed robust GC responses with significant induction of GC Tfh cells co-expressing chemokine receptors, CXCR3, and CCR6 exemplifying Th1/Th17 skewing of the GC with the DNAIP-10/IL-6 /ProCAF01 platform. Notably, strong Env-specific Tfh responses were elicited within lymph nodes. These data demonstrate the ability to tailor GC Tfh responses; ongoing studies are underway to determine whether induction of Th1/Th17 GC Tfh cells enhances rectal anti-Env IgA antibodies, and augments functional quality and durability of anti-Env IgG compared to a Th1 vaccine platform.

Basic Research

Author: **Yue (Yolanda) Wang, MD**
Contact: yue.wang@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Liyong Zhang, Vincent Chen, Swetha Nattu, Adrian V. Lee, Binfeng Lu, Rohit Bhargava, and Xiaosong Wang

Poster Number: 63

Role of BCL2L14-ETV6 gene fusions in triple-negative breast cancer immune evasion

Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype accounting for 10-20% of breast cancer morbidity but a disproportionate large number of breast cancer deaths. Chemotherapy remained the mainstay of intervention due to the lack of well-defined targets, and recent deep sequencing studies have revealed a paucity of TNBC-specific mutations. Most recently immune checkpoint inhibition (ICI) emerged as an effective therapy for advanced TNBC expressing PD-L1 for which the responses appear to be durable. However, the ICI response rates for unselected TNBC are only 5-10%. Discovering the genetic aberrations driving TNBC immune evasion represents an unmet clinical need. Here we identified the first TNBC-specific recurrent gene fusion between BCL2L14 and the prototype cancer gene ETV6. BCL2L14-ETV6 is preferentially detected in ~19% mesenchymal-like TNBC tumors that exhibit more aggressive histopathological features. Our preliminary studies suggest that BCL2L14-ETV6 fusions modulate the target genes of NFκB, a central mediator of inflammation, orchestrate cytokine contexture, endow epithelial mesenchymal transition, confer paclitaxel resistance, and dictate an immune microenvironment that lacks immune infiltrates. Our study showed BCL2L14-ETV6 fusions orchestrate immunosuppressive and protumor cytokine contexture, and impair immune cell infiltration. Elucidating the role of BCL2L14-ETV6 fusions in TNBC immune evasion could reveal unique, exploitable vulnerabilities to tackle this devastating disease, and illuminate new immunotherapeutic strategies. As the incidence of BCL2L14-ETV6 is comparable to that of ICI responders in unselected TNBC, such development could substantially expand the patient cohort that could benefit from new immunotherapeutic strategies to tackle this devastating disease.

Basic Research

Author: **Yue Wang, MD**
Contact: yuw130@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Mengying Hu, Olivera J Finn, and Xiao-Song Wang

Poster Number: 64

The association of tumor associated antigen burden with immune checkpoint blockade benefit in selected tumor entities with low T cell exhaustion and mutation burden

Tumor associated antigens (TAAs) have been studied as primary vaccine targets for more than two decades. However, vaccine trials against TAAs in unselected melanoma patients are largely disappointing. Recently, immune checkpoint blockade (ICB) emerged as an effective therapy for a subset of cancer patients, leading to durable responses. However, few generalizable associations between TAAs and ICB benefit have been reported, although most studies focus on melanoma that have highest tumor mutation burden (TMB) among all cancers. In this study, we developed a TAA burden (TAB) algorithm based on known and putative TAAs. Analysis of two clinical studies of urothelial carcinoma treated with anti-PD1/PD-L1 therapies revealed that high TMB diminishes association of TAB with ICB benefit. More interestingly, TAB shows increased correlation with ICB benefit with decreased CD8 T cell exhaustion state. This suggests that extensive T cell exhaustion may deplete the TAA-reactive T cell repertoire, thus diminish the association of TAB with ICB benefit. Similar observations are made in two neoadjuvant anti-PD1 trials for head and neck carcinoma, where TAB correlates with ICB benefit in tumors with unelevated T cell exhaustion state. This study challenges the current paradigm on the lack of association of tumor associated antigens with ICB response and calls for future studies on the immunogenicity of TAAs and potential TAA-based vaccine strategies in tumors with low T cell exhaustion and mutation burden.

Basic Research

Author: **Liyong Zhang, PhD**
Contact: liz45@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Yuehua Zhu, Suet Kee Loo, Rohit Bhargava, Adrian Lee, and Xiaosong Wang

Poster Number: 65

Role of Runt-related Transcription Factor 1 Intragenic Rearrangements in Cancers

We identified novel intragenic rearrangements (IGRs) involving Runt-related transcription factor 1 (RUNX1), a master transcription factor regulates hematopoiesis and a frequently mutated gene in leukemia. Our ICGC patient cohort, whole genome sequencing (WGS) and TCGA data analysis revealed that RUNX1 IGRs are highly enriched in esophageal cancer, lung cancer and triple-negative breast cancer (TNBC), which was further validated by the analysis of 89 primary TNBC tumors from UPMC patient cohort. Next, we examined the immune microenvironment of RUNX1 rearranged ICGC tumors, which exhibit a relative cold immune microenvironment compared to other TNBC tumors, featuring lack of CD8+ and CD4+ T cells, and comparatively low interferon γ signature.

We conducted RUNX1 knockdown experiments in cancer cell lines OE33, NCI-H1568, MCF7 and HCC1937 with siRNA targeting at different aberrant variants. The cell cycle analysis of these 4 cell lines by propidium iodide staining showed that the aberrant RUNX1 variant siRNAs induce dramatic apoptosis. Following mRNA-seq, upregulation of CXCL10 and downregulation of CCL5 on IGR knockdown group are identified. ELISA further confirmed the cytokine expression changes after knockdown. These findings suggest an immune suppressive role of RUNX1 IGR.

Taken together, we identified a novel intragenic rearrangement of RUNX1 protein in cancers. Understanding the function of this genetic aberration associated with immune dysfunction may illuminate new immunotherapeutic strategies to treat cancer patients or convert Immune checkpoint blockade therapy non-responders to responders.

Basic Research

Author: **Liyong Zhang, PhD**
Contact: liz45@pitt.edu
Mentor: Xiaosong Wang
Co-Authors: Ling Lin, Suet Kee Loo, Yuehua Zhu, and Xiaosong Wang

Poster Number: 66

Therapeutic targeting of ESR1–CCDC170 Rearrangements in Endocrine Resistant Breast Cancer

Endocrine therapy is the most common treatment for estrogen-receptor positive breast cancer, but its effectiveness is limited by high rates of intrinsic and acquired resistance, which accounts for the majority of devastating breast cancer deaths. Our lab discovered the first and most prevalent recurrent gene fusion in luminal breast cancer generated by cryptic rearrangements between ESR1 and its immediate neighbor gene CCDC170 that has an unknown function. ESR1-CCDC170 fusions are preferentially detected in 8-9% of more aggressive and lethal luminal breast cancers prone to develop endocrine resistance, as well as ~8% of metastatic luminal breast cancer. ESR1-CCDC170 fusions encode five distinct forms of N-terminally truncated CCDC170 proteins. Interestingly, this fusion appears to function through CCDC170, whereas ESR1 only contributes its constitutively active promoter. More importantly the significance of ESR1-CCDC170 is supported by multiple recent clinical studies: a neoadjuvant clinical trial detected ESR1-CCDC170 in 3 out of 27 tumors with reduced endocrine sensitivity (11%); another clinical study detected ESR1-CCDC170 in 29 out of 307 primary luminal breast cancer patients (9.4%) that experienced metastatic relapse, which significantly predicted worse disease-free survival.

Here our recent data suggest that ESR1-CCDC170 fusions endow breast cancer cell survival and reduced endocrine sensitivity in vitro and in vivo. Mechanistically ESR1-CCDC170 forms homodimers, physically binds to the receptor tyrosine kinase (RTK) protein complex containing HER2, HER3, and SRC, and activate their oncogenic signaling. This appears to engender a specific exploitable vulnerability in these deadly tumors by conferring increased sensitivities to HER2/SRC inhibitors. Our overarching hypothesis is that truncations of CCDC170 that delete its coiled-coil 1-2 domains may lead to altered protein interactions and cellular localization properties. ESR1-CCDC170 protein may facilitate HER2/HER3 heterodimerization through its ATP-driven autonomous homodimerization, leading to uncontrolled activation of their downstream signaling and increased sensitivity to neuregulin. Targeting this axis may be an effective therapeutic strategy to manage fusion-positive patients. This urge for further investigations to establish this druggable hypothesis and develop effective intervention against these deadly tumors. In this study, we further examined the responses of ESR1-CCDC170 expressing breast cancer cell lines to HER2/SRC inhibitors in the context of endocrine therapy, which could pave the way to a personalized treatment of ESR1-CCDC170-positive breast cancer patients that otherwise will have a devastating clinical outcome.

Clinical Research

Author: **Zeliha Cetin, MD**
Contact: cetinz@pitt.edu
Mentor: Alejandro Soto-Gutierrez
Co-Authors: Zehra N. Kocas-Kilicarslan*, Lanuza A.P. Faccioli, Nils Haep, Takashi Motomura, Sriram Amirneni, Ricardo Diaz-Aragon, Rodrigo M. Florentino, Kimberly N. Ortiz, Yiyue Sun, Zhiping Hu, Takeshi Kurihara, Guvanch Kakamyradov, Iris Pla-Palacin, Mengying Xia, Mark T. Miedel, Alina Ostrowska, D. Lansing Taylor, Jaideep Behari, Alejandro Soto-Gutierrez, and Edgar N. Tafaleng (* Equal authorship with Zeliha Cetin)

Poster Number: 67

Contribution of MAFLD-associated genetic variants to the progression of end-stage liver disease due to NASH or ALD

Chronic liver injury that results in cirrhosis and end-stage liver disease (ESLD) causes more than 1 million deaths annually worldwide. Metabolic dysfunction-associated fatty liver disease (MAFLD) and alcohol related liver disease (ALD) account for the majority of non-viral etiologies that lead to ESLD. There is variability in the severity and progression of ESLD and, although this has been historically attributed to differences in diet and exercise, there is growing evidence for a genetic component to this phenomenon. Here, we genotyped six previously published MAFLD-associated polymorphisms in healthy (n=123), nonalcoholic steatohepatitis (NASH) (n=145), NASH-associated ESLD (n=67), and ALD-associated ESLD (n=54) cohorts, and in combination with other clinical parameters [age, sex, BMI, type 2 diabetes (T2DM) status, ethnicity], analyzed their contribution to the progression of ESLD using multinomial logistic regression. The model revealed different sets of genetic and clinical risk factors associated with the progression from healthy to NASH, from NASH to NASH-associated ESLD, and from healthy to ALD-associated ESLD. PNPLA3 (p=0.005) and TM6SF2 (p=0.003) minor alleles, BMI (p=0.000), and age (p=0.021) were positive predictors for the progression from healthy to NASH. PNPLA3 minor allele (p=0.000), age (p=0.000), and having T2DM (p=0.005) were positive predictors while BMI (p=0.001) was a negative predictor for the progression from NASH to NASH-associated ESLD. PNPLA3 minor allele (p=0.000), age (p=0.001), and being male (p=0.002) were positive predictors while MBOAT7 minor allele (p=0.007) and BMI (p=0.007) were negative predictors for the progression from healthy to ALD-associated ESLD. The predictors identified to have significant associations are robust considering that the analysis was done with a limited sample size. The results suggests that, if all other parameters are kept equal, the PNPLA3 minor allele increases the overall susceptibility for the development of ESLD, the TM6SF2 minor allele increases susceptibility for the development of NASH, and the MBOAT7 minor allele protects against the development of ALD-associated ESLD. MAFLD-associated SNPs in combination with clinical risk factors, affect the progression of NASH- or ALD-associated ESLD. Genotyping of these SNPs in patients who are at risk for developing ESLD can provide a more accurate determination of susceptibility to ESLD and might encourage early lifestyle modifications and/or clinical intervention.

Limitations: The small sample size could have precluded the ability to detect other significant associations. T2DM status was excluded in some cohort comparisons due to incompleteness. Ethnicity was dropped from the analysis due to incompleteness and collinearity but the combination of genetics and BMI could serve as a proxy for ethnicity.

Clinical Research

Author: **Travis Stueber, BS**
Contact: tstueber77931@med.lecom.edu
Mentor: Erika Moore
Co-Authors: Vanya Jaitly and Erika M. Moore

Poster Number: 68

The Clinical Significance of Increased Large Cells in Marginal Zone Lymphoma

Marginal zone lymphoma is a primary, indolent small B-cell lymphoma. Subtypes include nodal, splenic, and those of extranodal mucosa-associated lymphoid tissue (MALT). These are slow growing and generally exhibit low rates of transformation to diffuse large B-cell lymphoma (DLBCL). At initial diagnosis, there can be large cells present that do not meet criteria for DLBCL. Prior studies have noted this finding but the clinical significance of these large cells has not been well established.

226 cases of marginal zone lymphoma from 1994-2021 were evaluated, including all subtypes. The number of large cells per high power field (hpf) was estimated as well as the Ki67 proliferation index and cases with <10 large cells/hpf were excluded. Other exclusionary criteria included cases where the large cells appeared to be in residual germinal centers and patients with a prior or concurrent history of DLBCL. Clinical information collected included patient demographics, staging, initial treatment and response, transformation to DLBCL, relapse, progression, progression free survival, and overall survival.

There were 33 cases with increased large cells with the majority containing >15 large cells/hpf (28/33) and 193 cases without increased large cells. Of the large cell cases, 25/28 also had Ki67>30% (Figure 1). Progression-free and overall survival were not significantly different between the two groups; however, MZL with increased large cells was associated with presentation at a higher stage ($p=0.0059$), was more likely to transform to DLBCL ($p=0.0008$), and had a greater frequency of relapse ($p=0.024$) (Table 1).

MZL with increased large cells had a significantly higher risk of transformation to DLBCL, were more likely to relapse, and were associated with a higher stage at diagnosis. Although larger studies are needed for validation, these results suggest that the presence of large cells in MZL may serve as a useful prognostic indicator and potentially help guide clinical decision making.

Clinical Research

Author: **Saurav Chopra, MD**
Contact: chopras6@upmc.edu
Mentor: Nathanael Bailey
Co-Authors: Nathanael Bailey

Poster Number: 69

Application of the International Consensus Classification and World Health Organization 5th Edition Classification to a Series of Myeloid Neoplasms

Introduction: Two new classifications of myeloid neoplasms have recently been published: the International Consensus Classification (ICC) and the 5th Edition of the World Health Organization (WHO5) Classification. We sought to examine the real-world impact of duelling classifications on patient diagnoses.

Methods: Institutional pathology database was searched and 237 specimens with a diagnosis of myeloid neoplasia were randomly selected. For each case, a classification as per the revised 4th edition of the WHO (WHOrev4), the 5th edition of the WHO (WHO5) and the ICC was assigned. The WHO5 and ICC diagnoses were compared to determine their degree of concordance.

Results: After applying the WHO5 and ICC diagnostic criteria, 134 (56.5%) cases were classified as concordant, 63 (26.6%) cases had terminological differences, 37 (15.6%) cases had minor diagnostic discrepancy, and 3 (1.3%) cases had major diagnostic discrepancy. Cases with minor diagnostic discrepancy included 25 cases of MDS, 10 cases of AML, and 2 cases of myeloid precursor lesions. Cases with major diagnostic discrepancy included 2 cases that were diagnosed as MDS, NOS, according to the ICC but classified as AML with mutated NPM1 and AML with RBM15::MRTFA according to the WHO5 and one case was characterized as chronic myelomonocytic leukemia according to the ICC and as AML with mutated NPM1 according to the WHO5.

Conclusion: This study confirms that a majority of cases are classified very similarly using the two systems. Given the overall similarity of the systems, future harmonization of the classifications should be pursued to avoid confusion and multiple diagnoses.

Clinical Research

Author: **Saurav Chopra, MD**
Contact: chopras6@upmc.edu
Mentor: Nathanael Bailey
Co-Authors: Nidhi Aggarwal and Nathanael Bailey

Poster Number: 70

Prognostic Significance of Different Mutations in Acute Myeloid Leukemia with Myelodysplasia-Related Gene Mutations

Background: Recent International Consensus Classification (ICC) of myeloid neoplasms introduced acute myeloid leukemia (AML) with myelodysplasia (MDS)-related gene mutations as a new diagnostic category, comprising AMLs with mutations in ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2 and without other genetic findings that confer better or adverse prognosis. This is an adverse-risk category of AML according to the 2022 European LeukemiaNet classification. There is limited data regarding the comparative prognosis of these individual mutations. We sought to compare the clinical outcomes in AML patients with MDS-related gene mutations, including patients currently classified as other AML subtypes.

Design: Cases of AML with mutations in MDS-related genes from 2015-21 were retrospectively identified and their clinical, histopathological, immunophenotypic, cytogenetic, and molecular data were reviewed. Study outcomes were overall survival (OS) and time to remission. Survival analysis and Cox proportional hazards regression were used to compare the study outcomes.

Results: A total of 182 patients were included in the study [mean (SD) age: 67.9 (14.3) years, female 44.5%]. Figure 1 shows the distribution of different MDS-related gene mutations. The mean (SD) follow-up period of the study was 500.5 (583.8) days. In the overall cohort, median OS was 312 days and median time to remission was 177 days. A comparison of OS and time to remission according to the number of MDS-related mutation is shown in Figure 2. Advanced age, history of other malignancy, TP53 mutation, and four MDS-related mutations (in reference to a single mutation) were associated with decreased survival, whereas stem cell transplantation and recurrent genetic abnormalities were associated with improved survival.

Conclusion: This study confirms a high mortality rate in AML with different MDS-related gene mutations and supports ICC inclusion of TP53-mutated cases into a separate category given especially poor outcomes. The study supports that the favorable prognosis of recurrent cytogenetic abnormalities may overcome the presence of MDS-related mutations. No clear survival difference was seen in the patients with both mutated NPM1 and MDS-related mutations versus MDS-related mutations alone. No clear differences in survival were seen between different MDS-related mutations, but a higher number of MDS-related gene mutations (>3) might be associated with decreased survival.

Clinical Research

Author: **Katelyn Davis, MD**
Contact: daviske8@upmc.edu
Mentor: Nathanael Bailey
Co-Authors: Nicholas R Jaeger and Nathanael Bailey

Poster Number: 71

Frequency of Clinical Diagnosis of IgG4-Related Disease After Positive Lymph Node Biopsy for Increased IgG4 Positive Plasma Cells

Background: IgG4-related disease (IgG4RD) pathologic diagnosis in lymph nodes has proven to be non-specific and challenging. Pathologic findings are better-defined in extranodal sites (dense lymphoplasmacytic infiltrate, storiform fibrosis, and phlebitis). The clinical features of IgG4RD are diverse and non-specific and can include lymphadenopathy, mass-forming lesions, autoimmune pancreatitis, and many more. Raising the suspicion for this disease as pathologists is important for clinical management as it is uniquely responsive to steroids. In this study, we evaluated lymph node biopsy specimens and sought to answer how frequently raising the suspicion for IgG4RD in lymph node pathology reports leads to a clinical diagnosis.

Design: All lymph node specimens that had been stained by immunohistochemistry for IgG4 were identified through a search of the institutional pathology report database. A total of 699 lymph node specimens with IgG4 staining were identified from 2008 to 2021. Specimens reporting negative IgG4 staining or low pathologic suspicion for IgG4 were excluded (599 cases). 100 cases underwent chart review including serum IgG4 levels, pathology reports, and clinical notes from the time of biopsy to date. 4 cases from duplicate patients were excluded. 16 cases with insufficient patient clinical documentation were excluded. 80 remaining cases were analyzed.

Results: Of the 80 cases analyzed, 83% mentioned IgG4 in the final diagnostic line of the pathology report; the remaining limited the discussion to the comment. 45% of cases (36/80) had a subsequent serum IgG4 level performed. Of those, 56% (20/36) were elevated (above 86 mg/dL). 20% (16/80) of cases had a malignant diagnosis associated with the lymph node biopsy, and none of these were subsequently diagnosed with IgG4RD. Approximately 19% (15/80) received a diagnosis of IgG4-related disease in clinical documentation after the biopsy. 80% (12/15) of those had the concern of IgG4 raised in the final diagnostic line of the pathology report versus in the comment only. 80% of those diagnosed had documentation of elevated serum IgG4 (range 119.2-492 mg/dL), and 80% of those diagnosed did not have clinical suspicion of IgG4RD prior to this biopsy.

Conclusion: A subset of cases with pathology-raised suspicion of IgG4RD led to a clinical diagnosis of IgG4RD (approximately 19%). Though this is a small subset of cases worked up for the disease, most (80%) had no clinical suspicion of the diagnosis prior to biopsy.

Clinical Research

Author: **Catherine Gestrich, MD**
Contact: gestrichck@upmc.edu
Mentor: Alyaa Al-Ibraheemi
Co-Authors: Jessica L. Davis, Laura Biederman, Ivy John, Rita Alaggio, Michael A. Arnold, Archana Shenoy, Amanda Tchakarov, and Alyaa Al-Ibraheemi

Poster Number: 72

ALK-rearranged Epithelioid Mesenchymal Neoplasm: Expanding the Spectrum of Tyrosine Kinase Altered Mesenchymal Tumors

The anaplastic lymphoma kinase (ALK) gene encodes a receptor tyrosine kinase and fusions involving this gene have been reported in a variety of mesenchymal neoplasms. ALK-altered tumors with epithelioid morphology have been described in epithelioid inflammatory myofibroblastic sarcoma, epithelioid fibrous histiocytoma, and recently reported in three mesenchymal tumors occurring in adult patients (PMID: 36125853). Herein, we are describing clinicopathologic features of 7 mesenchymal tumors with epithelioid morphology occurring predominately in the pediatric population. Clinical, histopathologic, and genomic data was collected from six hospitals. Next generation sequencing was performed on formalin fixed paraffin embedded samples. We identified 7 tumors occurring in 4 females and 3 males, with an age ranging from 1 month to 28 years (median: 17 years). Five tumors were superficial and solitary, while one presented with multiple peritoneal and omental nodules and one presented as a large mediastinal mass. The sizes ranged from <0.5 to 14.0 cm. Morphologically, all tumors were comprised of epithelioid cells arranged in sheets, anastomosing cords, or small clusters embedded in a myxohyaline stroma. The cells had slightly variably-sized ovoid nuclei with moderately-prominent nucleoli and abundant eosinophilic cytoplasm. Other morphologic features were: rhabdoid cytology (n=3), focal spindling (n=3), and round cell cytology (n=1). Mitotic figures were sparse in 4 cases and did not demonstrate necrosis. The remaining three tumors (2 deep, 1 superficial) had more than 10 mitoses per 10hpf as well as foci of necrosis. CD34 was positive in 2 cases and S100 was patchy/focally positive in 2 cases. ALK fusions were identified in all cases (table). One tumor recurred locally 2 years after initial resection, 1 patient had widely metastatic disease (mediastinal tumor). At time of last follow up (n=6): 4 patients were alive without evidence of disease, 1 died due to complications of therapy (peritoneal tumor), one was alive with disease. Our findings expand the spectrum of ALK-rearranged mesenchymal tumors. Our cases predominately occurred in older children and mainly exhibited epithelioid to round cell morphology, as opposed to spindle cell morphology and did not have characteristic features/fusions associated with epithelioid myofibroblastic sarcoma. Tumors in a deep location with high grade features follow a more aggressive clinical course.

Clinical Research

Author: **Terri Jones, MD**
Contact: jonest12@upmc.edu
Mentor: Samer Khader
Co-Authors: Daniel L Geisler, Swikrity Upadhyay Baskota, and Samer N Khader

Poster Number: 73

Accurately Making the Diagnosis of Mesothelioma Utilizing Serous Fluid Cytology Specimens: An Institutional Experience

The diagnosis of malignant mesothelioma (MM) has historically been challenging, especially on serous fluid cytology (SFC) specimens. Distinguishing between reactive and neoplastic mesothelial cells can be difficult on cytomorphologic evaluation. However, recent advances in immunohistochemistry (IHC) and fluorescence in-situ hybridization (FISH) studies have helped pathologists better determine the nature of atypical mesothelial proliferations, allowing for an accurate and reliable diagnosis of MM on these specimens. SFC has the advantages of being less invasive, not requiring general anesthesia, and frequently having a shorter turnaround time as compared to open biopsy and other surgical specimens. Additionally, SFC can yield enough material for ancillary studies, including IHC and FISH. SFC specimens diagnosed as MM, suspicious for MM (SMM), and atypical mesothelial cells (AMC) since 2010 were identified by querying the laboratory information system. Clinical data and pathologic parameters were gathered for each case. A total of 116 cases of MM, SMM, and AMC were identified. Of these, 65 cases had a definitive diagnosis of MM. Out of the 65 cases, 19 (29%) cases had an initial/primary diagnosis of MM made on SFC specimens. Ancillary studies were utilized in all 19 cases. Forty-six cases represented secondary diagnoses of MM on fluids for patients who carried a prior MM diagnosis, representing either persistent disease, recurrence, or metastasis. Average age at diagnosis was 65 years (23-87 years) with most patients being male (70%). Most cases had epithelioid morphology (80%), with biphasic morphology seen in 10 (15%) and sarcomatoid-only morphology seen in 3 (5%). In addition, 15 cases were diagnosed as SMM, with 7 of these cases representing secondary diagnoses. Thirty-nine cases were diagnosed as AMC, 23 representing secondary diagnoses. A diagnosis of MM can be made with accuracy on SFC with the appropriate cytomorphology and the aid of ancillary testing. Ancillary studies, such as mesothelial markers, BAP-1 IHC, and CDKN2A FISH can be utilized in adequately cellular specimens to make a definitive diagnosis of MM in atypical mesothelial proliferations.

Clinical Research

Author: **Dimitrios Korentzelos, MD**
Contact: korentzelosd@upmc.edu
Mentor: T. Rinda Soong
Co-Authors: Ziyu Huang, Brian Isett, Hyun Jung Park, Esther Elishaev, Ivy John, Douglas J. Hartman, Lindsey Seigh, Lauren Brilli Skvarca, Terri E Jones, Chengquan Zhao, Rohit Bhargava, Mirka W Jones, Sarah E Taylor, Anette U Duensing, and T Rinda Soong

Poster Number: 74

Integrated Clinicopathologic and Gene Expression Analysis to Profile Immune Prognostic Indicators in Uterine and Non-Uterine Leiomyosarcoma (LMS)

Background: LMS presents in a wide range of anatomic locations. Little is known about the immune microenvironments and sensitivities to immune checkpoint (IC) inhibitors across tumor sites. We aimed to characterize and compare the immune landscapes in uterine LMS (uLMS) and non-uterine/soft tissue LMS (stLMS) to identify prognostic immune markers.

Design: Immunohistochemical expression of PD-L1, TIM-3, Gal-9, LAG-3, CTLA4, MHCI, T cells and macrophages was evaluated by digital quantification in 41 uLMS and 37 stLMS. Cross-sectional analyses were performed with Fisher's exact tests and logistic regression. Log-rank tests and Cox proportional regression models with adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs) were used to assess overall survival (OS) and recurrence-free survival (RFS). Sixty-three cases yielded sufficient RNA for whole exome sequencing to study expression profiles of 12,000 genes.

Results: Median age was 58 years. More stLMS presented at higher stage than uLMS at diagnosis, and more uLMS (49%) exhibited PD-L1+ expression compared with stLMS (26%) (Fig.1). Expression of PD-L1, TIM-3, and Gal-9 was associated with high MHCI expression, as well as elevated CD8+ T cells and macrophages (Fig.1). PD-L1 CPS+ status also correlated with increased FOXP3+ T regulatory cells. Median follow-up time was 37 months (range: 1-246 months), with 41 deaths and 51 recurrences observed. Independent indicators for worse RFS included PD-L1 CPS≥5 (aHR: 3.0; 95% CI: 1.1-8.1) (Fig.2A), high MHCI expression (aHR: 5.0; 95% CI: 1.9-13.2) (Fig.2B) and stage III/IV disease (aHR: 3.1; 95% CI: 1.3-7.6) after adjusting for tumor site and confounders. PD-L1 CPS≥5 also predicted shorter OS (aHR: 3.3; 95% CI:1.1-10.4) after controlling for tumor features and adjuvant treatments. Upregulation of genes involved in IC-related functions and antigen processing was noted in LMS with PD-L1 CPS≥5 (Fig.2C). Differential expression of a subset of genes involved in DNA damage and repair (DDR) pathways was seen by different PD-L1 status (Fig.2D).

Conclusion: PD-L1 CPS≥5 was a poor prognostic indicator of LMS regardless of uterine or non-uterine tumor site. Our findings highlight differences of PD-L1 positivity in uLMS and stLMS with gene expression correlation. Differential expression of DDR genes was associated with PD-L1 status, supporting IC ligand expression and its possible interaction with the DDR pathways, suggesting a potential opportunity of combined DDR and IC targeting therapies for LMS to achieve greater treatment efficacy.

Clinical Research

Author: **Mason Marshall, MD**
Contact: marshallm5@upmc.edu
Mentor: Samer Khader and Esther Elishaev
Co-Authors: E. Elishaev and S. Khadr

Poster Number: 75

Correlation of the Detection Rate of Malignant Cells in Fluid Cytology with FIGO Stage in Primary Ovarian Clear Cell Carcinoma

Background: Ovarian clear cell carcinoma (OCCC) is a rare subtype of ovarian epithelial carcinoma. It is usually present as a minor component within more common epithelial patterns. OCCC is often unilateral and diagnosed at earlier stages when compared to other ovarian cancers. Malignant cells in pelvic or peritoneal washings, by itself, upstages the patient to at least FIGO 1C3 and indicates metastatic disease, conferring a poor prognosis. To date, there are no large studies looking at the diagnostic performance of pelvic and peritoneal fluid cytology in cases with pure OCCC.

Design: We performed a 20-year system-wide natural language search on CoPath (Cerner) to identify cases of pure OCCC with companion pelvic wash or peritoneal fluid cytology. We excluded patients with mixed epithelial carcinomas or carcinosarcomas. Cases were sorted by FIGO stage of the surgical specimen and cytologic detection rate was determined. For the cases with positive cytology the IHC profiles were recorded.

Results: Of 144 total cases, 55 had positive cytology (39% detection rate), and 4 were atypical/suspicious (2.7%). FIGO stage 1: 16/84 cases positive (19%), 0/43 1A (0%), 1 atypical (2.3%), 0/2 1B (0%), 1C 16/39 (41%), 1 atypical (2.6%). FIGO stage 2: 6/14 cases positive (43%), 1/3 2A (33%), 2B 0/6 (0%), 2C 5/5 (100%). FIGO Stage 3: 31/39 cases positive (79%), 3A 10/15 (66%), 1 atypical (6.6%), 3B 4/4 (100%), 3C 17/20 (85%). FIGO Stage 4: 2/2 cases positive (100%). There were an additional 3 cases without corresponding stage of the ovarian primary that all had negative cytology.
10 cases had IHC performed on cell blocks. BerEP4 and MOC31 were positive in 9/9 and 6/6 cases, while 0/6 and 0/3 were positive for calretinin and WT1. 3/4 were positive for PAX-8. 1/1 was positive for CK7. 4/4 were positive for HNF1-β. 1/2 cases were positive for CDX2, while 0/2 were positive for ER. TTF-1, GATA-3, CD-68, and CK20 were all negative in one case performed.

Conclusion: While there is variation within substages, OCCC detection rate in fluid cytology specimens increases as FIGO stage of the primary malignancy increases and allows for accurate staging. This is important in OCCC due to significant prognostic differences in early vs advanced lesions. Neoplastic cells were uniformly positive for epithelial markers and HNF1-β, while negative for mesothelial markers. Long-term surveillance is needed to allow appropriate sensitivity and specificity to be calculated as well as the prognostic impact of positive fluid cytology in OCCC.

Clinical Research

Author: **Simmi Patel, MD**
Contact: patelsj@upmc.edu
Mentor: Aatur D Singhi
Co-Authors: Katelyn Smith, Phoenix Bell, Christopher M. Heaphy,and Aatur D Singhi

Poster Number: 76

The Detection of Alternative Lengthening of Telomeres using a Novel Chromogenic in situ Hybridization Assay is a Poor Prognostic Biomarker for Patients with Pancreatic Neuroendocrine Tumors

Background: Alternative lengthening of telomeres (ALT) is a telomerase-independent telomere maintenance mechanism and defined by large ultra-bright, intranuclear foci of telomeric DNA using fluorescence in situ hybridization (FISH). The presence of ALT is an important prognostic biomarker for pancreatic neuroendocrine tumors (PanNETs) and correlates with early relapse-free survival (RFS). However, the routine adoption of ALT assessment within pathology laboratories has been limited due to the lack of widespread availability and technical challenges associated with telomere-specific FISH. To address these issues, chromogenic in situ hybridization (CISH) for telomeric DNA was developed and validated to assess for ALT in a large cohort of PanNETs.

Design: A chromogenic probe consisting of repetitive telomeric sequences (CCCTAA) was designed for an automated stainer and optimized using 60 leiomyosarcomas with known ALT FISH status. Similar to telomere-specific FISH, ALT by CISH was defined by large chromogenic signals within the neoplastic nuclei as compared to the nuclei within the surrounding non-neoplastic stroma. Upon optimization, 360 sporadic, primary PanNET surgical resection specimens were evaluated by telomere-specific CISH, and correlated with patient age, gender, tumor size, WHO grade, lymphovascular and perineural invasion, T- and N-stage, protein status of ATRX/DAXX and ALT by FISH, synchronous and metachronous distant metastases, and RFS.

Results: The presence of ALT by CISH was identified in 112 (31%) PanNETs and had 100% correlation with ALT status by FISH. ALT CISH correlated with large mean tumor size, increased WHO grade, advanced T- and N-stage, loss of ATRX/DAXX protein expression, and the presence of both synchronous and metachronous distant metastases ($p<0.01$). Among 271 patients without synchronous metastasis, the 5-year RFS rate for ALT CISH-positive patients was 39% as compared to 94% for ALT CISH-negative patients ($p<0.01$). By multivariate analysis, ALT CISH was a negative prognostic factor for RFS, and independent of tumor size, WHO grade, lymphovascular and perineural invasion, N-stage ($p<0.01$).

Conclusion: Using an automated stainer, a telomere-specific CISH assay has been successfully developed and can accurately detect ALT in surgically resected PanNETs. Further, ALT by telomere-specific CISH was associated with multiple adverse clinicopathologic features and was a negative, independent prognostic factor for RFS among patients with PanNETs.

Clinical Research

Author: **Pranav Patwardhan, MD**
Contact: patwardhanpp@upmc.edu
Mentor: Nidhi Aggarwal
Co-Authors: John Skaugen, Somak Roy, and Nidhi Aggarwal

Poster Number: 77

Incidental detection of JAK2 V617F mutations in next generation sequencing analysis of solid tumors – a case series

Background: Next generation sequencing (NGS) of solid tumors is rapidly expanding and is crucial for establishing or confirming a diagnosis, predicting disease prognosis, and identifying specific therapeutic targets. Occasionally, genomic alterations not associated with the targeted solid tumor may be identified, particularly those relating to hematopoietic elements that may also be present in the tissue. Several studies have reported the identification of alterations related to clonal hematopoiesis of indeterminate potential (CHIP) and myeloproliferative neoplasms (MPN). Here, we report the incidental finding of JAK2 V617F mutations in NGS of performed on several solid tumors.

Methods: Cases where JAK2V617F mutation was detected on solid tumor NGS performed in house between 2017-2021 were analyzed.

Results: Between 2017 and 2020, we identified 8 cases of solid tumor NGS performed inhouse with incidental JAK2 V617F mutations. The majority of cases (n = 7) were lung non-small cell carcinoma, with 1 additional case of renal cell carcinoma. The median patient age was 75.5 years (range 53-87). The median JAK2 variant allele frequency (VAF) was 4.7% (range 1.8-16.5). Seven patients also had solid-tumor associated mutations identified, which had significantly higher VAFs compared to those of JAK2. Three patients had a previously established diagnosis of PV. Four patients had historical laboratory data available for review, and all had elevations in hemoglobin/hematocrit (n = 2) or platelet count (n = 2). One patient underwent subsequent peripheral blood JAK2 V617F testing at an outside laboratory, which was positive. See Table 1 for additional case information.

Discussion: Identification of alterations associated with CHIP, MPN, or other hematologic disorders is uncommon but important to recognize in the setting of solid tumor NGS. Comparison of VAF to tumor-associated mutation VAF can be helpful in making the distinction, as well as review of tumor sections to identify hematopoietic elements. Additionally, many of the genes associated with CHIP or MPN are only rarely associated with solid tumors. Review of patient history and laboratory data, if available, may also be useful. Communication with clinicians, either directly or through clear language in the NGS report, may be beneficial to allow for additional work-up, if indicated.

Clinical Research

Author: **Pranav Patwardhan, MD**
Contact: patwardhanpp@upmc.edu
Mentor: TR Soong
Co-Authors: Elgohari BA, Skvarca LB, Jones TE, Garrett AA, O'Brien E, MacArthur EC, Elishaev E, Zhao C, Bhargava R, Jones MW, Vargo J, and Soong TR

Poster Number: 78

High TIM-3 Expression is an Independent Predictor of Improved Post-Radiation Therapy (RT) Clinical Outcomes of Vulvar Squamous Cell Carcinoma (SCCA)

Background: RT is a fundamental component of vulvar SCCA treatment. There has been growing appreciation on the potential of synchronizing RT and immune checkpoint (IC) inhibitors for greater treatment efficacy. We aimed to study the post-RT prognostic significance of IC molecules in vulvar SCCA, which has remained largely undefined.

Design: Immunohistochemical expression of TIM-3, PD-L1, Gal-9, MHC class I (MHCI), and extent of CD8+ tumor infiltrating lymphocytes (TILs) were evaluated in 90 vulvectomy specimens from patients who received RT. TIM-3, PD-L1 and Gal-9 expression were assessed via Tumor Proportion Score (TPS) and Combined Positive Score (CPS). Cross-sectional analyses were performed with Fisher's exact tests. Post-RT overall survival (OS) and recurrence-free survival (RFS) were examined via log-rank tests and Cox proportional regression models with adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs).

Results: Median age was 70 years. Over half were of FIGO stage 3 or 4 disease, with 43% being p16+ and 22% having lymph node metastases. TIM3 TPS+, PDL1 TPS+, and Gal9 TPS+ status was seen in 65%, 52% and 52% of cases. TIM-3 CPS ≥5 was observed in 68% of tumors, and was significantly correlated with larger (>2 cm) tumor size, high expression of PD-L1, Gal-9 and MHCI, as well as extent of CD8+ TILs (Table 1). No significant associations of PD-L1 or Gal-9 with characteristics other than MHCI expression or TIL density were seen. Most (62%) received RTs for adjuvant therapy, while others were treated for neoadjuvant (20%), definitive (12%), and palliative (6%) purposes. Median post-RT follow-up time was 26 mons (range: 4-130 mons). TIM-3 CPS ≥5 and age>60 years were correlated with improved post-RT OS and RFS, while p16+ status showed borderline associations (Figure 1). Only TIM-3 CPS≥5 independently predicted better post-RT OS after confounding adjustment. Statistically significant predictors for improved post-RT RFS included TIM-3 CPS ≥5 (aHR: 0.2; CI: 0.1-0.5) and receipt of chemotherapy (aHR: 0.2; CI: 0.1-0.7); while FIGO stage ≥3 (aHR: 3.0; 1.2-7.4) and age>60 years (aHR: 3.3; CI: 1.2-9.0) predicted worse post-RT RFS after controlling for other tumor features.

Conclusions: TIM-3 CPS≥5 is an independent indicator of improved post-RT prognosis and is expressed by a significant portion of vulvar SCCA. The findings warrant further assessment of TIM-3 as an alternative IC therapeutic target in combination with RT in patients who attain only partial response to RT.

Clinical Research

Author: **Louis Samson, MD**
Contact: samsonl@upmc.edu
Mentor: Miguel Reyes-Mugica
Co-Authors: Stephanie Worrell, Emilio Medina-Ceballos, Lauren B. Skvarca, Natalie I. Larsen, Nicole N. Balmer, Ona Marie Faye-Petersen, Robert Bendon, Stewart Cramer, Cláudia M. Salgado, and Miguel Reyes-Múgica

Poster Number: 79

Aberrant neural crest cells migration leads to melanocyte presence in the umbilical cord

Background: Systematic evaluation of the umbilical cord is an integral part of placental examination. Among the important parameters of this exam is the assessment of visible lesions such as knots and discoloration. The latter can be due to funisitis (white), meconium staining (green), vascular congestion (red-blue), or maternal hyperbilirubinemia (yellow). This case series presents twelve examples of umbilical cord lesions characterized by foci of brown-black discoloration caused by melanin deposition.

Methods: Placental pathology reports from our institution mentioning pigment, melanocytes or melanin were reviewed, and the slides pulled for histologic analysis by two or more members of the pathology team. Immunohistochemical (SOX10, Melan-A, PHOX2B, and CD56) and histochemical (Fontana-Masson) staining was performed retrospectively on the umbilical cord sections.

Results: Twelve cases of umbilical cord with grossly visible brown linear stippling or punctate discoloration were confirmed. Microscopic examination showed scattered SOX10-positive melanocytes in the amnion layer with melanin deposition and uptake by the amniocytes. Eight of the twelve infants and none of the mothers had congenital melanocytic lesions.

Conclusion: This is the first description of melanocytes and melanin pigment in the umbilical cord. We believe that an aberrant migration of neural crest cells into the umbilical cord, with subsequent differentiation into melanocytes, is the most likely pathogenesis.

Clinical Research

Author: **Kotaro Takeda, MD, PhD**
Contact: takedak@upmc.edu
Mentor: Gabriela M. Quiroga-Garza
Co-Authors: Sheldon Bastacky, Rajiv Dhir, and Gabriela M. Quiroga-Garza

Poster Number: 80

BAP1 is the most common disrupted gene in VHL mutation-negative advanced clear cell renal cell carcinoma

Background: Clear cell renal cell carcinoma (ccRCC) is the most common malignant renal neoplasm in adults, and an advanced pathological stage is associated with a poor prognosis. Approximately 90% of ccRCC cases are caused by VHL gene abnormalities, such as gene mutations and 3p loss. However, around 10% of ccRCC cases do not have VHL gene abnormalities. Additionally, other gene abnormalities, such as BRCA1-associated protein 1 (BAP1), one of the chromatin remodeling genes, are involved in tumor progression. We conducted a study to determine (1) the most commonly disrupted gene in VHL mutation-negative ccRCC and (2) the histologic features of BAP1-mutated ccRCC.

Design: We conducted a retrospective review of morphologically diagnosed ccRCC with an advanced pathological stage (pT3 or pT4) between January 2020 and July 2022 and analyzed VHL gene mutations, as well as an additional 161 commonly mutated genes in cancer, using Oncomine testing. The histologic features, the presence of sarcomatoid and rhabdoid morphology and lymphovascular invasion, were analyzed.

Results: We analyzed 68 cases of advanced ccRCC. Eight cases (12%) corresponded to VHL mutation-negative ccRCC. BAP1 was the most commonly disrupted gene in this group, with BAP1 abnormalities identified in four cases (50%): one case of BAP1 mutation and three cases of BAP1 copy number loss. Other gene abnormalities included ARID1A, SETD2, PTEN, TERT, and p53. In contrast, in 60 cases (88%) of VHL mutation-positive ccRCC, BAP1 abnormalities (mutations and copy number loss) were found in 16 cases (26.6%). When VHL mutation and BAP1 mutation coexist, ccRCC tended to have high-grade histologic features (such as rhabdoid features) and a more frequent papillary growth pattern, as well as more frequent lymphovascular invasion, compared to BAP1 mutation-negative ccRCC.

Clinical Research

Author: **Rachel Vanderschelden, MD**
Contact: rachelkvds@gmail.com
Mentor: Rohit Bhargava
Co-Authors: Jacob A Jerome, Daniel Gonzalez , Lindsey Seigh, Gloria J Carter, Beth Z Clark, Esther Elishaev, Jeffrey Louis Fine, Lakshmi Harinath, Mirka W. Jones, Tatiana M. Villatoro, T Rinda Soong, Jing Yu , Chengquan Zhao, Doug Hartman, and Rohit Bhargava

Poster Number: 81

Implementation of Digital Image Analysis in Assessment of Ki-67 Index in Breast Cancer

Background: The clinical utility of the proliferation marker Ki67 in breast cancer treatment and prognosis is an active area of research. A standard scoring method has not been established, and differences among scoring methods account for much of the variability in Ki67 reporting. In addition, Ki67 determination can be time intensive for the pathologist. Therefore, use of digital image analysis (IA) tools may help to both standardize reporting and improve workflow. IA-assisted scoring has been shown to improve intra-observer agreement compared to use of the “eyeballing” method. We were interested in comparing the performance of an IA-assisted model to a robust manual scoring technique in a real-world setting.

Design: Using a prospective approach, we analyzed breast core biopsies and resection specimens signed out at our institution from January through July 2022. Whole slide images were acquired at 40x (0.25 micron/pixel resolution), and breast pathologists at our institution digitally selected areas of the Ki67-stained slides to be analyzed. An image analysis algorithm was developed using the Leica Aperio Nuclear Staining Algorithm for Immunohistochemistry and performed on the selected regions. The pathologists then verified the IA output against the digital image and provided an “IA-assisted final sign out index”. We compared this “IA-assisted” index to a Ki67 index determined by manual count of 500 cells from the same pre-selected areas. The Intraclass Correlation Coefficient (ICC) was calculated using R studio, and GraphPad Prism was used for Bland-Altmann analysis.

Results: 280 specimens were analyzed, representing 252 patients. Patients ranged in age from 28 to 105 years. Tumors represented all molecular subtypes and Nottingham grades (Table 1). The average difference in Ki67 index between manual counting and IA was small at 0.14 (95% limits of agreement: -12.25 - 12.54) (Figure 1). The ICC was 0.96 (95% confidence interval: 0.95 - 0.97), indicating excellent agreement (ICC >0.9) between the two methods.

Conclusion: Our study demonstrates strong performance of an IA-assisted Ki67 quantitation method in a large cohort of breast cancer cases. Pathologists with varying expertise in IA were able to digitally annotate slides and verify Ki-67 values with minimal alteration to their existing workflow. This suggests that IA-assisted Ki67 analysis can be a useful tool to streamline workflow, and is accurate even when used in real-world clinical conditions.

Clinical Research

Author: **Rachel Vanderschelden, MD**
Contact: rachelkvds@gmail.com
Mentor: Kenichi Tamama
Co-Authors: Simmi Patel and Kenichi Tamama

Poster Number: 82

Determining novel biomarker candidates associated with chronic drug exposure by exploring existing unused data acquired through urine comprehensive drug screening

Substance abuse is a growing health concern in the United States. In clinical settings, drugs and their metabolites are primarily used as biomarkers of acute drug exposure. However, clinicians lack biomarkers for chronic drug exposure, which could help to identify patients who are at high risk for addiction and better predict disease trajectories and treatment outcomes.

We sought to identify novel substance abuse-associated biomarker candidates by exploring unused data previously obtained through untargeted liquid chromatography high resolution mass spectrometry for urine comprehensive drug screening performed at the UPMC Clinical Toxicology Laboratory. Through medical chart review, we analyzed 363 cases and categorized patients by the results of enzyme immunoassay-based urine drug screening panel and age. Preprocessing of the dataset (including peak identification, alignment, and putative annotation) was performed using MS-DIAL; further statistical analysis was performed using MetaboAnalyst 5.0.

The first five components identified by principal component analysis explain approximately 36% of the variance of the dataset, and show association with age; however they do not appear to separate cases based on enzyme immunoassay results. Point-biserial correlation analysis performed between variables and enzyme immunoassay results identified a set of variables correlated with prior exposure to several illicit drugs, including cocaine (Figure).

Untargeted liquid chromatography high resolution mass spectrometry data routinely obtained through comprehensive drug screening is a rich source of potential biomarker candidates for chronic drug exposure. Initial analysis identified a set of biomarkers associated with illicit drug exposure, and further chemical identification may elucidate patterns of drug addiction and treatment response.

Clinical Research

Author: **Megan Zilla, MD, PhD**
Contact: zillaml@upmc.edu
Mentor: Rana Naous
Co-Authors: Ivy John and Rana Naous

Poster Number: 83

Loss of Histone H3K27 Trimethylation (H3K27me3) Expression as a Potential Diagnostic Pitfall in Sarcomatoid Carcinoma

Background: Loss of histone H3K27 Trimethylation (H3K27me3) immunohistochemical expression is commonly used as an ancillary test and a surrogate marker for the diagnosis of malignant peripheral nerve sheath tumor (MPNST). A potential histological mimics of MPNST is sarcomatoid carcinoma. Prompted by an index case of sarcomatoid carcinoma with H3K27me3 loss and the lack of literature on such cases, we sought to determine the frequency of H3K27me3 loss of expression in a cohort of sarcomatoid carcinomas.

Design: Twenty-two cases of primary or metastatic sarcomatoid carcinoma with spindle cell morphology mimicking MPNST were prospectively and retrospectively retrieved from our institutional archives. Analysis of additional cases is ongoing. The selected cases were stained with an antibody to H3K27me3 (Cell Signaling Technology, C36B11). The staining was independently assessed by the authors and scored as retained (less than 25% of tumor cells with loss of H3K27me3), lost (more than 95% of tumor cells with loss of nuclear staining), or partially lost (25% to 95% of tumor cells with loss of nuclear staining). If there were differences in scoring, the majority score was used.

Results: The patient population was 41% female and 59% male with a mean age of 74 years (range 43-89). Fifteen cases were primary tumors and seven were metastatic tumors. The primary tumor cases included bladder (5), kidney (4), esophagus (2), prostate (2), forearm skin (1) and oral cavity (1). The metastatic sites included bone (3; 2 from kidney and 1 unknown primary), axilla (2; 1 from forearm skin and 1 unknown primary), thigh soft tissue (1; unknown primary), and forearm soft tissue (1; unknown primary). Evaluation of H3K27me3 immunostaining in our cohort demonstrated complete loss of staining in 2 cases (9%), partial loss in 10 cases (45%) and retention in 10 cases (45%), (Figure 1). The cases with loss of H3K27me3 staining were primary tumors and were from the oral cavity (1) and bladder (1).

Conclusion: Our preliminary studies indicate that loss of H3K27me3 immunostaining in sarcomatoid carcinomas may occur in approximately 9% of cases. This poses as a potential diagnostic pitfall especially in challenging cases with absent keratin staining. Our findings suggest that complete loss of H3K27me3 in sarcomatoid carcinoma should be interpreted cautiously in the setting of a broad immunopanel within the appropriate clinical context. Additionally, more studies are needed to establish the prognostic significance of these

Congratulations to
2022 Retreat Trainee Poster Award Winners!

General Session	*Sponsored by Department of Pathology*
Outstanding Poster Award	Richard Herron, Katherine Killian, Jeffrey Kleinberger, Philip Mannes, Akram Shalaby, and Sierra Wilson
Merit Poster Award	Daniel Geisler, Taylor Gatesman, Matthew Halbert, Ryan LeGraw, Azfar Neyaz, and Yuehua Zhu

Liver Research	*Sponsored by Pittsburgh Liver Research Center*
Outstanding Poster Award	Meghan Mooring
Merit Poster Award	Chhavi Goel

CATER T32 Training Program	*Sponsored by Dr. Paul Monga*
Outstanding Poster Award	Daniel Zuppo
Merit Poster Award	Alexis Nolfi